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Cent ans de déclin des bourdons en Belgique

Influence du climat et de l'occupation du sol

Dissertation présentée par

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en vue de l'obtention du diplôme de Docteur en Sciences

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« *La nature est un professeur universel et sûr pour celui qui l'observe*. »

Carlo Goldoni (1707-1793), auteur dramatique italien

« *C'est une triste chose de penser que la nature parle et que le genre humain n'écoute pas*. »

Victor Hugo (1802-1885)

« *La bibliothèque de la vie brûle et nous ne connaissons même pas les titres des livres*. »

Gro Harlem Brundtland (née en 1939), ex-Première Ministre norvégienne

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Chapitre 2

2. **Vray S.**, Lecocq T., Roberts S.P.M., Rasmont P. 2017. Endangered by laws: potential consequences of regulations against thistles on bumblebee conservation. Annales de la Société entomologique de France (N.S.) 53:33–41.

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II. Iserbyt S., **Vray S.**, Dendoncker N., Viart S., Rasmont P. 2015. High-resolution distribution of bumblebees (*Bombus* spp.) in a mountain area marked by agricultural decline. Annales de la Société Entomologique de France 51 : 375–391.

III. Maebe K., Meeus I., **Vray S.**, Claeys T., Dekoninck W., Boevé J.-L., Rasmont P., Smagghe G. 2016. A century of temporal stability of genetic diversity in wild bumblebees. Scientific Reports 6 : 38289.

IV. Marshall L., Biesmeijer J., Rasmont P., Vereecken N., Dvorak L., Fitzpatrick U., Francis F., Neumayer J., Ødegaard F., Paukkunen J.P.T., Pawlikowski T., Reemer M., Roberts S.P.M., Straka J., **Vray S.**, Dendoncker N. 2017. The interplay of climate and land use change affects the distribution of EU bumblebees. Global Change Biology 24 : 101-116.

Summary

It is widely acknowledged that climate change and habitat loss and fragmentation are among the most important drivers of global biodiversity decline. A deeper understanding of the mechanisms involved in the responses of populations and communities to these environmental changes is a prerequisite to project and mitigate the long-term effects of these changes on biodiversity. Bumblebees (*Bombus* genus), which play a widely recognized and essential role on the pollination ecosystem service, have seen their populations drastically decreasing for decades around the world. The causes advanced include the reduction of floral resources, the degradation and fragmentation of natural habitats through urbanization and agricultural intensification, and more recently climate change. In order to project and mitigate the consequences of these environmental changes on future assemblages of bumblebees, it is important to understand how these changes have influenced their assemblages in the past. Belgium is a particularly suitable country for this purpose, as it represents a typical example of the environmental changes observed in Western Europe, and its bumblebee fauna has been studied for more than 100 years.

The present work aims to determine what changes in Belgian bumblebee assemblages occurred during the last century and to assess the impact of environmental changes on these modifications. For this, four major questions are addressed in the form of separate chapters: (1) What changes in bumblebee assemblages can be observed over the last century, and which species are the most

affected by the decline? (2) What importance do thistles (*i.e*. Cardueae) have in the diet of bumblebees, and could the legislations against thistles be a threat for their conservation? (3) Could the changes in landscape composition and structure explain the changes in bumblebee assemblages at the local scale? (4) Can the historical distribution of bumblebee species at the country scale be defined by climate and land cover, and which variables most influence their distribution?

The modifications of Belgian bumblebee assemblages, discussed in Chapter 1, are studied between three time periods: 1910-1930, 1970-1989, and 1990-2016. Various indicators of species richness and diversity are evaluated and all show a global regression over the century, more or less accentuated depending on the regions. Population trend analyses of each bumblebee species, based on abundance and range size, show that between 68 and 88% of species have been declining over the last century. Only few species tend to increase their relative abundance and dominate the others, leading to a homogenization of the assemblages. These differences in population trends are correlated with the species ecological traits. Our results show that the most declining species are those with a longer tongue (and therefore more associated with plants with long corolla), a more restricted diet, a higher habitat specialisation with a preference for open habitats, an overground nesting, developing smaller colonies, and emerging later in the season.

In Chapter 2, our results reveal the great importance of thistles in the diet of male bumblebees. We show that a high number of species, many of which are rare in Belgium and Europe, depend largely on the four thistle species for which the destruction is legislatively mandatory in several European countries including

Belgium. Such laws could therefore negatively affect bumblebee populations, already greatly weakened by global environmental changes. We argue for the abolishment of these legislations in favour of alternative measures that reconcile the conservation of biodiversity and agricultural needs.

In Chapter 3, we compare current high-resolution land use and bumblebee assemblages in four ancient municipalities of Belgium to those of a century ago (1910-1930), before the great land use changes (*i.e*. urbanisation and agricultural intensification). We show that the landscape composition and structure can partly explain the composition and the species richness of the bumblebee assemblages. As expected, the most anthropogenic landscapes (*i.e*. having the highest density of settlement areas and crops) are those where the bumblebee richness declined the most during the century, and where agriculture has intensified the most. The only locality with a bumblebee assemblage similar to the past experienced the least landscape change and is still constituted of a large proportion of forests, orchards, grasslands, and hedgerows. We also suggest the likely positive impact of spontaneous spawning heaps in the most anthropogenic landscapes, as well as the importance of heathland for several species.

In Chapter 4, we model the Belgian distribution of bumblebee species over the last century (*i.e*. same time periods as in Chapter 1) using land cover and bioclimatic variables reflecting averages and extremes of temperature and precipitation. Overall, the models show a good level of performance, which seems however lower for species with a larger distribution area covering the majority of the country. Regarding the importance of land cover in defining the distribution of bumblebee

species, we note in particular the increasing influence of settlement areas. With the exception of the currently abundant species, the species spread across the country a century ago have now deserted the regions with a high density of settlement and cropland areas in the north of the country. As in Chapter 3, these results confirm the negative impacts of urbanisation and agricultural intensification on bumblebee assemblages shown by previous studies. Concerning the bioclimatic variables, our results show that (lack of) precipitation plays a predominant role in defining bumblebee distribution. In addition to the deleterious effects of high temperatures reported by other studies, this could suggest a significant negative impact of drought events on bumblebees.

In conclusion, this dissertation allowed to establish the state of conservation of each bumblebee species in Belgium by analysing the historical changes of their populations during the century. This work has also provided new insights into the impact of land cover and climate changes on bumblebee assemblages. It highlights the great value of long-term monitoring of species and environmental changes in order to be able to draw up a picture of their current state, project their future changes, and lay the groundwork for future studies.

For future research, we emphasize the importance of better knowing and understanding the ecological characteristics of species (*e.g*. dispersal abilities, habitat preferences, interactions between species), which can strongly influence their responses to global changes. We have seen that cropland area alone cannot account for intensification of agriculture and that other parameters reflecting the intensity of land use must be taken into account in impact assessment studies. More

generally, as species' responses to global changes depend on the intimate interactions between the different environmental factors, we advise future studies to consider all of these parameters together rather than in isolation in order to improve the understanding of the ecological processes at work and the likelihood of future projections.

Résumé

Il est communément admis que le changement climatique ainsi que la perte et la fragmentation des habitats sont parmi les moteurs les plus importants du déclin de la biodiversité mondiale. Une compréhension plus poussée des mécanismes impliqués dans la réponse des populations et des communautés à ces changements environnementaux est un prérequis pour la prévision et la réduction des effets à long terme de ces changements sur la biodiversité. Les bourdons (genre *Bombus*), dont le rôle primordial pour le service écosystémique de pollinisation est largement démontré, voient leurs populations drastiquement régresser depuis des décennies à travers le monde. Les causes avancées impliquent notamment la diminution des ressources florales, la dégradation et la fragmentation des habitats naturels au travers de l'urbanisation et de l'intensification agricole, et plus récemment le changement climatique. Afin de pouvoir prédire et mitiger les conséquences de ces changements environnementaux sur les assemblages de bourdons dans le futur, il est important de comprendre comment ces changements ont influencé leurs assemblages dans le passé. La Belgique est un pays particulièrement adéquat pour cette recherche, car elle représente un exemple typique des changements environnementaux observés en Europe Occidentale et sa faune de bourdons y est étudiée depuis plus de cent ans.

Le présent travail vise à déterminer quelles ont été les modifications dans les assemblages de bourdons belges durant le 20ème siècle et à évaluer l'impact qu'ont pu avoir les changements environnementaux sur ces modifications. Pour cela,

quatre grandes questions sont abordées sous forme de chapitres distincts: (1) Quels changements dans les assemblages de bourdons sont observés au cours du siècle dernier, et quelles espèces sont les plus touchées par le déclin? (2) Quelle importance ont les chardons (*i.e.* Cardueae) dans la diète des bourdons, et est-ce que les lois d'échardonnage pourraient menacer leur conservation? (3) Les changements dans la composition et la structure du paysage pourraient-ils expliquer les modifications des assemblages de bourdons à l'échelle locale? (4) La distribution historique des espèces de bourdons à l'échelle du pays peut-elle être définie par le climat et l'occupation du sol, et quelles variables influencent le plus leur distribution ?

Les modifications des assemblages belges de bourdons, abordées dans le Chapitre 1, sont étudiées entre trois périodes de temps : 1910-1930, 1970-1989, et 1990-2016. Divers indicateurs de richesse et de diversité sont évalués et montrent tous une régression globale au cours du siècle, plus ou moins accentuée selon les régions. Les analyses de tendance de population de chaque espèce de bourdon, basées sur les abondances et sur les aires de distribution, montrent qu'entre 68 et 88% des espèces sont en déclin depuis un siècle. Quelques-unes seulement tendent à augmenter leur abondance relative et dominer les autres, entrainant une homogénéisation des assemblages. Ces différences de tendances de populations sont corrélées aux caractéristiques écologiques des espèces. Nos résultats montrent que les espèces qui ont le plus décliné sont celles qui ont une plus longue langue (et donc plus associées aux plantes à longue corolle), une diète plus restreinte, une plus grande spécialisation de l'habitat avec une préférence pour les

habitats ouverts, qui nichent à la surface du sol, qui développent des colonies plus petites et qui émergent plus tard dans la saison.

Dans le Chapitre 2, nos résultats révèlent la grande importance des chardons dans la diète des mâles de bourdons. Nous montrons qu'un nombre élevé d'espèces, dont plusieurs sont rares en Belgique et en Europe, dépend en grande partie des quatre espèces de chardons dont la destruction est rendue obligatoire par des lois d'échardonnage dans plusieurs pays européens dont la Belgique. De telles lois peuvent donc négativement affecter les populations de bourdons, déjà très affaiblies par les changements environnementaux globaux. Nous préconisons l'abolissement de ces lois d'échardonnage en faveur de mesures alternatives qui concilient la conservation de la biodiversité et les besoins agricoles.

Dans le Chapitre 3, nous comparons l'occupation du sol et les assemblages de bourdons actuels dans quatre anciennes communes de Belgique à ceux d'il y a un siècle (1910-1930), c'est-à-dire avant les grands changements d'occupation du sol (*i.e*. urbanisation et intensification agricole). Nous montrons que la composition et la structure du paysage peuvent expliquer en partie la composition et la richesse spécifique des assemblages de bourdons. Comme attendu, les paysages les plus anthropiques (*i.e*. présentant la plus haute densité de zones urbaines et de cultures) sont ceux où la richesse en bourdons a le plus décliné au cours du siècle, et où l'agriculture s'est la plus intensifiée. La seule localité arborant une faune de bourdons similaire au passé est celle dont le paysage a le moins changé et est toujours constitué d'une grande proportion de forêts, de vergers, de prairies et de bocage. Nous suggérons également l'impact positif probable des terrils en

succession spontanée dans les paysages les plus anthropiques, ainsi que l'importance des landes pour plusieurs espèces.

Dans le Chapitre 4, nous avons modélisé la distribution belge des espèces de bourdons au cours du siècle dernier (*i.e.* mêmes périodes que le Chapitre 1) en utilisant des variables d'occupation du sol et des variables bioclimatiques reflétant les moyennes et les extrêmes de température et de précipitations. Dans l'ensemble, les modèles montrent un haut niveau de performance, qui semble néanmoins plus faible pour les espèces présentant une grande aire de distribution couvrant la majorité du pays. En ce qui concerne l'importance de l'occupation du sol dans la définition de la distribution des espèces de bourdons, nous remarquons en particulier l'emprise croissante des zones urbaines. A l'exception des espèces restées abondantes, les espèces répandues à travers le pays il y a un siècle ont aujourd'hui déserté les régions à forte densité urbaine et agricole présentes dans le nord du pays. Comme dans le Chapitre 3, ces résultats tendent à confirmer les répercussions négatives de l'urbanisation et de l'intensification agricole sur les assemblages de bourdons montrées par les études précédentes. En ce qui concerne les variables climatiques, nos résultats montrent que les (ou le manque de) précipitations ont un rôle prédominant dans la définition de la distribution des bourdons. En plus des effets délétères des températures élevées rapportés par d'autres études, ceci pourrait suggérer un impact négatif non négligeable des épisodes de sécheresse sur les bourdons.

En conclusion, cette dissertation a permis d'établir l'état de conservation de chaque espèce de bourdon en Belgique en analysant les changements historiques

de leurs populations au cours du siècle. Ce travail a également apporté de nouveaux éléments dans la compréhension de l'impact qu'ont pu avoir les changements d'occupation du sol et du climat sur les assemblages de bourdons dans le passé. Il met en évidence le grand intérêt du monitoring à long terme des espèces et des changements environnementaux pour pouvoir dresser un tableau de leur état actuel, prévoir leurs changements à venir, et établir des bases auxquelles les études futures pourront se référer.

Pour les recherches futures, nous soulignons l'importance de mieux connaitre et comprendre les caractéristiques écologiques des espèces (*e.g*. capacités de dispersion, préférences d'habitat, interactions entre espèces), qui peuvent fortement influencer leurs réponses aux changements globaux. Nous avons vu que, puisque la surface en cultures ne peut à elle seule relater l'intensification de l'agriculture, d'autres paramètres reflétant l'intensité d'utilisation du sol doivent être pris en compte lors des études d'impact. Plus globalement, étant donné que les réponses des espèces aux changements globaux dépendent des interactions intimes entre les différents facteurs environnementaux, nous conseillons aux études futures de considérer tous ces paramètres ensemble plutôt qu'isolément afin d'améliorer la compréhension des processus écologiques à l'œuvre et la vraisemblance des projections futures.

Introduction générale

Introduction générale

1. Les grands facteurs de déclin de la biodiversité

Notre planète subit actuellement des changements environnementaux globaux, engendrant extinctions et modifications de l'aire de distribution des espèces d'une grande variété de taxons, et affectant l'intégrité même des écosystèmes (Vitousek 1994 ; Pimm *et al.* 1995 ; Walther *et al.* 2002 ; Parmesan & Yohe 2003 ; Millennium Ecosystem Assessment 2005 ; Tylianakis *et al.* 2008 ; Butchart *et al.* 2010). Les principaux moteurs de ces changements globaux sont connus et regroupés sous la dénomination de « *global environmental change* » (GEC), qui incluent notamment pour les écosystèmes terrestres :

- les dépôts d'azote assimilable, responsables de l'eutrophisation des milieux naturels et de l'homogénéisation de la flore ;
- l'enrichissement atmosphérique en dioxyde de carbone et les changements climatiques qui en découlent en partie ;
- les changements d'occupation et d'utilisation du sol, dont découlent destruction, dégradation et fragmentation des habitats naturels ;
- les perturbations des interactions biologiques, y compris les invasions par des espèces exotiques envahissantes ;
- les exploitations et extractions des ressources naturelles.

Il est communément admis que ces moteurs principaux interagissent entre eux pour former un cocktail anthropogène néfaste pour la biodiversité (Travis 2003 ; Memmott *et al.* 2007 ; Brook *et al.* 2008 ; Tylianakis *et al.* 2008 ; Oliver & Morecroft

2014 ; Scherber 2015), et que leurs effets seront de plus en plus importants tant que l'exploitation humaine se prolongera (Pimm *et al.* 1995 ; Sala *et al.* 2000 ; Pereira *et al.* 2010). En Europe, la synergie des changements du climat et de l'occupation du sol a déjà causé le déclin de populations d'une multitude de taxons (Travis 2003) et ces deux moteurs sont actuellement reconnus comme les menaces les plus sérieuses pour la biodiversité (Sala *et al.* 2000 ; Oliver & Morecroft 2014).

1.1. Perte et fragmentation des habitats

Plusieurs définitions existent pour qualifier l'habitat d'une espèce (Hall *et al*. 1997). Une des définitions les plus récentes et utilisées désigne l'habitat d'une espèce comme étant l'ensemble des ressources et conditions biophysiques nécessaires à l'accomplissement de son cycle de vie, y compris sa reproduction (Hall *et al*. 1997 ; Dennis *et al*. 2003). Il peut être constitué de différents types de milieu qui répondent chacun à un besoin particulier de l'espèce en ressources au cours de son cycle de vie, que ce soit par exemple en termes de ressources alimentaires, de partenaires sexuels, ou de sites de nidification. La taille d'une parcelle d'habitat doit être suffisamment grande pour contenir la quantité de ressources et de conditions favorables nécessaires à la persistance d'une population. De plus, la connectivité de cette parcelle d'habitat avec les parcelles d'habitat voisines doit pouvoir permettre les déplacements (*i.e.* la dispersion) d'individus entre les différentes populations hébergées par ces parcelles (Correa Ayram *et al.* 2016), constituant ainsi une métapopulation (Hanski & Gilpin 1991). Ces échanges d'individus entre populations permettent des flux de gènes qui assurent une structure génétique

diversifiée et donc de plus grandes possibilités adaptatives face aux changements environnementaux globaux.

Cependant, les modifications de l'occupation du sol et de la structure du paysage engendrées par les activités humaines peuvent causer une perte, une détérioration ou une fragmentation des habitats naturels (Fischer & Lindenmayer 2007), générant un grand nombre d'effets négatifs sur la biodiversité (Fahrig 2003). En plus d'impacter directement l'abondance et la distribution des populations (Lande 1987 ; Hanski *et al.* 1996 ; Venier & Fahrig 1996), la perte d'habitat affecte la richesse spécifique et les interactions interspécifiques au sein des communautés (Steffan-Dewenter *et al.* 2002 ; Polus *et al.* 2006 ; Bergerot *et al.* 2010). Résultant fréquemment de la perte d'habitat, la fragmentation de l'habitat est un processus à l'échelle du paysage au cours duquel une surface d'habitat est transformée en un certain nombre de parcelles d'habitat de superficie réduite, isolées les unes des autres par une matrice différente de l'habitat initial (Wilcove *et al.* 1986 ; Lord & Norton 1990). Les espèces qui survivent dans les parcelles d'habitat résiduelles sont donc confrontées à un environnement de surface réduite, une augmentation de l'isolement, et à de nouvelles frontières et interactions écologiques (Figure 1 ; Fahrig 2002 ; Fischer & Lindenmayer 2007). La fragmentation de l'habitat affecte donc le fonctionnement des populations de deux manières : d'une manière directe en réduisant l'étendue d'habitat disponible et donc le nombre d'individus hébergés, et d'une manière indirecte en réduisant les échanges d'individus (et donc d'allèles) entre les populations. Les petites populations isolées qui en résultent sont donc intrinsèquement plus vulnérables

aux extinctions locales dues aux événements stochastiques environnementaux, démographiques et génétiques (Gilpin & Soulé 1986 ; Andrén 1994 ; Frankham *et al.* 2002 ; Brook *et al.* 2002 ; Fischer & Lindenmayer 2007 ; Krauss *et al.* 2010). Ces extinctions locales de populations sont les premières étapes vers l'extinction complète d'une espèce à l'échelle globale (Ceballos & Ehrlich 2002 ; Sinervo *et al.* 2010 ; Collen *et al.* 2011).

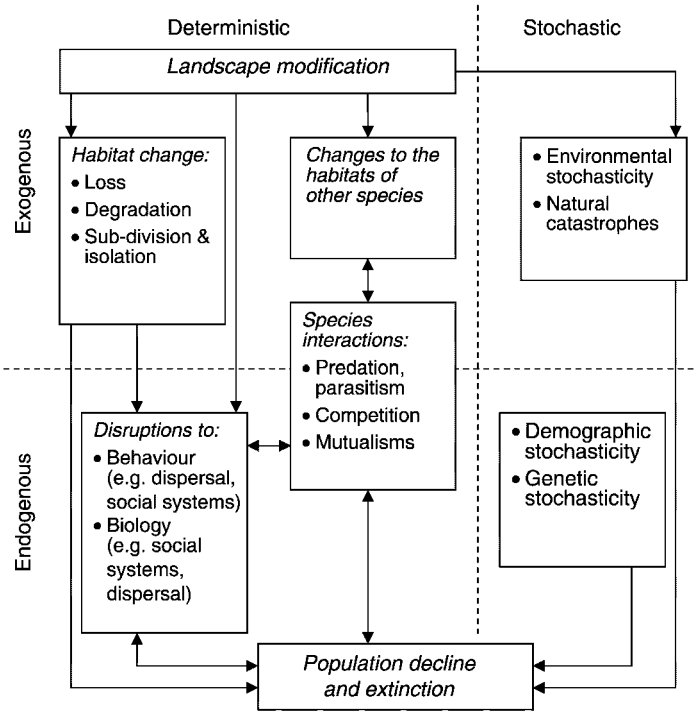

Figure 1. Menaces provenant d'une modification du paysage pour une espèce en déclin. Les processus déterministes conduisent à un déclin, tandis que les processus stochastiques sont issus d'évènements aléatoires qui peuvent aggraver ce déclin. Au contraire des processus endogènes, les processus exogènes sont externes à la biologie d'une espèce. D'après Fischer & Lindenmayer 2007.

1.2. Changements climatiques

Depuis 100 ans, la littérature scientifique s'intéresse à l'influence du climat sur la distribution géographique des espèces (Grinnell 1917 ; Andrewartha & Birch 1954 ; MacArthur 1972 ; Vitousek 1994). Le fait que le climat et les phénomènes

météorologiques extrêmes affectent les espèces végétales et animales est largement reconnu (voir une synthèse dans Parmesan 2006). Chaque espèce possède sa propre gamme de conditions climatiques sous lesquelles elle peut persister (*i.e.* sa niche climatique). Si les conditions climatiques de son lieu de vie changent et lui deviennent défavorables, trois types de réponse lui sont possibles : suivre le déplacement de sa niche climatique par la dispersion, rester sur place et s'adapter (comportement, phénologie, génétique), ou disparaître. La réponse de l'espèce au changement dépendra en grande partie de la vitesse et de l'amplitude de ce changement (Figure 2 ; Huntley *et al.* 2010).

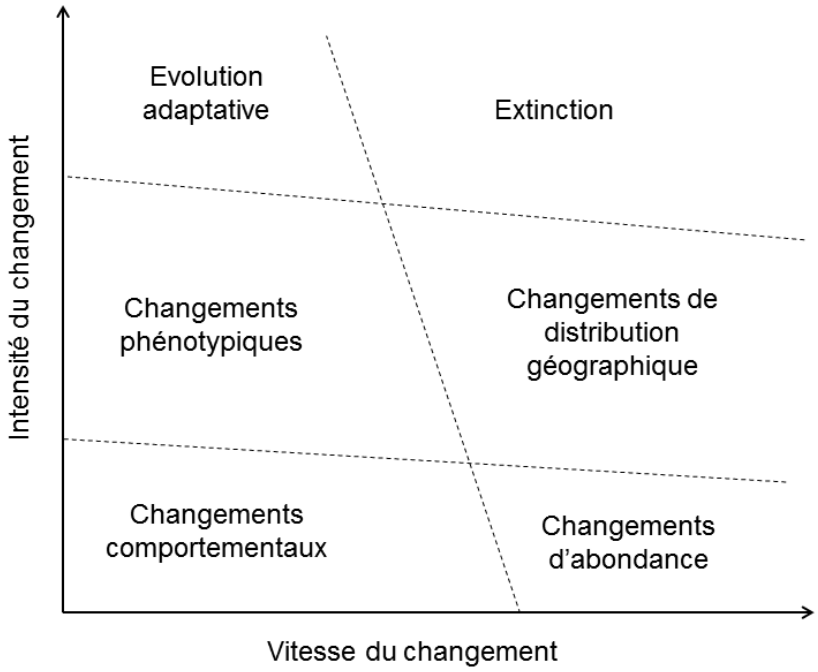

Figure 2. Représentation schématique des réponses d'une espèce face aux changements climatiques. La réponse prédominante dépend de la combinaison entre intensité et vitesse de ces changements. Les réponses spatiales, c'est-à-dire les changements dans la distribution de l'espèce, sont prédominantes pour des changements rapides et modérément intenses, comme les changements climatiques attendus pour ce siècle. Adapté à partir de Huntley *et al.* 2010.

Or, les changements climatiques engendrés par le développement industriel, c'est-à-dire l'augmentation des températures et des évènements météorologiques extrêmes surtout à partir des années 1970, agissent sur une échelle de temps beaucoup plus rapide que précédemment (IPCC 2014). Ces changements rapides du climat ont déjà affecté une multitude d'espèces dans la plupart des groupes taxonomiques et continueront d'agir dans le future (Parmesan *et al.* 2000 ; Walther *et al.* 2002, 2005 ; Root *et al.* 2003 ; Parmesan & Yohe 2003 ; Thomas 2005 ; Parmesan 2006 ; Visser 2008 ; Huntley *et al.* 2010). Les principaux changements observés sont des modifications de la phénologie, et en conséquence des désynchronisations potentielles entre espèces en interaction, ainsi que des contractions, expansions et déplacements d'aire de distribution géographique vers les pôles et en altitude, ainsi que l'extinction d'espèces polaires et de montagne (revue dans Parmesan 2006).

2. L'influence des traits écologiques sur le déclin des espèces

Les diverses réponses des espèces face aux facteurs de déclin sont influencées par leurs traits écologiques (Rasmont & Mersch 1988 ; Goulson *et al.* 2005 ; Bommarco *et al.* 2010 ; Williams *et al.* 2010 ; De Palma *et al.* 2015). En général, les corrélats d'un risque d'extinction accru sont une aire de distribution géographique restreinte, une rareté naturelle, et une spécialisation écologique (Brook *et al.* 2008). Par exemple, la sensibilité d'une espèce face aux différents effets de la fragmentation de l'habitat peut être accrue par une faible capacité de dispersion, un haut niveau trophique ou un mutualisme, une largeur de niche écologique

réduite et une rareté initiale (Laurance 1991 ; Holt *et al.* 1999 ; Davies *et al.* 2000 ; Tscharntke *et al.* 2002 ; Henle *et al.* 2004 ; Ewers & Didham 2005). Pour étudier les impacts des changements environnementaux sur des assemblages ou des communautés d'espèces, il importe donc de considérer notamment les différences interspécifiques de sensibilité et de réponses à ces changements plutôt que de les généraliser lors de la mise en place de plans de conservation.

3. Le service écosystémique de pollinisation

Le rôle des écosystèmes dans le développement des sociétés humaines est admis depuis des centaines d'années (Westman 1977 ; Daily 1997). Les contributions directes et indirectes des écosystèmes au bien-être humain sont légions, qu'il s'agisse de services culturels, d'approvisionnement direct de biens, ou de régulation (TEEB 2010). La pollinisation fourni non seulement un service de régulation des écosystèmes (Ollerton *et al.* 2011), mais également un service d'approvisionnement futur en contribuant à la production de fruits et légumes (Klein *et al.* 2007). En effet, une multitude d'espèces d'insectes, d'oiseaux et de chauves-souris contribuent à la pollinisation de nombreuses espèces cultivées (Allen-Wardell *et al.* 1998 ; Klein *et al.* 2007 ; Kremen *et al.* 2007). Parmi les plantes les plus cultivées à l'échelle mondiale, 87 sur 115 dépendent de la pollinisation animale, ce qui représente plus de 35% de la production globale (Klein *et al.* 2007). La valeur économique des pollinisateurs varie grandement entre les études et a été estimée entre 112 et 200 milliards USD (~ 98 à 175 milliards d'euros) par an à l'échelle globale (Costanza *et al.* 1997 ; Kearns *et al.* 1998 ; Klein *et al.* 2007 ; Gallai

et al. 2009). La plus grande partie du service de pollinisation est assurée par l'abeille mellifère et surtout par les abeilles sauvages (Corbet *et al.* 1991), contribuant respectivement à une moyenne de 2 913 USD (~ 2 552 €) et de 3 251 USD (~ 2 848 €) par hectare dans la production de 20 cultures dépendantes des abeilles (Kleijn *et al.* 2015). En Belgique, une récente étude a estimé la production alimentaire fournie par les insectes pollinisateurs à plus de 250 millions d'euros en 2010, ce qui représente 11% de la valeur de production totale des cultures (Jacquemin *et al.* 2017). Les cultures qui dépendent le plus des insectes pollinisateurs sont les fruits, les légumineuses et les légumes (Jacquemin *et al.* 2017). Cependant, le service de pollinisation est mondialement menacé par le déclin des pollinisateurs, résultant principalement des pratiques modernes de l'agriculture (pesticides, engrais, exploitation intensive des cultures et prairies), de la réduction en ressources florales, de la dégradation des habitats naturels et du changement climatique (Allen-Wardell *et al.* 1998 ; Kremen *et al.* 2002, 2007 ; Steffan-Dewenter *et al.* 2002 ; Ghazoul 2005 ; Memmott *et al.* 2007 ; Steffan-Dewenter & Westphal 2007 ; Ricketts *et al.* 2008 ; Potts *et al.* 2010 ; Kerr *et al.* 2015).

A la manière de l'IPCC (« *Intergovernmental Panel on Climate Change* » ou GIEC en français) sur les changements du climat, l'IPBES (« *Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services* »), créé en 2012, est un organisme intergouvernemental indépendant destiné à fournir aux décideurs des évaluations scientifiques objectives sur l'état des connaissances concernant la biodiversité et les écosystèmes de la planète. Parmi les évaluations thématiques,

l'IPBES a tout récemment publié un rapport sur le service écosystémique de pollinisation (« *Deliverable 3(a): Thematic assessment of pollinators, pollination and food production* » ; Potts *et al*. 2016). Ce rapport regroupe les résultats et les avis d'une multitude d'experts à l'échelle mondiale sur la valeur des pollinisateurs et de la pollinisation, les statuts et tendances des pollinisateurs (notamment les résultats des listes rouges de l'IUCN), les facteurs de déclin et les risques associés, ainsi que les options politiques et de gestion (Potts *et al*. 2016). Ils mettent notamment en évidence le besoin urgent d'une surveillance internationale ou nationale à long terme des pollinisateurs et de la pollinisation pour fournir des informations sur l'état et les tendances de la plupart des espèces dans la plupart des régions du monde.

4. Diversité et écologie des bourdons

Les bourdons sont de grands insectes hyménoptères appartenant à la superfamille des Apoïdea, plus communément appelée « abeilles » au sens large (Michener 2000), et à la famille des Apidae, qui inclue également l'abeille mellifère (*Apis mellifera*). Ils appartiennent tous au genre *Bombus* Latreille 1802, divisé en 15 sous-genres (Tableau 1) et qui comprend environ 250 espèces dans le monde (Williams 1998 ; Cameron *et al.* 2007 ; Williams *et al.* 2008 ; Hines 2008). Ils sont naturellement présents sur tous les continents, à l'exception de l'Antarctique, l'Océanie et l'Afrique subsaharienne (Williams 1998 ; Hines 2008). De par leur grande taille, leur épaisse fourrure et leur endothermie facultative, les bourdons sont particulièrement adaptés aux faibles températures (Heinrich 1975, 1979). De

ce fait, les bourdons sont les plus abondants et diversifiés dans les zones tempérées, alpines et arctiques de l'Hémisphère Nord, où ils représentent les pollinisateurs les plus ubiquistes (Williams 1988, 1998 ; Rasmont *et al.* 2015).

Tableau 1. Sous-genres de *Bombus* et nombres d'espèces proposés par Williams *et al.* (2008), ainsi que les espèces observées actuellement ou dans le passé en Belgique (Rasmont & Pauly 2010).

Sous-genre	Nombre d'espèces	Espèces observées en Belgique
Alpigenobombus Skorikov	7	*B. wurflenii* Radoszkowski, 1859
Alpinobombus Skorikov	5	-
Bombias Robertson (ou *Confusibombus* Ball)	3	*B. confusus* Schenck, 1859
Bombus sensu stricto	10	*B. cryptarum* (Fabricius, 1775) *B. lucorum* (L., 1761) *B. magnus* Vogt, 1909 *B. terrestris* (L., 1758)
Cullumanobombus Vogt	23	*B. cullumanus* (Kirby, 1802)
Kallobombus Dalla Torre	1	*B. soroeensis* (Fabricius, 1793)
Megabombus Dalla Torre	22	*B. hortorum* (L., 1761) *B. ruderatus* (Fabricius, 1775)
Melanobombus Dalla Torre	17	*B. lapidarius* (L., 1758)
Mendacibombus Skorikov	12	-
Orientalibombus Richards	3	-
Psithyrus Lepeletier (espèces inquilines)	30	*B. barbutellus* (Kirby, 1802) *B. bohemicus* Seidl, 1837 *B. campestris* (Panzer, 1801) *B. norvegicus* (Sparre Schneider, 1918) *B. quadricolor* (Lepeletier, 1832) *B. rupestris* (Fabricius, 1793) *B. sylvestris* (Lepeletier, 1832) *B. vestalis* (Fourcroy, 1785)
Pyrobombus Dalla Torre	50	*B. hypnorum* (L., 1758) *B. jonellus* (Kirby, 1802) *B. pratorum* (L., 1761)
Sibiricobombus Vogt	7	-
Subterraneobombus Vogt	10	*B. distinguendus* Morawitz, 1869 *B. subterraneus* (L., 1758)
Thoracobombus Dalla Torre	50	*B. humilis* Illiger, 1806 *B. muscorum* (L., 1758) *B. pascuorum* (Scopoli, 1793) *B. pomorum* (Panzer, 1805) *B. ruderarius* (Müller, 1776) *B. sylvarum* (L., 1761) *B. veteranus* (Fabricius, 1793)

4.1. Socialité, cycle de vie et phénologie

Les bourdons sont des espèces eusociales primitives (Michener 1969). La structure eusociale est le plus haut niveau de spécialisation entre des individus en coopération (Wilson 1971 ; Crespi & Yanega 1995). Dans ce type de société, il existe plusieurs distinctions entre les individus. Premièrement, il y a une division entre ceux ayant accès à la reproduction (castes reproductrices) et ceux dont la reproduction est inhibée (caste non reproductrice). Deuxièmement, au sein de la caste non reproductrice, il peut y avoir une division du travail (soins aux jeunes, approvisionnement en ressources alimentaires, défense du nid…). Les sociétés de bourdons sont constituées de trois castes : la reine et les mâles (les reproducteurs), et les ouvrières formant la caste non reproductrice. Les bourdons sont dits eusociaux « primitifs » car, contrairement aux espèces eusociales comme l'abeille mellifère ou les fourmis, ils présentent une phase solitaire (Michener 1969, 1974). Cette phase correspond à la période d'hibernation et de fondation de la colonie par la reine seule (voir ci-dessous). Les colonies sont le plus souvent monogynes (une reine unique) et monandres (la reine ne s'accouple qu'avec un seul mâle). Comme chez tous les hyménoptères, le déterminisme du sexe se fait par haplodiploïdie (Cook & Crozier 1995) : les mâles sont (en très grande majorité) haploïdes (*i.e.* un jeu de chromosomes) car issus d'œufs non fécondés, qui peuvent être pondus par la reine mais aussi par les ouvrières lorsque la colonie est en fin de cycle. A l'inverse, les femelles sont diploïdes (*i.e.* deux jeux de chromosomes) car issues d'œufs fécondés pondus par la reine, la seule à s'accoupler avec un mâle.

Le cycle des bourdons est annuel, initié au printemps par la fondation de la colonie par la reine sortie d'hibernation. Elle construit deux pots en cire, l'un pour accueillir le pollen et l'autre pour accueillir le nectar. Elle pond ensuite une dizaine d'œufs qu'elle couve jusqu'à leur éclosion pour donner des larves, en général dans les quatre jours. A ce stade, la reine est seule pour approvisionner le couvain et a donc besoin d'une quantité suffisante de fleurs disponibles à proximité du nid. Après quatre stades et au bout d'une dizaine de jours, les larves se tissent un cocon dans lequel elles se transforment en pupes. Elles éclosent ensuite au bout d'une quinzaine de jours, donnant toutes des ouvrières, dont certaines se chargent de collecter les ressources florales et les autres d'aider la reine à nourrir les nouvelles larves. La reine cesse alors de collecter les ressources et se consacre entièrement à la ponte et à la couvaison. A partir de ce point, la croissance en taille de la colonie s'accélère si les ressources florales sont suffisantes, jusqu'à une taille qui peut atteindre entre 100 et 400 ouvrières chez *B. terrestris* et *B. lapidarius*, et entre 20 et 100 chez les espèces du sous-genre *Thoracobombus* (Alford 1975 ; von Hagen & Aichhorn 2014). Lorsque la colonie a atteint un nombre d'ouvrières suffisant, elle commence à produire des mâles et de nouvelles reines (« *switch point* », Duchateau & Velthuis 1988). La colonie comptant un très grand nombre d'ouvrières, le contrôle chimique exercé par la reine qui inhibe le développement des ovaires des ouvrières n'est plus suffisant et les ouvrières commencent elles aussi à produire des œufs non fécondés qui engendrent des mâles (« *competition point* », Keller & Nonacs 1993 ; Le Conte & Hefetz 2008).

A la fin de l'été, les jeunes reines et les mâles émergent et quittent le nid au bout de quelques jours, alors que la reine fondatrice et le reste de la colonie dégénèrent et meurent. Les mâles se consacrent à chercher du nectar pour se nourrir et à chercher des reines vierges à féconder. Plusieurs stratégies nuptiales existent chez les mâles de bourdons (Bergman 1997). La stratégie la plus commune, présente chez la plupart des espèces, est un comportement de « patrouille » du mâle (« *patrolling behaviour* »). Ce dernier délimite un circuit qu'il marque de phéromones, que ce soit sur les feuilles, les pierres ou encore les troncs d'arbre, et dont la localisation diffère selon les espèces (Bringer 1973; Kullenberg *et al.* 1973; Svensson 1979). Il parcourt ensuite ce circuit à la recherche de jeunes reines attirées par ses phéromones. Chez d'autres espèces, comme *B. subterraneus*, *B. muscorum* et *B. ruderarius*, le mâle surveille la sortie d'un nid autre que le sien et se précipite sur la première jeune reine qui en sort (« *nest waiting behaviour* »). Chez d'autres encore, comme *B. confusus*, le mâle choisi un point d'observation en hauteur d'où il guette le passage d'une jeune reine (« *perching behaviour* »).

Après s'être accouplée avec un (ou rarement plusieurs) mâle(s), la jeune reine cherche alors un endroit propice pour son hibernation, comme un talus au sol meuble où elle creuse un petit tunnel de quelques centimètres terminé par une chambre dans laquelle elle passera l'hiver. La survie de la reine durant cette période de dormance dépend grandement de la quantité de graisse qu'elle a pu accumuler à la fin de l'été (Beekman *et al.* 1998).

La date de sortie d'hibernation des reines dépend grandement de l'espèce. Certaines espèces sont dites précoces, émergeant en mars, comme par exemple *B.*

pratorum ou *B. terrestris*, alors que d'autres sont dites tardives, comme *B. sylvarum* qui peut émerger aussi tard que mai (Alford 1975). La durée du cycle de la colonie est elle aussi dépendante de l'espèce, en plus de la température et de l'approvisionnement en ressources (Alford 1975). Les cycles les plus courts sont rencontrés chez *B. pratorum* et *B. hortorum* (environ 14 semaines) et les plus longs chez *B. pascuorum* (environ 25 semaines, Goodwin 1995). La plupart des espèces de bourdons sont univoltines, c'est-à-dire qu'elles ne développent qu'une génération par an. Certaines espèces (*i.e. B. jonellus*, *B. pratorum* et *B. hypnorum*), peuvent par contre parfois présenter deux générations par an (Alford 1975).

D'une manière générale, le succès d'une colonie, c'est-à-dire lorsque la colonie parvient à produire de nouvelles reines ou des mâles, varie énormément selon le niveau de parasitisme, la disponibilité en ressources florales tout au long du cycle, ou même certaines pratiques agricoles qui peuvent détruire les nids, comme le fauchage ou le labourage.

4.2. Le cas des *Psithyrus*

La vie en société procure de nombreux avantages : soins collectifs aux jeunes, augmentation de l'efficacité dans la récolte de ressources alimentaires et dans la défense du nid (défense collective contre les compétiteurs ou les prédateurs) et donc réduction de la pression de prédation. Cependant, la socialité engendre également des coûts, dont notamment la grande part de temps et d'énergie dédiée à la construction du nid et aux soins parentaux. En conséquence, un individu « tricheur » (le parasite), exploitant les soins parentaux fournis par un autre

individu ou une société (l'hôte) afin de porter à terme sa propre descendance, se voit bénéficier des avantages de la société sans ses coûts énergétiques, dans la mesure où l'hôte ne réprime pas le phénomène. Ce type de parasitisme est appelé « parasitisme de ponte ». Le cas le plus connu du grand public est le coucou gris (*Cuculus canorus*). Lorsqu'il s'agit d'un individu parasitant une société et pas un seul autre individu, on parle de « parasitisme social » ou « inquilinisme ». Chez les bourdons, il existe des espèces exerçant un inquilinisme facultatif, où les reines usurpent une colonie déjà existante dans certaines conditions (Sladen 1912 ; Bohart 1970 ; Alford 1975). Mais il existe aussi des espèces inquilines obligatoires, ou « bourdons-coucous », qui ont perdu toute capacité à fonder une colonie et exploitent la force ouvrière d'autres espèces de bourdons pour l'élevage de leur progéniture, allouant leur énergie seulement à leur reproduction. Ces bourdons-coucous font essentiellement partie du sous-genre *Psithyrus* (Løken 1984). Les espèces de *Psithyrus*, comme tous les parasites sociaux, ont coévolué avec leurs espèces hôtes ce qui assure leur succès de parasitisme. En général, l'interaction parasite-hôte est spécifique : une espèce de *Psithyrus* parasite une espèce ou un groupe restreint d'espèces hôtes (Tableau 2).

Tableau 2. Espèces parasites du sous-genre *Psithyrus* et leurs espèces hôtes présentes en Belgique. Les espèces entre parenthèses indiquent des hôtes occasionnels (Løken 1984 ; Williams 2008 ; Lhomme 2009).

Psithyrus	*Bombus* hôtes
B. barbutellus	*B. hortorum* (*B. hypnorum*) (*B. ruderatus*)
B. bohemicus	*B. lucorum*
B. campestris	*B. humilis* *B. pascuorum* *B. pomorum* *B. pratorum*
B. norvegicus	*B. hypnorum* (*B. jonellus*)
B. quadricolor	*B. soroeensis*
B. rupestris	*B. lapidarius* (*B. pascuorum*) (*B. sylvarum*)
B. sylvestris	*B. pratorum* (*B. jonellus*)
B. vestalis	*B. terrestris*

La femelle *Psithyrus* émerge d'hibernation un peu plus tard que son ou ses espèces hôtes et passe d'abord une grande partie de son temps à se nourrir. Une fois ses ovarioles bien développées, elle cherche un jeune nid d'une espèce hôte (Alford 1975), qu'elle peut reconnaitre selon l'odeur des individus qui le constituent (Cederberg 1979 ; Fisher *et al.* 1993). Une fois le nid trouvé, deux stratégies alternatives sont utilisées par les femelles *Psithyrus* pour se faire accepter par la colonie : la stratégie discrète et la stratégie agressive. Dans la stratégie discrète, comme chez *B. sylvestris* et *B. campestris*, la femelle commence par se cacher dans le nid et s'imprègne de l'odeur du couvain, ce qui permet de supprimer toute animosité de la part des ouvrières (Küpper & Schwammberger

1995 ; Dronnet *et al.* 2005). Une fois acceptée, la femelle pond ses œufs, qui sont élevés par les ouvrières au même titre que ceux de la reine hôte avec qui elle cohabite. Au contraire, dans la stratégie agressive, comme chez *B. vestalis* et *B. rupestris*, la reine inquiline tue la reine fondatrice ainsi que ses œufs et larves, et prend sa place au sein de la colonie qui élève alors uniquement sa propre progéniture (Fisher 1988). Le reste du cycle de vie des *Psithyrus* reste semblable à celui des autres espèces. Les femelles sont cependant beaucoup moins fréquemment rencontrées que les mâles, qui sont communément observés sur les chardons à la fin de l'été (Goulson 2010).

4.3. Régime alimentaire

Les bourdons, comme toutes les abeilles, sont phytophages et se nourrissent de nectar comme source de sucre et d'eau, et de pollen comme source de protéines. Pour faciliter la récolte de ces ressources florales, les adultes sont dotés d'adaptations morphologiques : un long proboscis pour aspirer le nectar et des corbeilles sur les pattes postérieures pour récolter le pollen (Michener 1974), à l'exception des *Psithyrus* qui ne collectent pas de pollen. Le nectar est principalement consommé par les adultes, tandis que le pollen est réservé aux larves en développement. Par conséquent, seules les femelles récoltent du pollen pour le ramener à la colonie, alors que les mâles, qui ne participent pas à l'élevage des larves, ne collectent que du nectar.

La plupart des bourdons, comme la majorité des insectes pollinisateurs, ont une diète relativement flexible et peuvent visiter une gamme plus ou moins large

d'espèces de fleurs. Le choix des fleurs visitées dépend du type de ressource collectée et de ses qualités nutritives (Somme *et al.* 2015). Certaines familles de plantes sont plus visitées pour le pollen, comme les Fabaceae, et d'autres pour le nectar comme les Asteraceae (Figure 3).

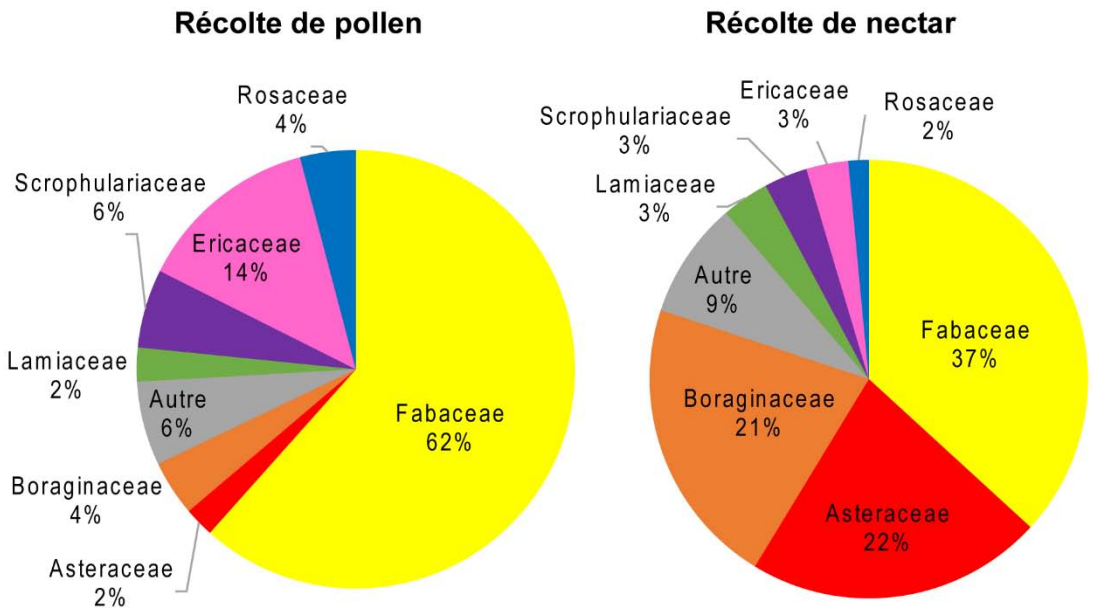

Figure 3. Pourcentage de visites des bourdons sur les familles de plantes au Royaume-Uni, selon la récolte de pollen et la récolte de nectar, toutes espèces et castes confondues. D'après Goulson *et al.* 2005.

Figure 4. Diversité de plantes butinées : (a) *B. pascuorum* sur *Lamium album* (Lamiaceae), (b) *B. lapidarius* sur *Trifolium repens* (Fabaceae), (c) *B. lapidarius* sur chardon (Asteraceae), (d) *B. lucorum* sur *Origanum vulgare* (Lamiaceae), (e) *B. hortorum* sur *Echium vulgare* (Boraginaceae), (f) *B. pascuorum* sur *Symphytum officinale* (Boraginaceae), (g) *B. lapidarius* sur *Knautia* sp. (Dipsacaceae), (h) *B. terrestris* sur *Centaurea cyanus* (Asteraceae), (i) *B. hortorum* sur *Trifolium pratense* (Fabaceae), (j) *B. terrestris* sur *Buddleja davidii* (espèce exotique envahissante, Buddlejaceae), (k) *B. terrestris* sur *Vicia cracca* (Fabaceae), (j) *B. pratorum* sur *Vaccinium myrtillus* (Ericaceae). Photos : S. Vray.

Deux catégories d'espèces de bourdon peuvent être séparées selon la manière d'alimenter les larves et la stratégie de butinage des fleurs (Sladen 1912). Chez les « *pollen-storers* » (par exemple *B. lapidarius*, *B. lucorum* et *B. terrestris*), le pollen est stocké dans un pot de cire comme le nectar et les larves sont nourries par du pollen fermenté de quelques jours. Les ouvrières butinent des fleurs qui peuvent être différentes selon qu'elles aient besoin de remplir les réserves en nectar ou en pollen. Chez les « *pocket-makers* », au contraire, il n'y a pas de stockage de pollen comme pour le nectar et les larves sont approvisionnées directement en pollen fraichement récolté. Les ouvrières récoltent en même temps sur les mêmes fleurs le pollen et le nectar. Elles ne butinent donc pratiquement que des fleurs fournissant à la fois du nectar et du pollen. C'est notamment le cas de *B. distinguendus*, *B. hortorum*, *B. humilis*, *B. pascuorum*, *B. ruderarius* et *B. ruderatus*.

Le choix des espèces florales dépend également de l'espèce, chacune ayant ses propres préférences (Kleijn & Raemakers 2008). Ces différences de préférences permettent en partie de réduire la compétition interspécifique, et donc la cohabitation de plusieurs espèces dans un même milieu (Heinrich 1976 ; Inouye 1978 ; Ranta & Lundberg 1980 ; Ranta 1984 ; Harder 1985). Les préférences florales sont en partie dictées par la longueur de la langue des bourdons (Brian 1957 ; Hobbs 1962 ; Inouye 1980 ; Ranta & Lundberg 1980 ; Harder 1985). Les espèces ayant une langue plus longue, comme *B. hortorum* et *B. ruderatus* (environ 14 mm), peuvent plus facilement atteindre le nectar des fleurs à longues corolles que les espèces à plus courte langue, comme *B. terrestris* (environ 8.5mm), qui ont tendance à visiter des fleurs à courte corolle (Prys-Jones 1982). Certaines espèces

à courte langue, particulièrement *B. terrestris* et *B. lucorum*, ont cependant appris à exploiter le nectar des fleurs à plus longues corolles en les perçant à leur base (« *nectar robbing* », Inouye 1983 ; Leadbeater & Chittka 2008).

Par ailleurs, toutes les espèces n'ont pas la même largeur de diète. Par exemple, *B. terrestris* et *B. pratorum* ont une diète plus diversifiée que *B. hortorum* et *B. humilis* qui collectent du pollen majoritairement sur les Fabaceae (Rasmont & Mersch 1988 ; Kleijn & Raemakers 2008 ; Goulson *et al.* 2008b). D'une manière générale, les espèces de pollinisateurs les plus spécialisées sont plus vulnérables aux perturbations (*i.e.* extinctions) dans les réseaux plantes – pollinisateurs (Memmott *et al.* 2004).

Cependant, il est important de noter que la plupart des études ciblent les choix floraux des ouvrières, rencontrées en plus grand nombre sur le terrain, et donc la collecte de pollen pour le développement de la colonie. Ces études ont montré l'importance indiscutable des Fabaceae (comme les trèfles, *Trifolium* spp.) dans la diète des bourdons (Rasmont & Mersch 1988 ; Rasmont *et al*. 1993, 2005 ; Goulson *et al.* 2005, 2008b). Les Asteraceae, dont le pollen est bien moins favorable pour les bourdons (Vanderplanck *et al.* 2016), ne sont pratiquement pas visitées pour le pollen. Leur rôle dans la diète des bourdons est par conséquent bien moins reconnu, alors qu'elles constitueraient pourtant une très grande part des visites pour le nectar selon Goulson *et al.* 2005. Elles pourraient donc être d'une importance tout aussi capitale pour les mâles adultes, qui ne se nourrissent que de nectar. Il semblerait que les chardons (surtout *Cirsium* et *Carduus* spp.) et centaurées (*Centaurea* spp.) seraient parmi les Asteraceae les plus visitées par les

mâles dans certaines régions (Croxton *et al.* 2002 ; Pywell *et al.* 2005 ; Carvell *et al.* 2006 ; Vray *et al*. 2017), mais peu d'études ont exploré le sujet. Or, afin de concevoir des mesures de conservation adéquates, qu'elles soient directes (par exemple le semis de bandes fleuries) ou indirectes (conservation des plantes « clés »), il est crucial de connaître les plantes les plus importantes pour la diète et l'écologie de l'ensemble des castes de bourdons. Les chardons tout particulièrement sont soumis à des lois d'arrachage dans plusieurs pays dont la Belgique (Crémer *et al.* 2008). Si leur importance pour les mâles de bourdons était avérée, les lois d'échardonnage pourraient constituer une menace pour les populations de bourdons (Vray *et al*. 2017).

4.4. Habitats

L'habitat des bourdons peut inclure quatre différents types de milieu qui contiennent chacun une ressource nécessaire à un stade particulier de leur cycle de vie : le premier pour l'hibernation de la jeune reine, le deuxième pour la nidification et l'établissement de la colonie, le troisième pour la collecte des ressources florales, et le quatrième pour la parade nuptiale des mâles. Puisque nos inventaires ont principalement ciblé les spécimens butinant des fleurs, nous abordons plus longuement les sites de collecte des ressources florales.

4.4.1. Sites d'hibernation

Les sites d'hibernation des jeunes reines sont assez mal connus. Les quelques données disponibles indiquent que la plupart des espèces hibernent le plus souvent dans le sol sur les pentes bien drainées et orientées au nord ou au nord-ouest, à

l'ombre d'un arbre, ou plus rarement dans une souche d'arbre ou sous la mousse (Sladen 1912 ; Bols 1939 ; Alford 1969).

4.4.2. Sites de nidification

Les sites de nidification varient d'une espèce à l'autre (Sladen 1912 ; Svensson *et al.* 2000 ; Kells & Goulson 2003 ; Goulson 2010). Par exemple, les reines de *B. terrestris*, *B. lapidarius*, *B. sylvarum* et *B. subterraneus* tendent à préférer les terrains ouverts pour installer leur nid, tandis que celles de *B. lucorum* et de *B. pascuorum* ont une préférence pour les lisières de forêt (Svensson *et al.* 2000). La plupart des espèces, appelées « *renter bees* », nichent dans des cavités préexistantes, sous le sol ou en surface. La majorité choisit un nid souterrain accessible par un petit tunnel. C'est notamment le cas de *B. terrestris* et *B. lucorum*, qui nichent souvent dans les terriers abandonnés de rongeurs. *B. hypnorum* niche en hauteur, dans les cavités des arbres, les vieux nids d'oiseaux ou même les nichoirs artificiels. *B. pratorum* est plus opportuniste et peut nicher dans le sol, à sa surface, ou encore en hauteur. D'autres espèces, appelées « *carder bees* », nichent à la surface du sol, sous la végétation (le plus souvent dans les touffes d'herbe), dans un nid qu'elles aménagent elles-mêmes à l'aide de matériel végétal déchiqueté (Sladen 1912). C'est le cas de *B. humilis, B. muscorum, B. pascuorum*, *B. ruderarius*, *B. sylvarum* et *B. veteranus*, qui sont également toutes des « *pocket-makers* » (Sladen 1912).

4.4.3. Sites de collecte des ressources florales

Les ouvrières butinent en général les fleurs autour de la colonie dans un rayon plus ou moins grand selon les espèces, appelé « *foraging range* » en anglais et

pouvant être traduit par « aire de butinage ». Les sous-habitats pour la nidification et pour les ressources florales étant souvent différents, il est donc indispensable pour la survie d'une colonie que les parcelles contenant les ressources florales soient suffisamment disponibles au sein de son aire de butinage, c'est-à-dire à une distance facilement atteignable par les ouvrières. Malgré l'importance primordiale de l'aire de butinage dans la conservation des espèces de bourdons, elle est encore relativement mal connue. De plus, dû aux différentes méthodes d'estimation et à la difficulté de l'estimer, elle varie entre les études (revue dans Goulson 2010). En Allemagne, une étude employant la méthode de capture-marquage-recapture a indiqué que les ouvrières de *B. terrestris* butinaient dans un rayon maximal de 800 mètres autour du nid, dont 40% dans un rayon de 100 mètres (Wolf & Moritz 2008). Dans les Pyrénées françaises, les distances de recapture des ouvrières de *B. terrestris* s'étalaient de 1 à 330 mètres (Namur 2005). Les techniques de radio-tracking, quant-à-elles, ont montré que la majeure partie des ouvrières butinaient dans les 500 à 700 mètres du nid (Osborne *et al.* 1999). Par ailleurs, les études s'attardant sur plusieurs espèces ont montré des différences notables d'aire de butinage (Walther-Hellwig & Frankl 2000 ; Kreyer *et al.* 2004 ; Knight *et al.* 2005 ; Westphal *et al.* 2006). Les espèces du sous-genre *Thoracobombus* ont tendance à butiner dans les 500 mètres autour du nid (1 km au maximum), alors que celles comme *B. lapidarius* et *B. terrestris* peuvent butiner beaucoup plus loin, jusqu'à 2,5 et 3 km respectivement. La taille de la colonie et la taille du corps des individus pourraient expliquer ces différences, les espèces développant de grandes colonies et dont les ouvrières ont une taille plus importante se déplaçant plus loin pour

butiner (Alford 1975 ; Greenleaf *et al.* 2007). Ces différences d'aire de butinage pourraient très probablement avoir des implications directes dans la réponse des espèces aux changements du paysage, comme c'est le cas pour les différences de capacités de dispersion (Darvill *et al.* 2010). Les espèces avec une grande aire de butinage sont susceptibles d'être moins sensibles à la perte et à la fragmentation des habitats, car elles peuvent se déplacer dans une plus grande zone que les espèces à plus petite aire de butinage qui nécessitent des parcelles de fleurs plus proches et moins fragmentées. En général, les individus de colonies installées dans des paysages plus fragmentés ont tendance à se déplacer plus loin que celles dans des paysages moins fragmentés (Carvell *et al.* 2012), ce qui implique un plus grand coût énergétique. Qui plus est, la répartition et la quantité des parcelles florales dans le paysage environnant une colonie peut varier selon les périodes de floraison, une quantité suffisante de fleurs n'étant pas toujours disponible tout au long de la vie de la colonie (Carvell *et al.* 2004, 2006). Ceci ajoute une difficulté supplémentaire quant à l'évaluation de la qualité des habitats des bourdons et à la gestion du paysage en leur faveur.

4.4.4. Sites de parade nuptiale des mâles

Comme nous l'avons vu au point 4.1., les mâles de bourdons exécutent des parades nuptiales qui varient selon les espèces (Bringer 1973; Kullenberg *et al.* 1973; Svensson 1979). Ces parades sont effectuées dans des sites qui peuvent différer de ceux mentionnés précédemment. Le circuit des mâles qui exercent un comportement de « patrouille » et de marquage phéromonal peut inclure une

multitude d'éléments du paysage (feuilles et troncs d'arbre, buissons, poteaux de clôture, pierres, ou tout autre repère bien visible), dont le type et la hauteur varient en fonction des espèces (Bringer 1973 ; Svensson 1979 ; Fussell & Corbet 1992). Par exemple, *B. lapidarius* choisi en général les points les plus élevés comme la cime des arbres, *B. hortorum* patrouille à moins d'un mètre au-dessus du sol et tend à choisir des creux sombres, et *B. terrestris* visite un éventail de points de hauteur variée le long des haies d'arbustes et des arbres (Bringer 1973 ; Svensson 1979 ; Fussell & Corbet 1992). L'habitat des mâles, qui passent le plus clair de leur temps dans la parade nuptiale, peut donc être très différent des autres types d'habitat nécessaires à l'hibernation de la reine ou à la fondation de la colonie. De plus, puisque le comportement de « patrouille » nécessite beaucoup d'énergie, les mâles ont besoin de ressources florales à proximité de leur circuit.

5. L'importance des bourdons pour le service écosystémique de pollinisation

De par leur régime alimentaire et la nécessité de collecter les ressources florales pour approvisionner leur colonie, les bourdons participent activement à la pollinisation des plantes (Ollerton *et al.* 2011). Ils sont parmi les pollinisateurs les plus importants pour la pollinisation de nombreuses plantes de production agricole et horticole (Holm 1966 ; Corbet 1987 ; Plowright & Laverty 1987 ; Corbet *et al.* 1991 ; Goulson 2003). En effet, une large gamme de plantes d'intérêt économique dépend des bourdons pour une pollinisation efficace, qu'il s'agisse de plantes fourragères comme la luzerne (*Medicago sativa*) et les trèfles (*Trifolium* spp.)

(Holm 1966), de plantes oléagineuses comme le colza (*Brassica napus*) et le tournesol (*Helianthus annuus*), ou encore de la grande majorité des fruits : fraises (*Fragaria* spp.), cerises (*Prunus avium*), pommes (*Malus domestica*), poires (*Pyrus communis*), kiwi (*Actinidia deliciosa*), pastèque (*Citrullus lanatus*), myrtilles (*Vaccinium* spp.), poivrons (*Capsicum annuum*) et tomates (*Lycopersicon esculentum*) pour n'en citer que quelques-uns (Goulson 2010). Pour ces dernières espèces, comme pour toutes les Solanaceae, la pollinisation ne peut être effectuée qu'en faisant vibrer la fleur. Seuls les bourdons en sont capables grâce à leur comportement de « buzzing » (Banda & Paxton 1990 ; van Ravestijn & van der Sande 1991). De plus, contrairement à l'abeille mellifère, les bourdons sont toujours actifs dans les conditions météo froides et pluvieuses (Corbet *et al.* 1993) et sont aussi plus rapides et plus efficaces (Poulsen 1973 ; Free 1993 ; Stanghellini *et al.* 1998, 2002 ; Stubbs & Drummond 2001 ; Artz & Nault 2011).

En plus de leur importance dans la pollinisation des cultures, le service de pollinisation fourni par les bourdons s'étend bien entendu à la flore sauvage. Ils sont les principaux agents pollinisateurs d'une grande partie des fleurs sauvages des zones tempérées, arctiques et alpines de l'hémisphère Nord (Corbet *et al.* 1991). Sans les bourdons, la diversité floristique serait fortement amoindrie dans ces régions (Biesmeijer *et al.* 2006).

6. Le déclin et la conservation des bourdons

Une multitude d'études montrent un déclin dramatique des bourdons dans le monde entier (Williams 1982 ; Rasmont *et al.* 1993 ; Biesmeijer *et al.* 2006 ; Kosior

et al. 2007 ; Goulson *et al.* 2008a ; Colla & Packer 2008 ; Cameron *et al.* 2011 ; Nieto *et al.* 2014 ; Potts *et al.* 2015). Parmi les abeilles, le genre *Bombus* inclut le plus haut pourcentage d'espèces en risque d'extinction en Europe (Nieto *et al.* 2014). Sur les 68 espèces d'Europe, 31 sont en déclin, 20 sont stables et 9 sont en expansion (Figure 5, Nieto *et al.* 2014). Dès les années 80, plusieurs causes complémentaires ont été avancées pour expliquer cette régression alarmante. Nous les citons ici par ordre chronologique, en commençant par les causes identifiées et étudiées dès le début des années 1980 et en terminant par les facteurs qui ont commencé à être investigué à partir des années 2000.

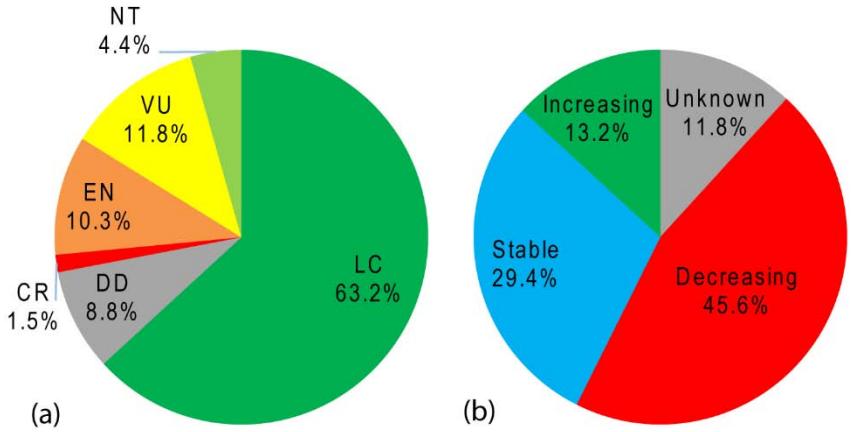

Figure 5. Statuts de conservation des listes rouges de l'IUCN (a) et tendances des populations (b) de *Bombus* spp. en Europe (Nieto *et al.* 2014). DD = données insuffisantes (« *Data Deficient* »), LC = préoccupation mineure (« *Least Concerned* »), NT = quasi menacé (« *Near Threatened* »), VU = vulnérable (« *Vulnerable* »), EN = en danger (« *Endangered* »), et CR = en danger critique d'extinction (« *Critically Endangered* »).

6.1. La perte et la fragmentation de l'habitat

La première cause de déclin des bourdons est la fragmentation et la perte de leur habitat, se traduisant par une disponibilité amoindrie en paysages ouverts en raison de l'intensification du boisement, de l'urbanisation et surtout de l'agriculture

(Williams 1986 ; Rasmont & Mersch 1988 ; Carvell 2002 ; Goulson *et al.* 2008a ; Williams & Osborne 2009 ; Ahrné *et al.* 2009 ; Le Féon *et al.* 2010 ; Potts *et al.* 2010 ; Ollerton *et al.* 2014). A la sortie de la Première Guerre Mondiale (années 1920s-1930s), les industries et matériaux utilisés auparavant pour la guerre sont recyclés en grande partie dans l'agriculture : les usines qui produisaient les nitrates utilisés dans la fabrication des bombes sont recyclées dans la production des premiers engrais chimiques azotés, les gaz de combat sont transformés en pesticides (*e.g*. l'ypérite en DDT, un insecticide, et l'agent orange du Vietnam en herbicide), les barbelés remplacent les haies, certains tanks sont transformés en tracteurs, etc. Le même phénomène suit la Seconde Guerre Mondiale, mais cette fois-ci démultiplié par le Plan Marshall signé entre l'Europe et les Etats-Unis d'Amérique, qui encourage fortement l'importation de produits industriels (*e.g*. machines agricoles motorisées, outils, engrais, pesticides) et agricoles (*e.g*. variétés de blé, maïs hybrides, tabac). Dès les années 1950s, la complète modernisation de l'agriculture commence à modifier le faciès des paysages agricoles d'Europe occidentale, notamment *via* le remplacement des bovins et chevaux de trait par des tracteurs et moissonneuses-batteuses, l'arrachage des haies, l'agrandissement et la spécialisation des cultures, le remplacement des légumineuses en tête de rotation par des cultures industrielles (*e.g*. betterave sucrière), etc. (Mazoyer & Roudart 2006). Dans les années 1970s, le Plan Mansholt provoque une accélération de l'intensification agricole. Le remembrement des parcelles induit la transformation des petites polycultures diversifiées en larges monocultures et la suppression du bocage, les rendements s'accroissent, les élevages de bétail augmentent leur

productivité, et un grand nombre de vergers est détruit (Christians 1998). Tous ces changements ont profondément modifié et homogénéisé la structure du paysage (Christians 1998 ; Robinson & Sutherland 2002 ; Mazoyer & Roudart 2006). Les champs et pâtures intensifs, les prés en fauchage précoce et l'absence de haies rendent les paysages agricoles bien plus hostiles pour les bourdons qu'il y a un siècle. Comme dit précédemment, la plupart des bourdons nichent sur le sol dans les touffes de végétation ou dans un ancien terrier de rongeur (Alford 1975 ; Kells & Goulson 2003 ; Goulson *et al.* 2010), ce qui les rend vulnérables aux perturbations du sol couramment rencontrées dans les paysages agricoles modernes (labour, fauchage et ensilage). Seules les zones agricoles présentant des éléments du paysage non perturbés (talus, prés ou bandes herbeuses en fauchage tardif, haies, alignements d'arbres, lisière de bois, etc.) peuvent héberger plus durablement et en plus grand nombre les colonies de bourdons (Banaszak 1992 ; Svensson *et al.* 2000 ; Mänd *et al.* 2001 ; Croxton *et al.* 2002 ; Osborne *et al.* 2008a). Par ailleurs, la lutte contre les rongeurs et le manque de fleurs sauvages produisant des graines réduisent en conséquence la disponibilité en sites de nidification pour les bourdons. En Europe occidentale, le déclin des bourdons a été plus accru dans les régions des plaines agricoles que dans les régions méditerranéennes et montagnardes, où l'agriculture est restée relativement extensive (Rasmont *et al.* 2005 ; Iserbyt *et al.* 2008), voire même en déprise (voir Iserbyt *et al.* 2015 en Annexe II). Au Royaume-Uni, la plupart des espèces rares ne se retrouvent plus maintenant que dans les zones côtières et les camps d'entrainement militaires, épargnés par l'agriculture intensive (Goulson & Darvill 2004 ; Goulson 2010).

L'urbanisation des terres a également considérablement réduit la disponibilité en habitats favorables aux bourdons (Ahrné *et al.* 2009 ; Deguines *et al.* 2016). Cependant, les jardins et les parcs urbains peuvent être favorables aux bourdons s'ils offrent suffisamment de ressources florales et de sites de nidification (Tommasi *et al.* 2004 ; McFrederick & LeBuhn 2006 ; Osborne *et al.* 2008b ; Goulson *et al.* 2010 ; Garbuzov & Ratnieks 2014 ; Normandin *et al.* 2017), surtout dans les régions agricoles intensives ou les régions fortement boisées (Winfree *et al.* 2007 ; Samnegård *et al.* 2011 ; Diaz-Forero *et al.* 2013 ; Baldock *et al.* 2015).

Parmi les abeilles, les bourdons sont les plus affectés par la fragmentation de leurs habitats (Aguirre-Gutiérrez *et al.* 2015). Comme dit précédemment, une colonie doit pouvoir accéder à une quantité suffisante de ressources florales au sein de son aire de butinage, bien souvent inférieure à 1 km de rayon (Carvell *et al.* 2012). Les réseaux de haies et de bandes en fauchage tardif sont non seulement importantes pour les sites de nidification et les ressources florales (Croxton *et al.* 2002 ; Sepp *et al.* 2004 ; Osborne *et al.* 2008a ; Morandin & Kremen 2013), mais aussi pour l'orientation et les déplacements des bourdons qui ont tendance à suivre les éléments linéaires du paysage (Cranmer *et al.* 2012). En plus des complications pour leurs déplacements de butinage, la fragmentation des habitats peut aussi affecter la dispersion des bourdons entre populations (Darvill *et al.* 2010), entrainant perte en diversité génétique et vulnérabilité accrue aux extinctions locales (Osborne *et al.* 1991 ; Brook *et al.* 2002 ; Dauber *et al.* 2003 ; Steffan-Dewenter *et al.* 2006 ; Le Féon *et al.* 2010 ; Jha & Kremen 2013). En résumé, plus un paysage est diversifié et riche en habitats naturels, plus il peut accueillir une grande

diversité en bourdons et plus généralement en insectes (Steffan-Dewenter 2003 ; Pywell *et al.* 2006).

6.2. La réduction en ressources florales

Une seconde cause de déclin, intimement liée à la première et également étudiée dès les années 1980, est la rupture dans la disponibilité en ressources florales, tant spatiale que temporelle. L'utilisation massive d'engrais chimiques essentiellement dès 1950 a permis le remplacement de la tête de rotation agricole, auparavant consacrée aux légumineuses (trèfle, luzerne, sainfoin, etc. ; Figure 6) qui enrichissent le sol en azote assimilable, par des cultures de rapport (*e.g*. la betterave sucrière ; Christians 1998 ; Mazoyer & Roudart 2006). Ce remplacement a fait disparaître une ressource alimentaire majeure pour de nombreuses espèces de bourdons (Rasmont 2007). En Europe, la diminution drastique des cultures de légumineuses semble suffisante pour expliquer la régression de la plupart des espèces de bourdons (Rasmont & Mersch 1988 ; Goulson & Darvill 2004 ; Goulson *et al.* 2005 ; Rasmont *et al.* 2005 ; Carvell *et al.* 2006). Les engrais azotés et les herbicides utilisés dans les champs et les prairies affectent indirectement les bourdons en réduisant la diversité de plantes (Kleijn *et al.* 2009 ; Roulston & Goodell 2011). De plus, l'homogénéisation des cultures ne permet plus de fournir des ressources florales tout au long de la vie des colonies de bourdons. Très peu de cultures fleurissent à la fin de l'hiver, moment crucial pour l'établissement de la colonie par la jeune reine sortie d'hibernation. En outre, le passage de la production de foin à l'ensilage des prés de fauche a entrainé un fauchage de plus en plus

précoce et a réduit à néant la disponibilité des fleurs d'été, constituées d'une grande part de légumineuses (Rasmont 1988). Par ailleurs, l'eutrophisation des milieux naturels résultant de l'utilisation en grande quantité d'engrais azotés a engendré le déclin des légumineuses également dans la flore sauvage car défavorisées par une haute teneur en azote assimilable du sol. Or, il a été constaté que la majorité des espèces de bourdons en déclin sont des espèces à longue langue, autrement dit butineuses spécialistes de plantes à longues corolles comme les Fabaceae et Lamiaceae (Rasmont & Mersch 1988 ; Rasmont *et al.* 1993, 2005 ; Goulson *et al.* 2008b). Le déclin de ces espèces plus spécialisées pourrait donc avoir un lourd impact sur la prospérité des plantes dépendantes de ces espèces pour leur reproduction, qu'elles soient sauvages ou cultivées.

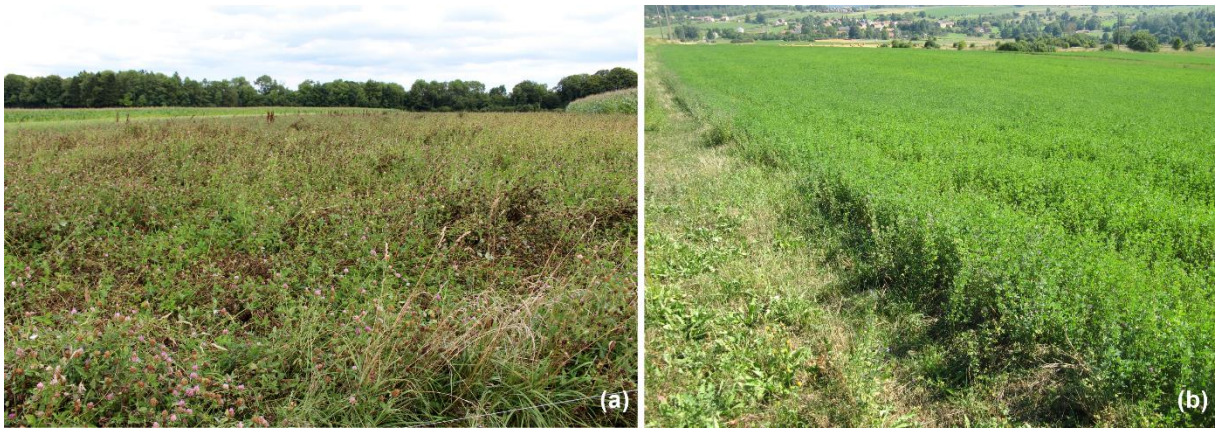

Figure 6. Cultures de légumineuses à Torgny, en Gaume (province du Luxembourg): (a) trèfle rouge (*Trifolium pratense*), (b) luzerne (*Medicago sativa*). Photos : S. Vray.

6.3. Les pesticides

En plus des deux facteurs précédents, l'intensification de l'agriculture a également apporté son lot de pesticides dès la seconde moitié du 20e siècle, la majorité d'entre eux ayant été développés à la sortie de la Première et de la Seconde Guerres

Mondiales. La plupart des études d'impacts toxicologiques des pesticides ayant été conduites sur l'abeille mellifère, les conséquences pour les bourdons sont encore relativement mal connues et supposées être différentes en raison des différences de comportement de butinage (Thompson & Hunt 1999 ; Thompson 2001). De plus, les mesures de réduction de l'incidence des pesticides ne prennent généralement en compte que l'abeille mellifère. Un exemple est la pulvérisation très tôt le matin ou tard le soir, périodes de plus faible activité de l'abeille mellifère, mais malheureusement périodes de grande activité des bourdons (Thompson & Hunt 1999 ; Thompson 2001). Similairement, les pulvérisations en début de saison présentent très certainement un impact beaucoup plus important chez les bourdons, au moment de l'émergence des jeunes reines et de la fondation des colonies, que chez l'abeille mellifère (Thompson 2001).

Depuis les années 2000, de plus en plus d'études se penchent sur les bourdons et révèlent des effets négatifs des pesticides sur le comportement de butinage des ouvrières (Morandin *et al.* 2005 ; Mommaerts *et al.* 2006 ; Feltham *et al.* 2014), sur la productivité des colonies (Gels *et al.* 2002), sur la vitesse de développement et la production de reines (Whitehorn *et al.* 2012). Parmi la gamme des pesticides ayant un impact sur les abeilles, les plus connus (et médiatisés) sont les pyréthrinoïdes et les néonicotinoïdes (Thompson & Hunt 1999 ; Goulson 2013). Ces derniers sont des neurotoxiques provoquant paralysie et mort des insectes (Tomizawa & Casida 2005). A leur toxicité aigüe s'ajoute leur grande rémanence dans les sols, les bourdons nichant à proximité étant exposés sur une longue durée (Goulson *et al.* 2015), et leur accumulation dans les ressources florales comme le nectar (Krupke

et al. 2012). Cependant, deux autres pesticides bien moins médiatisés, le diflubenzuron et le teflubenzuron, sont parmi les plus toxiques pour les bourdons et pourtant largement utilisés (Mommaerts *et al.* 2006). C'est également le cas de la deltaméthrine, bien connue pour sa haute toxicité envers les abeilles (*e.g*. Moncharmont *et al*. 2003 ; Dai *et al*. 2010).

6.4. Les changements climatiques

Plus récemment, le changement climatique a été avancé comme facteur non négligeable du déclin des bourdons (Williams *et al.* 2007 ; Iserbyt & Rasmont 2012 ; Rasmont & Iserbyt 2012 ; Bartomeus *et al.* 2013b ; Kerr *et al.* 2015 ; Rasmont *et al.* 2015). En effet, en plus des espèces spécialisées, plusieurs espèces initialement abondantes et largement répandues sont maintenant en régression, même dans les régions encore riches en habitats favorables. Les évènements extrêmes de sécheresses et de vagues de chaleur semblent avoir un impact important sur la disponibilité en ressources florales et sur les populations de bourdons, y compris dans les régions favorables en termes d'habitat (Iserbyt & Rasmont 2012 ; Rasmont & Iserbyt 2012 ; Ploquin *et al.* 2013 ; Herrera *et al.* 2014). Les températures à partir de 40°C qui surviennent lors des canicules provoquent une mortalité rapide chez les bourdons mâles (Martinet *et al.* 2015). Les sécheresses peuvent causer une forte mortalité de la végétation herbacée (Brochet 1977 ; Thomson 2016), et donc indirectement affecter les colonies de bourdons en réduisant la disponibilité des ressources florales (Memmott *et al.* 2007 ; Thomson 2016 ; Ogilvie *et al*. 2017). À

l'inverse, les inondations peuvent également entrainer une forte mortalité chez les espèces de bourdon qui nichent dans une cavité souterraine (Goulson *et al.* 2015).

En plus de ces évènements extrêmes ponctuels, les modifications graduelles et continues du climat ont également des conséquences dramatiques sur les bourdons (Kerr *et al.* 2015 ; Rasmont *et al.* 2015). De nombreuses espèces présentent des phénomènes de déplacement de leurs populations (Ploquin *et al.* 2013) et plus globalement de changement de leur distribution géographique, résultant pour la plupart en un décalage vers les latitudes Nord ou une contraction de leur aire de distribution (Bartomeus *et al.* 2013a ; Aguirre-Gutiérrez *et al.* 2016). Le récent « *Climatic Risk and Distribution Atlas of European Bumblebees* » (Rasmont *et al.* 2015) estime les distributions futures des espèces européennes selon leur niche climatique et prévoit un déclin et une contraction d'aire de répartition pour la plupart des espèces pour les horizons 2050 et 2100.

De plus, ces changements climatiques graduels peuvent provoquer un décalage de phénologie entre l'émergence des bourdons et la floraison de leurs plantes nourricières, ceux-ci ne répondant pas forcément aux mêmes stimuli, et altérant ainsi le fonctionnement des réseaux plantes-pollinisateurs (Memmott *et al.* 2007 ; Hegland *et al.* 2009 ; Bartomeus *et al.* 2013b ; Forrest 2014 ; Pyke *et al.* 2016 ; Schleuning *et al.* 2016 ; Ogilvie *et al*. 2017).

7. Le cas des bourdons de Belgique

Très peu de régions présentent des informations détaillées et remontant loin dans le temps sur les bourdons, ce qui rend difficile l'estimation précise du déclin de ces

espèces. Deux pays font exception : le Royaume-Uni (Williams 1982 ; Goulson 2010) et la Belgique (Rasmont 1988). La faune des bourdons de Belgique est étudiée depuis plus de cent ans. Les premiers à s'intéresser aux bourdons de Belgique furent Meunier (1888) et surtout F. J. Ball, qui réalisa un immense travail de collecte de données au début des années 1910s et principalement durant la Première Guerre Mondiale (Ball 1914, 1920). A cette époque, 31 espèces étaient présentes en Belgique. Depuis, plusieurs rapports ont indiqué qu'une multitude d'espèces étaient en déclin depuis les années 1950 (Rasmont & Mersch 1988 ; Rasmont *et al.* 1993), et ont continué à régresser après les années 1990 à l'inverse d'autres espèces de pollinisateurs comme les syrphes (Carvalheiro *et al.* 2013).

8. Objectifs et questions de recherche

Cette dissertation a pour ambition d'**affiner la compréhension des processus responsables de la régression des populations de bourdons** en Belgique, ce qui permettra à plus long terme de **développer des stratégies de conservation en faveur de ces pollinisateurs indispensables** pour les écosystèmes naturels et leurs services rendus à la production agricole, qui tiennent compte d'une compréhension fine de ces processus. La thèse s'articule autour de quatre chapitres analysant les changements d'abondance et de distribution des espèces de bourdons ainsi que leurs liens avec les différentes causes de déclin (Figure 7).

Le premier objectif (**Chapitre 1**) est de mettre à jour les connaissances sur les statuts et tendances des espèces de bourdons en Belgique, en combinant et analysant des données anciennes nouvellement disponibles avec des données

actuelles récemment collectées. Nous étudierons à la fois les changements d'abondance et de taille de l'aire de distribution de chaque espèce à l'échelle de la Belgique. De plus, les différents traits écologiques des espèces seront étudiés afin de déterminer quels types de traits sont corrélés avec un plus fort déclin. **Cette étude constitue la première étape dans l'établissement de la liste rouge des espèces de bourdons de Belgique.**

Le deuxième objectif (**Chapitre 2**) est de clarifier l'importance des différentes familles de plantes dans la diète de chaque caste de bourdons. L'accent sera mis en particulier sur la diète des mâles, dont les quelques rares études disponibles attestent l'importance des Asteraceae et plus particulièrement des chardons. L'importance des espèces de chardons ciblées par les lois d'échardonnage sera évaluée pour chaque espèce de bourdon, afin d'estimer la potentielle menace que de telles lois pourraient avoir sur leurs populations.

Le troisième objectif (**Chapitre 3**) est d'identifier les changements d'occupation et d'utilisation du sol qui pourraient expliquer les modifications des assemblages de bourdons *à l'échelle locale*, en étudiant en détail quatre localités de Belgique bien échantillonnées il y a cent ans et actuellement, et ayant des dynamiques d'occupation et d'utilisation du sol contrastées.

Enfin, le quatrième et dernier objectif (**Chapitre 4**) est d'assembler les données de bourdons, les données d'occupation du sol et les données climatiques dans un seul et même jeu de données afin de modéliser les distributions de chaque espèce de bourdon *à l'échelle de la Belgique* durant les trois mêmes périodes de temps que celles considérées au Chapitre 1. Ceci permettra d'identifier les facteurs ayant joué

un rôle dans les changements passés des populations de bourdons à une échelle plus large que celle étudiée au sein Chapitre 3. Ces résultats permettront d'identifier les variables clés et les défis à relever pour les modélisations des aires de distribution futures des espèces de bourdons.

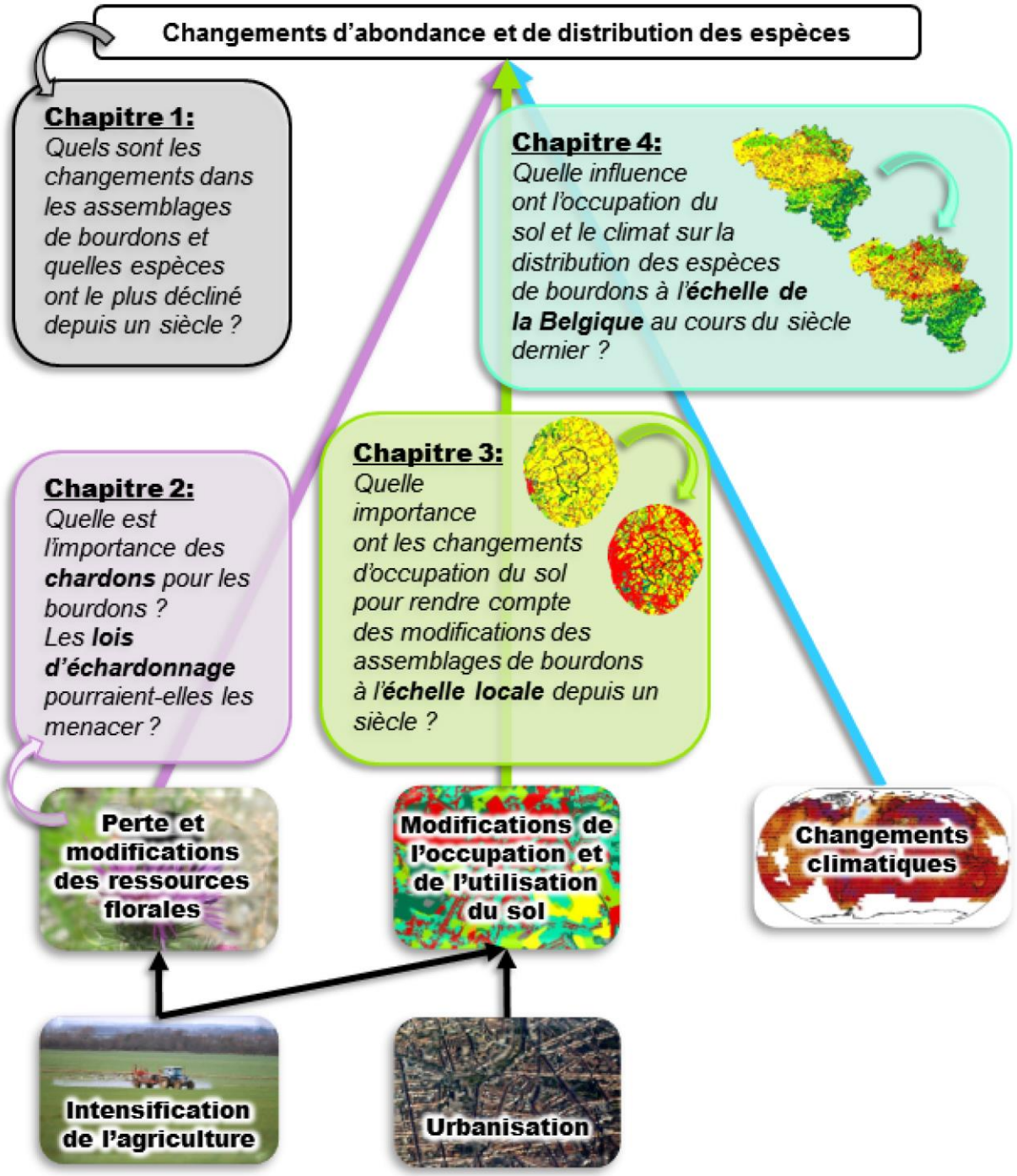

Figure 7. Structure de la thèse, organisée en quatre chapitres.

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Cadre méthodologique général

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1. Le projet BELBEES

Cette thèse s'inscrit dans le cadre du projet fédéral BELBEES (« *Multidiciplinary assessment of BELgian wild BEE decline to adapt mitigation management policy* », www.belbees.be). Le projet BELBEES est un projet de recherche de conservation financé par la Politique Scientifique Fédérale (BELSPO; BR/132/A1/BELBEES) qui vise à réaliser une estimation multidisciplinaire du déclin des abeilles sauvages de Belgique afin d'adapter les politiques de conservation. Ce projet rassemble plusieurs partenaires : l'Université de Mons (Pierre Rasmont, le coordinateur du projet), l'Institut Royal des Sciences Naturelles de Belgique (Jean-Luc Boevé), l'Université de Gand 1 (Dirk de Graaf), l'Université de Namur (Nicolas Dendoncker), l'Université de Liège Gembloux Agro Bio-Tech (Marc Dufrêne), l'Université de Gand 2 (Guy Smagghe), et les associations naturalistes Natagora (Wallonie) et Natuurpunt (Flandre).

Figure 1. Logo du projet BELBEES.

Le projet BELBEES a pour buts de 1) rassembler et analyser les données sur les changements des populations d'abeilles sauvages de Belgique, et 2) mesurer les rôles respectifs des différents facteurs responsables de leur déclin afin d'identifier les pistes d'actions pour le service écosystémique de pollinisation. Il se subdivise en six « *working packages* », dont :

- WP2 : collecte des données d'abeilles sauvages anciennes et actuelles, encodage et analyses de tendances et de distributions des espèces.
- WP3 : test des différentes hypothèses de déclin :

1) Perte en ressources florales
2) Fragmentation de l'habitat et perte de diversité génétique
3) Emergence de maladies
4) Développement des pesticides
5) Changements climatiques

- WP4 : méta-analyse et modélisation.

Dans le cadre de cette dissertation, la partie concernant les bourdons sera abordée dans le **Chapitre 1** pour le WP2, le **Chapitre 2** pour le WP3.1, le **Chapitre 3** ainsi que l'**Annexe III** (Maebe *et al.* 2016) pour le WP3.2, et le **Chapitre 4** ainsi que l'**Annexe IV** (Marshall *et al*. 2017) pour le WP4.

2. Echelles de travail

Les changements environnementaux se produisent à différentes échelles spatiales et temporelles, et la manière dont les organismes répondent à ces changements dépend de leurs échelles (Turner 1989). Par conséquent, les processus écologiques analysés dans une étude dépendent des échelles et de la résolution choisies (Turner 1989 ; Rahbek 2005). Par exemple, une échelle spatiale de haute résolution couvrant une petite étendue permet l'étude de l'influence des facteurs locaux (*e.g.* fragmentation de l'habitat) sur les populations et communautés locales. Par contre, une échelle plus globale couvrant une large étendue décèle par exemple les

changements de distribution d'une espèce liés aux changements environnementaux globaux (*e.g.* changements climatiques). L'approche multi-échelle de cette étude permet donc de déceler différents processus agissant à différentes échelles.

Les échelles spatiales les plus fines sont étudiées dans le **Chapitre 3**, où les modifications des assemblages de bourdons sont comparées aux changements de composition du paysage et d'intensité de l'utilisation du sol dans quatre localités belges. Deux échelles spatiales sont abordées : une zone « tampon » (« *buffer* » en anglais) de un kilomètre et une de trois kilomètres autour de l'ancienne commune (voir les détails au point 3.4.2). La résolution spatiale est la plus haute possible et correspond à la résolution des cartes topographiques (*i.e.* 1:20.000^{e}, un centimètre de la carte représente 200 mètres). Deux périodes de temps sont étudiées : 1910-1930 et 2013-2015.

L'échelle de la Belgique est étudiée dans les **Chapitres 1 et 4**, qui s'attardent sur les changements de distribution des bourdons, de l'occupation du sol et du climat à travers le pays. La résolution spatiale est ici plus faible et correspond à des carrés de 5x5 km (ou de 10x10km pour les indices de diversité du Chapitre 1). Les deux chapitres étudient trois périodes de temps : 1910-1930, 1970-1989 et 1990-2016.

Enfin, l'échelle spatiale la plus large concerne le **Chapitre 2**, qui englobe non seulement la Belgique, mais aussi la France, les Pays-Bas, et le Royaume-Uni. Dans

ce chapitre, l'entièreté des données de bourdons et de plantes visitées est exploitée sans découpage spatial et sans distinction de l'année, allant de 1878 à 2015.

3. Les jeux de données

3.1. Les données faunistiques

Les données faunistiques ont été accumulées en plusieurs étapes plus ou moins successives (Figure 2). L'identification des spécimens de bourdons a été réalisée en suivant la clé d'identification de Rasmont & Terzo (2010). L'encodage et la gestion des données ont été réalisés dans le programme Data Fauna-Flora version 5.0.6 (Barbier *et al.* 2015) et ont permis de compléter la *Base de Données Fauniques de Gembloux et de Mons* (BDFGM) au départ du jeu de données présenté par Rasmont *et al*. 2015.

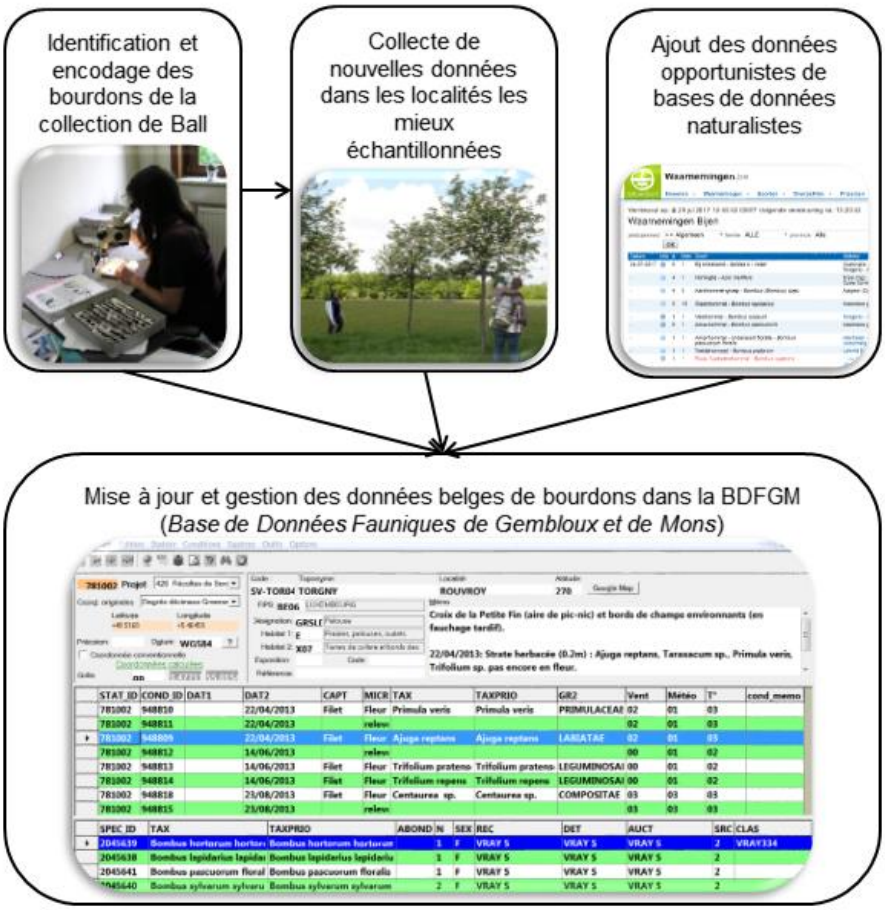

Figure 2. Méthodes d'accumulation et de gestion des données fauniques de sources différentes. Photos : S. Vray.

La première étape a été de (ré)identifier et d'encoder chaque spécimen de bourdon de la collection de F.J. Ball (Ball 1914, 1920), conservée à l'Institut Royal des Sciences Naturelles de Belgique (IRSNB), et couvrant majoritairement la période de 1910 à 1930. Même si tous les spécimens avaient été identifiés par Ball, l'identification de chacun a dû être vérifiée et parfois corrigée. En effet, en plus des quelques erreurs d'identification, Ball ignorait la diversité des espèces du sous-genre *Bombus*, présentée par Rasmont (1984), ainsi que la présence de *Bombus cullumanus* (Rasmont 1982). De ce fait, une nouvelle étiquette de détermination (Figure 3) a été ajoutée aux étiquettes originelles (Figure 4). Une partie (environ

21 300 spécimens) de la collection a été encodée durant la thèse de P. Rasmont (Rasmont 1988). Le reste (environ 41 000 spécimens) a été encodé durant les premières années de cette thèse. Tous les spécimens ont pu être encodés à l'exception des quelques 16 000 ouvrières des quatre espèces du sous-genre *Bombus* (*B. cryptarum*, *B. lucorum*, *B. magnus*, *B. terrestris*), pratiquement impossibles à différencier sur base morphologique de manière fiable avec les méthodes actuelles. Pour chaque spécimen, la localité, la date et le récolteur, mentionnés sur les étiquettes (Figure 4), ont été encodés. Des coordonnées géographiques conventionnelles, correspondant au clocher du village, ont été assignées à chaque localité. La plus petite division administrative qui correspond à ces coordonnées est l'ancienne commune.

Det. P. RASMONT
& S. VRAY 2015
Bombus hortorum
hortorum (L.)

Figure 3. Exemple d'étiquette de détermination ajoutée aux étiquettes originelles.

Figure 4. Exemple de boite de la collection de F.J. Ball, et exemples d'étiquettes accompagnant chaque spécimen. Deux étiquettes sont présentes pour chaque individu. La première indique la localité, la date (ou à défaut l'année), et le récolteur. La deuxième mentionne l'espèce identifiée, la caste, ainsi que le nom du déterminateur et l'année de l'identification. Photos : S. Vray.

La collection de F.J. Ball incluait à l'origine la collection du Baron P. De Moffarts et la collection Tosquinet. Elle a ensuite été complétée par une enquête que le Musée Royal d'Histoire Naturelle de Bruxelles a entreprise en 1913 en se limitant au territoire belge (Ball 1914). Très peu d'informations quant-aux techniques d'échantillonnage sont disponibles, mais F.J. Ball parle dans son manuscrit de 1914 de « chasses méthodiques faites dans diverses régions dissemblables du pays » et de « recherche et élevage des nids ». Durant la seule année 1913, 10 000 bourdons ont ainsi été collectés (Ball 1914). Dans son manuscrit de 1920, F.J. Ball annonce que les « chasses méthodiques entreprises sous la direction du Musée dans certaines localités représentatives des diverses parties du pays » ont continué les années suivantes et ont permis d'accroître considérablement le nombre de spécimens collectés (Ball 1920). Au total, la collection de F.J. Ball rassemble plus de 78 000 spécimens, dont environ 62 000 qui ont pu être encodés dans notre base de données. Selon nos données, environ 40 récolteurs ont participé aux collectes de cette collection. Ceux qui y ont le plus contribué sont F.J. Ball lui-même (37% des spécimens dont le nom du récolteur est connu), G. Severin (27%), L. Grenson (6%), A. Koller (5%), A. Boone (5%), R. Derick (4%), J. Destage (3%), P. Mertens (2%), V. Vanshepdael (2%), A. Honoré (2%), et De Hennin (2%).

Une fois les données centenaires encodées, il a été possible d'identifier les localités qui ont été les plus échantillonnées à cette époque (Figure 5). Parmi les mieux échantillonnées, 29 anciennes communes ont été sélectionnées pour les collectes actuelles dans le cadre du projet BELBEES. Parmi elles, six anciennes

communes ont été ré-échantillonnées dans le cadre de cette thèse : Moorsel (province de Flandre Orientale, en région de Flandre sablo-limoneuse), Trivières et Saint-Vaast (province du Hainaut, en région limoneuse hennuyère), Francorchamps (province de Liège, en Ardenne), ainsi que Torgny et Lamorteau (province du Luxembourg, en Lorraine gaumaise). Les anciennes communes de Trivières et Saint-Vaast ont été fusionnées (sous le nom de « Trivières »), ainsi que celles de Torgny et Lamorteau (sous le nom de « Torgny »), afin d'obtenir des surfaces comparables pour les analyses. Les données de ces quatre localités sont exploitées dans le **Chapitre 3** et les spécimens de ces collectes ont été utilisés pour les analyses génétiques de l'étude présentée dans l'**Annexe III** (Maebe *et al.* 2016).

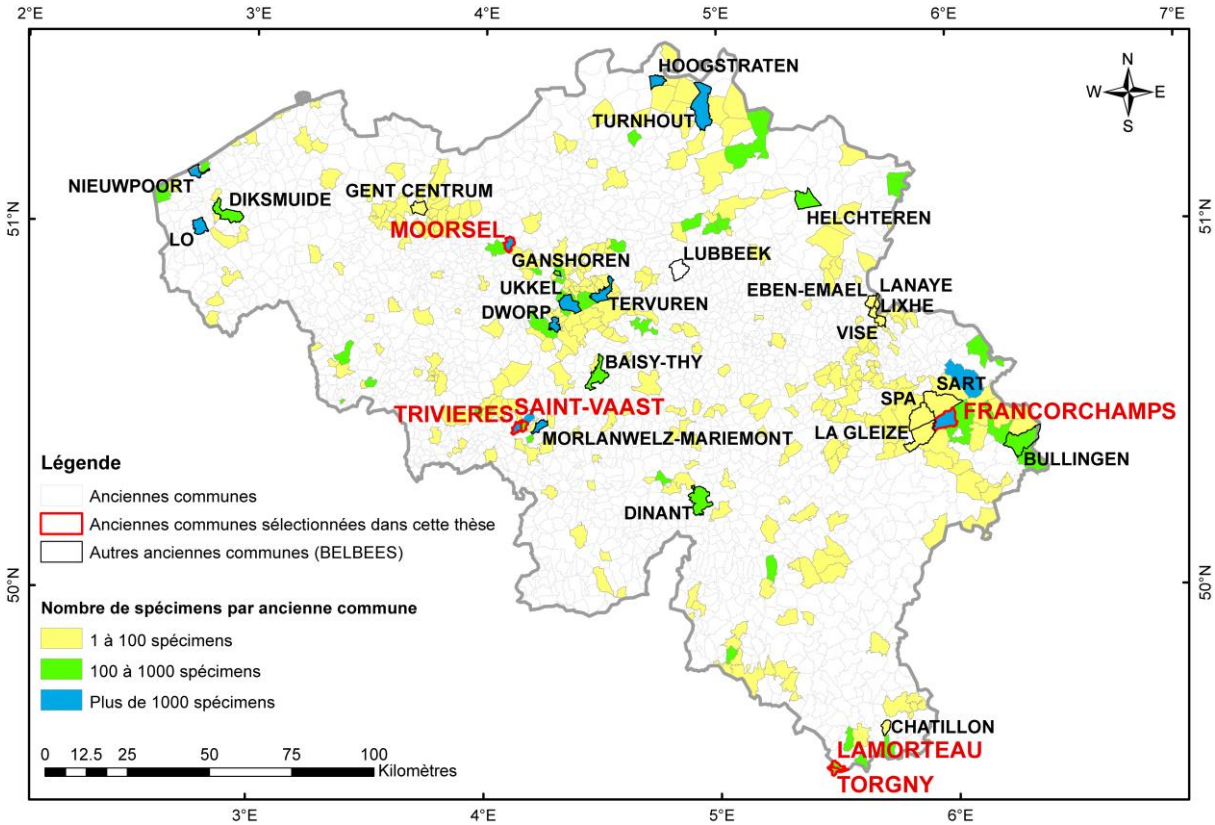

Figure 5. Nombre de spécimens anciens (1910-1930) par ancienne commune, et anciennes communes sélectionnées. Les anciennes communes encadrées en rouge sont celles ré-échantillonnées dans le cadre de cette thèse, celles encadrées en noir sont les autres sélectionnées dans le cadre du projet BELBEES.

Les nouveaux échantillonnages de chacune des quatre localités se sont déroulés durant trois années consécutives, à raison de trois jours par localité en 2013, trois en 2014 (quatre pour Francorchamps), et quatre en 2015 (Figure 6). Les jours d'échantillonnage se sont étalés de mars à septembre afin de capturer à la fois les espèces à émergence précoce et à émergence tardive. Au total, plus de 3900 spécimens ont été collectés par 15 récolteurs sur 41 jours, dont 31 jours (76%) avec un seul récolteur (Figure 6). Onze stations ont été échantillonnées dans Moorsel, 10 dans Trivières (et Saint-Vaast), 19 dans Francorchamps et 13 dans Torgny (et Lamorteau).

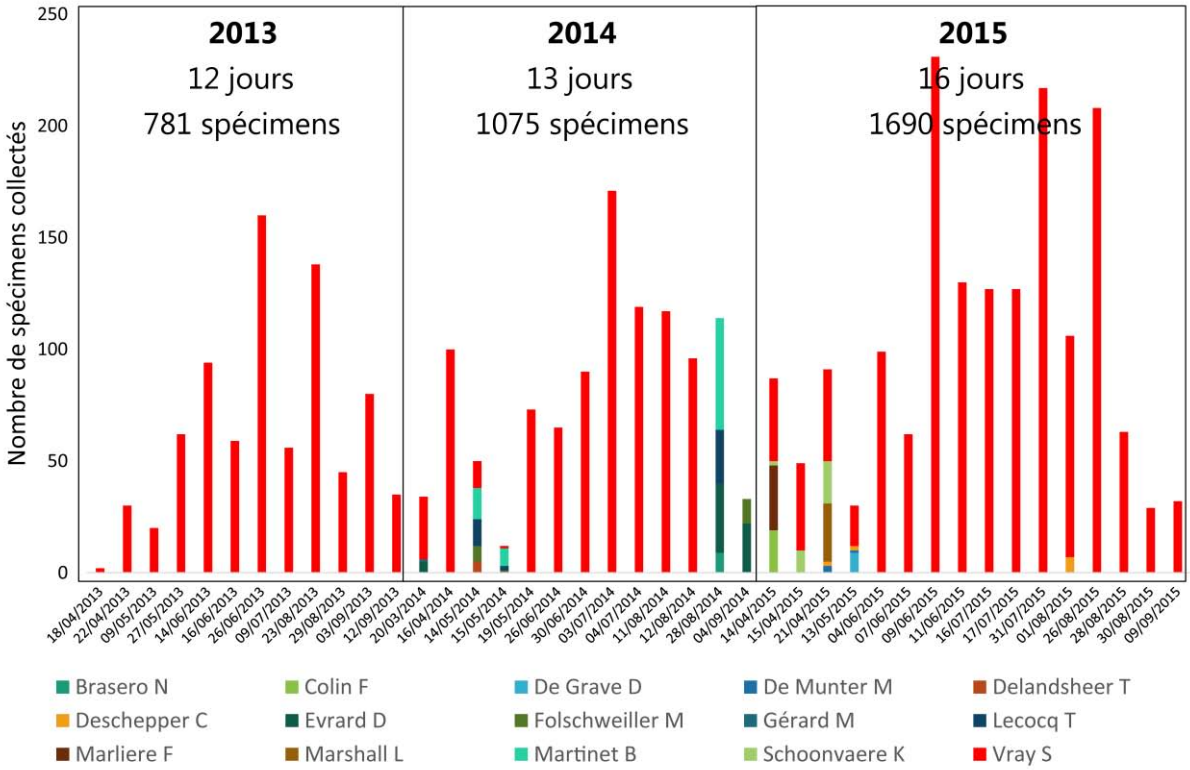

Figure 6. Distribution temporelle des jours d'échantillonnage des quatre localités (Trivières (et Saint-Vaast), Moorsel, Francorchamps, Torgny (et Lamorteau)) étudiées dans le Chapitre 3 et dans l'Annexe III (Maebe *et al*. 2016).

L'échantillonnage de chaque ancienne commune a été effectué suivant le protocole de Westphal *et al.* (2008) et a consisté en des transects (des marches en ligne droite) où tous les bourdons rencontrés ont été capturés à l'aide d'un filet, durant des conditions météorologiques favorables pour les bourdons (temps sec, température d'au moins 15°C, vent de force inférieure à 6 sur l'échelle de Beaufort). Pour chaque visite dans chaque station, un relevé phytosociologique a été effectué, ainsi que toute observation jugée utile (par exemple le type de champs à proximité, l'intensité de pâture, si le pré ou le bord de route vient d'être fauché, etc.). Pour chaque spécimen, plusieurs informations ont été notées : l'identification de l'espèce, la caste, le récolteur, la date, la plante sur laquelle se trouvait le bourdon ou les conditions de capture (en vol, sur le sol, etc.). L'identification de chaque individu a ensuite été confirmée sous binoculaire au Laboratoire de Zoologie de l'UMONS suivant la clé de Rasmont & Terzo (2010).

Enfin, en plus des données centenaires et des données collectées sur le terrain, la BDFGM a été complétée par la base de données naturalistes de Observations.be/Waarnemingen.be appartenant à Natagora/Natuurpunt, partenaires du projet BELBEES. Cette base de données reprend les données opportunistes de centaines d'observateurs à travers le pays (majoritairement en Flandre).

La base de données résultante, intégrée dans la BDFGM, est exploitée dans chaque chapitre de cette thèse ainsi que dans les diverses études des partenaires du projet BELBEES.

3.2. Les données floristiques

Les données floristiques de la BDFGM ont principalement été exploitées dans le **Chapitre 2**, qui aborde l'importance des chardons dans la diète des bourdons et la potentielle menace des lois d'échardonnage. Afin de disposer de suffisamment de données, les données de visites de fleurs ont été accumulées à partir de la BDFGM pour les quatre pays concernés par une loi d'échardonnage : la Belgique, la France, le Royaume-Uni et les Pays-Bas.

Dans le **Chapitre 3**, un relevé des plantes butinées par les bourdons a été réalisé lors de chaque visite dans chaque station échantillonnage des quatre localités d'étude. Ces données floristiques ont permis d'ajouter l'information sur la diversité floristique utilisée par les bourdons dans chaque site pour la période 2013-2015.

3.3. Les données des traits écologiques spécifiques

Les données des traits écologiques de chaque espèce ont été compilées à partir de la BDFGM et de plusieurs autres sources. Il s'agit de traits divers sur l'inquilinisme, la stratégie de nidification (von Hagen & Aichhorn 2014), la longueur de la langue (Goulson & Darvill 2004), les préférences en familles et genres de plantes, la largeur de diète (nombre de familles et de genres visités), le nombre et les préférences d'habitats (Pittioni & Schmidt 1942; Reinig 1972; Rasmont 1988), la phénologie, et la rareté en Belgique ainsi qu'en Europe (statuts IUCN européens). Les traits écologiques utilisés et leurs sources sont détaillés dans le **Chapitre 1**.

3.4. Les données d'occupation du sol

Toutes les manipulations de cartes et analyses cartographiques ont été réalisées à l'aide du logiciel ArcGIS version 10 (ESRI 2011). Différentes sources de données ont été utilisées selon l'échelle de l'étude (Figure 7).

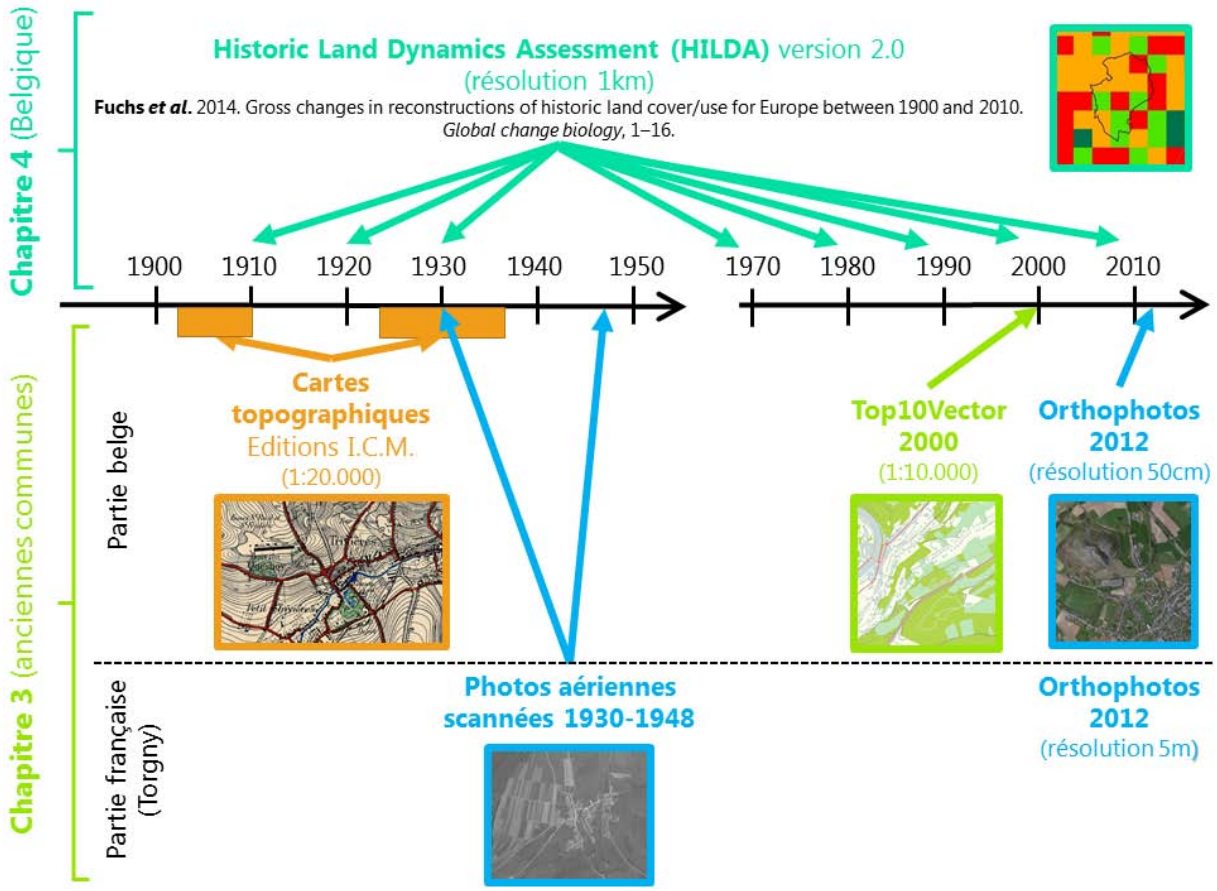

Figure 7. Sources des données d'occupation du sol utilisées dans les Chapitres 3 et 4.

3.4.1. L'échelle de la Belgique

Les données d'occupation du sol utilisées pour l'étude à l'échelle de la Belgique (**Chapitre 4**) ont été obtenues par le projet "*Historic Land Dynamics Assessment*" (HILDA) version 2.0 (Fuchs *et al.* 2013, 2014). Ce projet vise à reconstituer l'occupation du sol à l'échelle de l'Europe de 1900 à 2010, à partir de données issues

de la télédétection, des inventaires nationaux, des photographies aériennes, des statistiques d'occupation du sol et des cartes historiques. La base de données est disponible pour chaque décade à une résolution spatiale de 1 km^2, sur le site du « *Laboratory of Geo-information Science and Remote Sensing* » de l'Université de Wageningen (www.wur.nl/en/Expertise-Services/Chair-groups/Environmental-Sciences/Laboratory-of-Geo-information-Science-and-Remote-Sensing/Models/Hilda.htm).

L'occupation du sol est classée en six catégories (Figure 8):

1) Les bois et forêts, y compris les zones de transition entre buissons et forêt, les pépinières d'arbres, les zones de reboisement pour la foresterie ;
2) Les prairies, y compris les prairies naturelles, les zones humides et les pâturages ;
3) Les terres cultivées, y compris les vergers ;
4) Les installations humaines : bâtiments, routes, chemins de fer… y compris jardins et zones urbaines vertes ;
5) L'eau (mers, rivières, lacs, marais, etc.) ;
6) Une catégorie « autre » regroupant les zones de végétation rudérale, les plages, les sols nus, les rochers, etc.

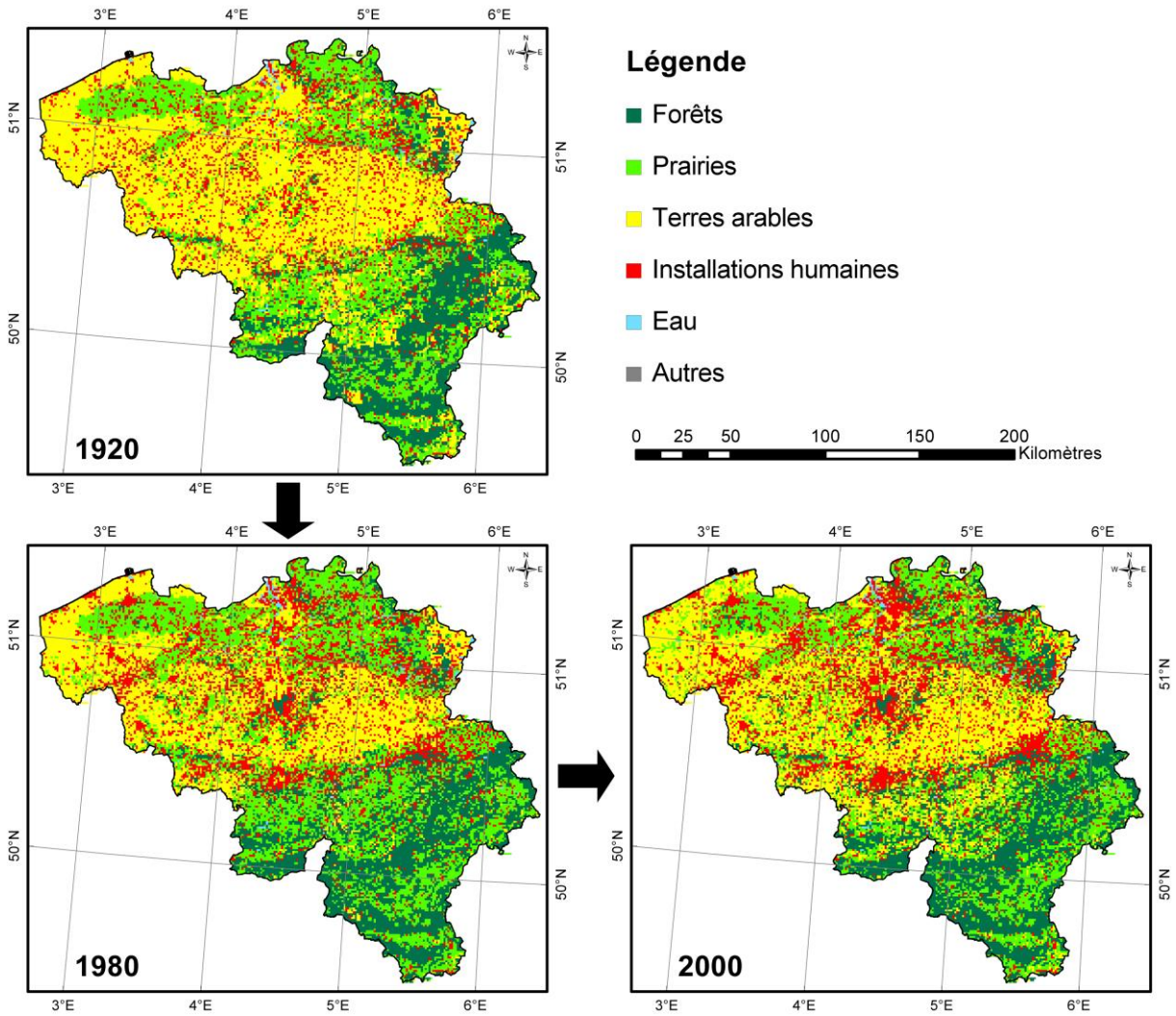

Figure 8. Occupation du sol en 1920 (centre de la période 1910-1930), en 1980 (centre de la période 1970-1989) et en 2000 (centre de la période 1990-2016).

3.4.2. L'échelle des anciennes communes

La résolution de 1 km² des données HILDA n'étant pas assez précise pour l'étude à l'échelle des anciennes communes (**Chapitre 3**), d'autres sources ont été utilisées pour les deux périodes de temps envisagées (1910-1930 et 2013-2015 ; Figure 7). Comme les effets de la structure et de la composition du paysage sur les bourdons et les pollinisateurs en général varient selon l'échelle spatiale (Steffan-Dewenter *et al.* 2002; Westphal *et al.* 2006), deux zones « tampon » autour des limites des

anciennes communes ont été considérées : 1 km, représentant la distance de butinage moyenne ou maximale selon les espèces de bourdons, et 3 km, représentant la distance maximale de la grande majorité des espèces et permettant de prendre en compte le contexte environnemental des sites (Walther-Hellwig & Frankl 2000; Kreyer *et al.* 2004; Knight *et al.* 2005; Greenleaf *et al.* 2007; Osborne *et al.* 2008; Wolf & Moritz 2008; Hagen *et al.* 2011; Carvell *et al.* 2012).

Pour la période de 1910 à 1930, des cartes topographiques à l'échelle 1:20.000 issues de l'Institut Cartographique Militaire, actuel Institut Géographique National (IGN) belge, ont été utilisées. Celles-ci ont été géoréférencées et vectorisées manuellement (exemple de Francorchamps sur la Figure 9a). Comme l'ancienne commune de Torgny se situe à la frontière française (Figure 5), des photographies aériennes de l'an 1930 issues de l'IGN français ont également été géoréférencées et vectorisées (Figure 9c). Au total, huit catégories d'occupation du sol ont pu être distinguées par photo-interprétation (Figures 9b et d):

1) Les terres arables, incluant également les pépinières, les oseraies et les petites cultures maraichères ;
2) Les vergers ;
3) Les prairies, incluant les pâturages, les prés de fauche et les prairies marécageuses ;
4) Les landes, incluant les bruyères et prés-sarts, les landes buissonneuses, les landes associées à des conifères ou à des feuillus, les tourbières, les fanges et terrains fangeux ;

5) Les jardins, incluant également les parcs, les pelouses entretenues, les cimetières et les potagers ;
6) Les installations humaines, incluant les bâtiments, les routes, les chemins de fer, les serres et toute autre surface stérile ;
7) Les forêts, qu'elles soient de feuillus, de conifères ou mixtes et incluant les plantations forestières et les zones buissonneuses ;
8) Une catégorie « autre » reprenant l'eau, les marais et marécages, les rochers, les excavations, les carrières, les tertres, les sablières, les schorres et le sable.

Pour la période récente (2013-2015), la carte numérique vectorielle « Top10Vector » issue de l'IGN a été utilisée. Cette carte est un jeu de données contenant les données topographiques vectorielles de Belgique à l'échelle 1:10.000 pour l'année 2010 et consistant en 37 catégories d'occupation du sol. Ces dernières ont été reclassées dans les huit mêmes classes d'occupation du sol que pour les données anciennes. Pour la partie française de Torgny, des orthophotos à la résolution de 5 mètres de l'année 2012 ont été vectorisées et classées manuellement par photo-interprétation. Les cartes et résultats des analyses cartographiques sont présentés dans le Chapitre 3.

En complément de l'occupation du sol, différentes statistiques agricoles à l'échelle des communes et anciennes communes ont été collectées pour les deux périodes de temps étudiées. Celles-ci permettent de donner une idée de l'utilisation des parcelles agricoles (cultures et prairies). Ces statistiques incluent les surfaces relatives de différents types de culture (légumineuses, céréales, pommes de terre,

betterave sucrière), la surface relative de jachères, les rendements à l'hectare de cultures (orge, épeautre, pomme de terre, betterave sucrière), ainsi que le nombre et la densité de bétail (bovins, chèvres, chevaux, moutons).

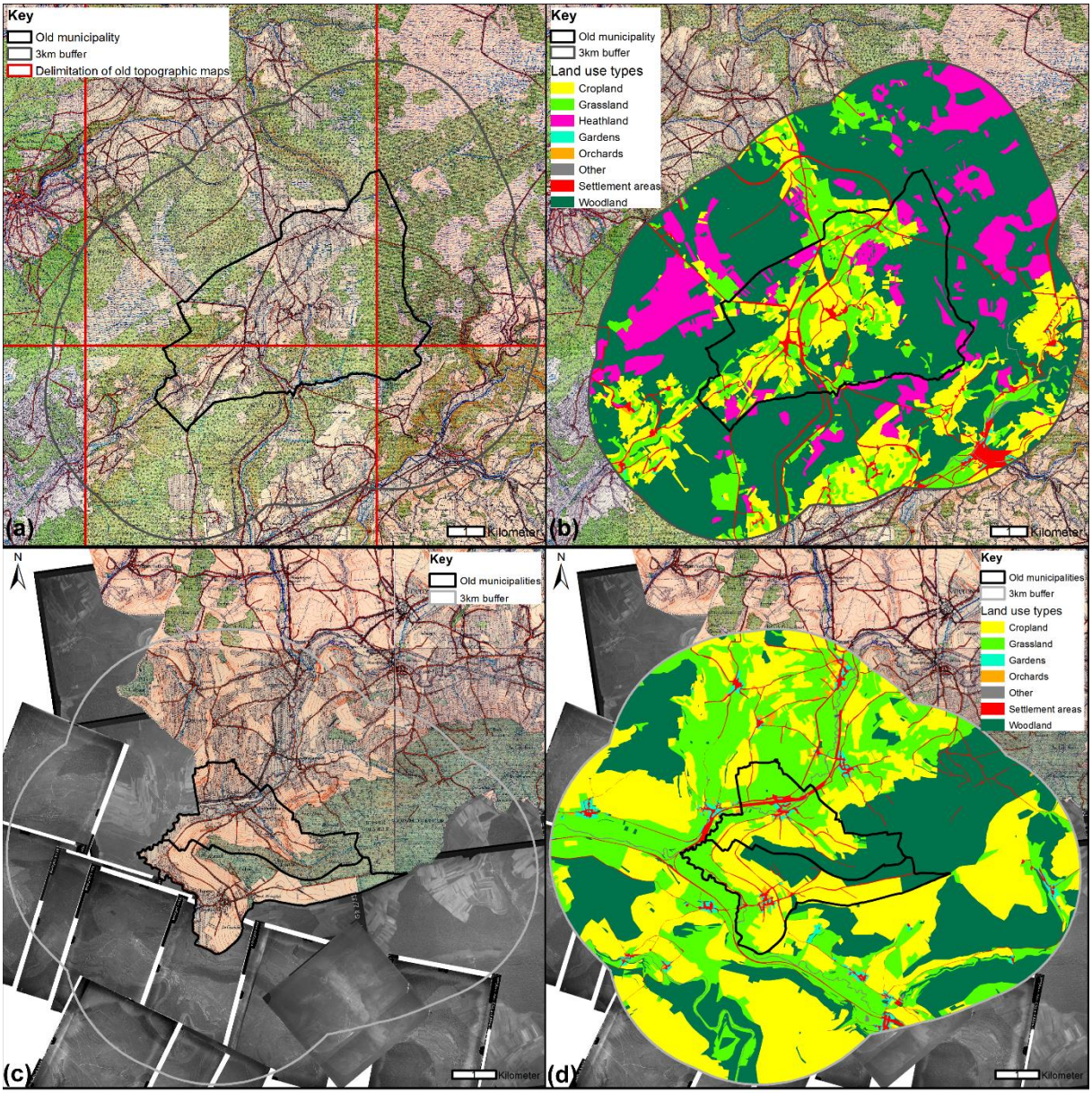

Figure 9. Processus de classification par photo-interprétation en classes d'occupation du sol à partir de cartes topographiques numérisées pour Francorchamps (a et b) et de cartes topographiques (pour la partie belge) et photographies aériennes (pour la partie française) numérisées pour Torgny (c et d), pour la période 1910-1930.

3.5. Les données climatiques

Les données climatiques ont été générées à partir du programme ClimateEU version 4.63, disponible à l'adresse « http://tinyurl.com/ClimateEU ». Ces données sont issues du projet "*ClimateEU: historical and projected climate data for Europe*" (Wang *et al.* 2012; Hamann *et al.* 2013), et la méthodologie employée est décrite par Hamann *et al.* (2013). Ce programme permet de générer toute une gamme de variables climatiques (Tableau 1) pour un ensemble de coordonnées géographiques, de l'année 1900 à nos jours.

Tableau 1. Données de températures et de précipitations générées par le programme ClimateEU.

Températures (°C)	
Tave01 → 12	Température moyenne du mois de janvier (01) à décembre (12)
Tmax01 → 12	Température maximale du mois de janvier (01) à décembre (12)
Tmin01 → 12	Température minimale du mois de janvier (01) à décembre (12)
Tave_wt	Température moyenne de l'hiver (décembre à février)
Tave_sp	Température moyenne du printemps (mars à mai)
Tave_sm	Température moyenne de l'été (juin à aout)
Tave_at	Température moyenne de l'automne (septembre à novembre)
Tmax_wt	Moyenne des températures maximales de l'hiver (décembre à février)
Tmax_sp	Moyenne des températures maximales du printemps (mars à mai)
Tmax_sm	Moyenne des températures maximales de l'été (juin à aout)
Tmax_at	Moyenne des températures maximales de l'automne (septembre à novembre)
Tmin_wt	Moyenne des températures minimales de l'hiver (décembre à février)
Tmin_sp	Moyenne des températures minimales du printemps (mars à mai)
Tmin_sm	Moyenne des températures minimales de l'été (juin à aout)
Tmin_at	Moyenne des températures minimales de l'automne (septembre à novembre)
MAT	Température annuelle moyenne
MWMT	Température moyenne du mois le plus chaud
MCMT	Température moyenne du mois le plus froid
TD	Différence de température entre MWMT et MCMT
Précipitations (mm)	
PPT01 → 12	Précipitations du mois de janvier (01) à décembre (12)
PPT_wt	Précipitations de l'hiver (décembre à février)
PPT_sp	Précipitations du printemps (mars à mai)
PPT_sm	Précipitations de l'été (juin à aout)
PPT_at	Précipitations de l'automne (septembre à novembre)
MAP	Précipitations annuelles moyennes

A partir de ces données, nous avons pu dériver les variables bioclimatiques communément utilisées en écologie, présentées dans le tableau 2. Nous avons ensuite effectué une sélection de ces variables à utiliser pour les modélisations d'aire de distribution de chaque espèce de bourdon, expliquées dans le Chapitre 4.

Tableau 2. Variables bioclimatiques. Seule une sélection de ces variables a été utilisée dans le Chapitre 4.

Températures (°C)	
BIO1	Température annuelle moyenne
BIO2	Amplitude thermique quotidienne moyenne (différence moyenne entre la température maximale et la température minimale mensuelle)
BIO3	Isothermalité (= (BIO2 / BIO7)*100)
BIO4	Saisonnalité des températures (écart-type * 100)
BIO5	Température maximale du mois le plus chaud
BIO6	Température maximale du mois le plus froid
BIO7	Amplitude annuelle de température (= BIO5 – BIO6)
BIO8	Température moyenne du quadrimestre le plus humide
BIO9	Température moyenne du quadrimestre le plus sec
BIO10	Température moyenne du quadrimestre le plus chaud
BIO11	Température moyenne du quadrimestre le plus froid
Précipitations (mm)	
BIO12	Précipitations annuelles
BIO13	Précipitations du mois le plus humide
BIO14	Précipitations du mois le plus sec
BIO15	Saisonnalité des précipitations (coefficient de variation)
BIO16	Précipitations du trimestre le plus humide
BIO17	Précipitations du trimestre le plus sec
BIO18	Précipitations du trimestre le plus chaud
BIO19	Précipitations du trimestre le plus froid

4. Analyses statistiques

4.1. Indices de diversité et de richesse spécifique

Divers indices mathématiques permettent de calculer le niveau de richesse et de diversité en espèces d'un assemblage ou d'une communauté. Dans les **Chapitres 1**

et 3, nous nous sommes concentrés sur les indices les plus connus et utilisés, que nous détaillons ici.

4.1.1. Les indices de Shannon et de Simpson

Les indices de Shannon et de Simpson sont la plupart du temps présentés ensemble car complémentaires. Ils évaluent à la fois la richesse spécifique, c'est-à-dire le nombre d'espèces (n), et l'égalité des espèces au sein de l'assemblage (*i.e.* régularité de distribution des espèces dans l'assemblage). L'indice de Shannon (1948) est un indice dérivé de la théorie de l'information qui mesure l'entropie au sein d'un échantillon. Elle estime l'incertitude que l'espèce tirée au hasard dans un échantillon soit i, selon la formule :

$$H = -\sum_{i=1}^{n} \frac{N_i}{N} \ln\left(\frac{N_i}{N}\right)$$

Où N_i est le nombre d'individus de l'espèce i et N le nombre total de spécimens toutes espèces confondues. L'entropie H augmente à mesure que le nombre d'espèce croît. La valeur de 0 indique que l'assemblage ne contient qu'une seule espèce. Pour un nombre donné d'espèces, H est maximum quand toutes les espèces sont équitablement représentées dans l'assemblage.

L'indice de Simpson (1949), quant à lui, est une mesure de concentration qui estime la probabilité que deux individus sélectionnés aléatoirement dans un échantillon donné soient de la même espèce. La formule de l'indice de concentration de Simpson est :

$$Concentration = \frac{\sum_{i=1}^{n} N_i\ (N_i - 1)}{N\ (N-1)}$$

L'indice varie entre 0 et 1. Plus il se rapproche de 1, plus les chances d'obtenir des individus d'espèces différentes sont faibles, et donc plus la diversité de l'échantillon est faible. Afin de mesurer la diversité de l'assemblage, et ainsi rendre plus facile la comparaison avec l'indice de Shannon, nous avons considéré son opposé (D = 1 – *Concentration*), ce qui représente donc la probabilité que deux individus tirés au hasard soient d'une espèce différente. Dans ce cas, comme pour l'indice de Shannon, plus l'indice est élevé et plus l'assemblage est diversifié.

La mesure de H est plus sensible aux variations d'abondance des espèces rares, tandis que D est plus sensible aux variations d'abondance des espèces dominantes de l'assemblage.

4.1.2. L'indice de Berger-Parker

L'indice de Berger-Parker (Berger & Parker 1970) représente la proportion de l'assemblage représentée par l'espèce la plus abondante. Il s'agit donc de l'abondance relative de l'espèce la plus dominante de l'assemblage : $d = \max\left(\frac{N_i}{N}\right)$. Plus cet indice est élevé, plus l'espèce la plus abondante domine l'assemblage d'espèces.

4.1.3. Richesse spécifique et indice de Hurlbert

La richesse spécifique est simplement le nombre d'espèces enregistré dans un lieu donné à un moment donné (n). Comparer la richesse spécifique entre échantillons

de taille différente est peu recommandé car elle dépend de l'effort d'échantillonnage (Gotelli & Colwell 2001). En effet, tracer une courbe d'accumulation qui représente le nombre d'espèces en fonction du nombre de sous-échantillons (« courbe basée échantillon ») ou du nombre de spécimens collectés par sous-échantillon (« courbe basée individu ») permet de constater que la richesse spécifique augmente avec la taille de l'échantillon (Figure 10). Si la taille de l'échantillon n'est pas suffisante, l'échantillonnage a peut-être raté les espèces plus rares de l'assemblage. Il est possible d'estimer si l'échantillonnage est suffisant en analysant cette courbe d'accumulation. Si elle atteint un plateau, c'est que l'échantillonnage a très probablement pu collecter toutes les espèces présentes sur le site. Afin de « lisser » cette courbe, il est possible d'utiliser une courbe de raréfaction (Figure 10), qui est obtenue en sous-échantillonnant aléatoirement dans l'échantillon complet des sous-échantillons de toutes tailles et en calculant le nombre moyen d'espèces trouvé pour chacun. La courbe de raréfaction représente donc l'espérance statistique de la courbe d'accumulation (Gotelli & Colwell 2001).

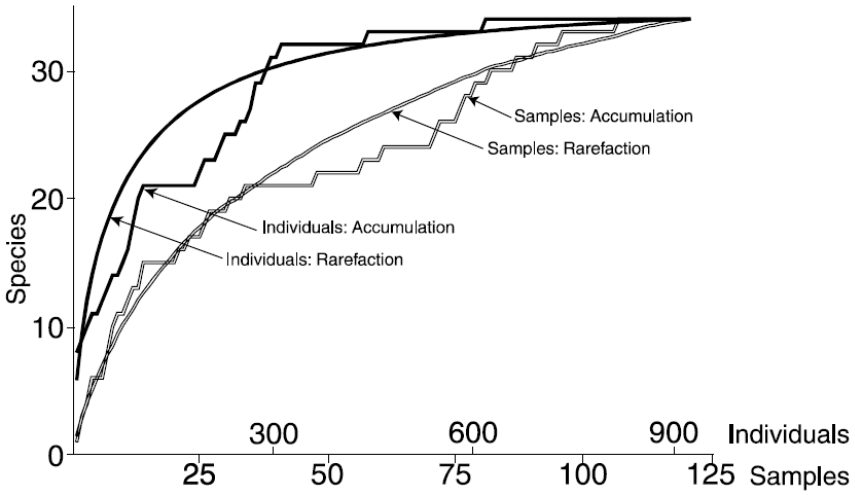

Figure 10. Courbes d'accumulation et de raréfaction basées sur le nombre d'échantillons (« *samples* ») ou d'individus (« *individuals* »). Les courbes basées échantillon sont généralement inférieures aux courbes basées individu à cause de l'agrégation spatiale des espèces (Gotelli & Colwell 2001).

Nous avons estimé l'effort d'échantillonnage *via* les courbes de raréfaction basées individu pour chaque ancienne commune échantillonnée en 1910-1930 et en 2013-2015 dans le **Chapitre 3**. Pour le **Chapitre 1**, le nombre de spécimens dans chaque période étant très élevé, toutes les courbes ont atteint un plateau et ne sont donc pas présentées.

De plus, dans ces deux chapitres, nous avons utilisé la même technique de raréfaction qui consiste à calculer le nombre d'espèces que contiendraient les échantillons s'ils étaient ramenés à la même taille. Ici, nous avons estimé la richesse spécifique espérée (ou raréfiée) dans un sous-échantillon de 100 spécimens. L'effectif de 100 est le nombre moyen de spécimens couramment collectés au cours d'une journée d'échantillonnage (selon nos données). Nous avons utilisé pour cela l'espérance de Hurlbert (1971), qui estime n' comme le nombre espéré d'espèces dans un sous-échantillon standardisé à N' spécimens (ici 100) issu de l'échantillon N contenant n espèces :

$$E(n') = \sum_{i=1}^{n} \left[1 - \frac{C_N^{N'} - N_i}{C_N^{N'}} \right]$$

Avec C qui désigne le nombre de combinaisons possibles : $C_N^{N'} = \frac{N!}{N'!(N-N')!}$.

4.2. Technique des rangs

Dans les **Chapitres 1 et 3**, nous avons utilisé une technique qui limite en partie les biais liés aux différences de taille d'échantillon pour comparer les abondances et la dominance de chaque espèce dans chaque période : la technique des rangs. Nous avons estimé le rang de chaque espèce dans chaque période basé sur son abondance relative (N_i/N). L'espèce la plus dominante de l'assemblage a un rang de 1, la deuxième plus dominante un rang de 2, et ainsi de suite. Dans le Chapitre 1, afin de comparer les changements d'abondance relative des espèces entre les périodes deux à deux, nous avons calculé le delta-rang (*e.g*. rang en P1 – rang en P2). Lorsque le delta-rang est négatif ou positif, l'abondance relative de l'espèce a respectivement décliné ou augmenté entre les deux périodes. Similairement, nous avons estimé le ratio des tailles relatives d'aire de distribution basées sur le nombre relatif de carrés occupés par l'espèce entre deux périodes (*e.g*. rang P2 / rang P1). Dans ce cas, un ratio inférieur ou supérieur à 1 signifie respectivement un déclin ou une expansion de la taille relative de l'aire de distribution de l'espèce.

4.3. Méthode de Stroot & Depiereux (1989)

Dans le **Chapitre 1**, complémentairement aux techniques de rang, nous avons estimé les tendances de population des espèces selon la méthode de Stroot &

Depiereux (1989) utilisée dans Rasmont *et al*. (1993). Cette méthode se base sur le test du Chi-carré (Tableau 3).

Tableau 3. Estimation des tendances de population d'espèces basées sur la méthode de Stroot & Depiereux (1989). p et T peuvent être soit l'abondance (le nombre de spécimens), soit le nombre de carrés où l'espèce a été enregistrée. D'après Rasmont *et al*. (1993).

Espèces	Nombres observés			Nombres attendus		Chi²	Tendance
	P1	P2	Total	P1	P2		
Espèce 1	p_{11}	p_{12}	T_1	e_{11}	e_{12}	I_1	-
Espèce 2	p_{21}	p_{22}	T_2	e_{21}	e_{22}	I_2	+
Espèce *i*	p_{i1}	p_{i2}	T_i	e_{i1}	e_{i2}	I_i	…
Total	$T_{.1}$	$T_{.2}$	T	$T_{.1}$	$T_{.2}$		

La méthode estime le nombre attendu de spécimens (ou de carrés) pour chaque espèce *i* dans chaque période *j* selon la formule :

$$e_{ij} = \frac{T_{i.} * T_{.j}}{T}$$

Pour chaque espèce, la statistique du Chi-carré est calculée selon la formule :

$$I_i = \sum_{j=1}^{2} \frac{\left(p_{ij} - e_{ij}\right)^2}{e_{ij}}$$

La statistique du Chi-carré obtenue (I) est ensuite comparée à la valeur théorique de la distribution du Chi-carré avec un degré de liberté de 1 (comparaison d'une espèce entre deux périodes) et pour une probabilité donnée (0.05, 0.01 ou 0.001). Si le Chi-carré calculé est inférieur au Chi-carré théorique, il n'y a pas de différence significative entre le nombre observé (p_{ij}) et le nombre attendu (e_{ij}) et l'espèce est considérée comme stable. Si par contre il est supérieur au Chi-carré théorique, il y a une différence significative et l'espèce peut suivre deux tendances :

- si $p_{ij} > e_{ij}$: il y a plus d'individus observés que ce qui serait attendu selon l'effort d'échantillonnage, la tendance de l'espèce est considérée positive (croissance) ;
- si $p_{ij} < e_{ij}$: le nombre d'individus est anormalement bas comparé à ce qui serait attendu selon l'effort d'échantillonnage, la tendance de l'espèce est considérée négative (déclin).

Dans le cas où le nombre attendu est inférieur à 5, les conditions pour effectuer un test du Chi-carré ne sont pas respectées (Legendre & Legendre 2012) et la tendance est alors renseignée entre parenthèses.

4.4. Coefficients de dissimilarité entre assemblages d'espèces

Un coefficient de dissimilarité (ou distance) permet de mesurer l'association entre deux objets sur base de descripteurs. Dans le cas du **Chapitre 3**, le coefficient mesure l'association entre assemblages d'espèces de sites différents et est calculé à partir de la matrice d'abondance [sites × espèces]. Le coefficient le plus connu et le plus utilisé est la distance euclidienne. Elle est calculée par la formule de Pythagore qui définit entre les points une relation d'espace euclidien à *n* dimensions :

$$D_1 = \sqrt{\sum_{i=1}^{n} (y_{i1} - y_{i2})^2}$$

Avec y_{i1} = valeur du descripteur *i* pour l'objet 1 et *n* = le nombre de descripteurs.

De ce fait, elle prend en compte l'information des doubles zéros et est très sensible aux valeurs élevées. Comme l'information « double zéro » n'est pas forcément une

base valide de comparaison de deux assemblages (deux sites ne sont pas forcément similaires si une espèce y est doublement absente), elle est peu recommandée pour les descripteurs d'abondance d'espèces ou de fréquences (Legendre & Legendre 2012). Pour ces types de descripteur, il est recommandé d'utiliser un coefficient qui ne compte par la double absence comme indicatrice de ressemblance.

Dans le **Chapitre 2**, nous avons utilisé la distance du Chi-carré pour estimer les dissemblances entre les visites des trois castes de bourdon sur les différentes familles de plantes. La distance du Chi-carré est une distance euclidienne où les carrés sont pondérés par la somme des valeurs des descripteurs à travers leur fréquence relative par rapport à tous les autres :

$$D_{16} = \sqrt{\sum_{i=1}^{n} \frac{y_{++}}{y_{i+}} \left(\frac{y_{i1}}{y_{+1}} - \frac{y_{i2}}{y_{+2}} \right)^2}$$

Avec y_{i+}= somme des valeurs du descripteur i pour tous les objets ; y_{+1} = somme des valeurs de tous les descripteurs pour l'objet 1 et y_{++} = somme des valeurs de tous les descripteurs pour tous les objets. Contrairement à la distance euclidienne, la distance du Chi-carré est une distance qui ne tient pas compte des doubles zéros dans l'estimation de la ressemblance, et pour qui les différences entre abondances élevées contribuent moins à la distance que les différences entre abondances faibles.

Dans le **Chapitre 1**, la première étape était d'identifier des groupes de décades qui représentaient le mieux la distribution du nombre de carrés échantillonnés pour chaque espèce et chaque période. Dans le **Chapitre 3**, un des objectifs était de

quantifier la dissemblance entre les assemblages des différentes localités et périodes basée sur les différences d'abondance de chaque espèce. Pour ces deux chapitres, nous avons décidé d'utiliser le coefficient de dissimilarité de Bray-Curtis (Bray & Curtis 1957) car nos jeux de données comportaient beaucoup de zéros. En plus de ne pas prendre en compte les doubles zéros, ce coefficient, contrairement à d'autres (comme par exemple la distance du Chi-carré ou le coefficient de Canberra), permet qu'une différence entre valeurs d'une espèce abondante ait la même contribution que la même différence entre valeurs d'une espèce rare (Legendre & Legendre 2012). Pour Bloom (1981), ce coefficient est le plus représentatif de la dissemblance réelle entre deux assemblages d'espèces. Le coefficient de Bray-Curtis calcule la dissimilarité d'assemblage d'espèces entre deux sites (1 et 2) selon la formule :

$$D_{14} = \frac{\sum_{i=1}^{n} |y_{i1} - y_{i2}|}{\sum_{i=1}^{n} (y_{i1} + y_{i2})} = 1 - \frac{2W}{A + B}$$

Avec y_{i1} = effectif de l'espèce *i* au site 1 et y_{i2} = effectif de l'espèce *i* au site 2. A et B représentent la somme des abondances des espèces des sites 1 et 2, et W la somme des plus petites abondances des espèces communes aux deux sites. Ce coefficient est compris entre 0 et 1. Une valeur de 0 indique une similarité parfaite des assemblages entre sites et une valeur de 1 des sites n'ayant aucune espèce en commun.

4.5. Techniques d'ordination en espace réduit

Afin de mieux visualiser les résultats issus d'une matrice de distance/dissimilarité, il est possible de les représenter sous la forme d'un graphique en utilisant une technique dite d'ordination. Les techniques d'ordination permettent l'exploration multidimensionnelle de la structure des données et représentent une approche descriptive non-supervisée. Elles consistent à transposer un nuage de points-objets, situés dans l'espace initial des descripteurs, dans un espace de dimension réduite où les ressemblances entre objets sont représentées sur deux ou trois axes hiérarchisés et indépendants (orthogonaux). En d'autres mots, ceci permet de dégager les grandes tendances de la variabilité de son jeu de données pour l'ensemble des descripteurs étudiés qui sont résumés en deux (ou trois) axes principaux. Ces axes peuvent être vus comme l'expression de gradients écologiques (provenant d'une combinaison de descripteurs) qui expliquent au mieux la dispersion des données. La technique d'ordination la plus connue est l'analyse en composantes principales (ACP). Il s'agit d'une méthode linéaire qui préserve la distance euclidienne entre les sites et qui est basée sur le calcul des corrélations (ou des covariances) entre les variables. Elle est adaptée aux données écologiques quantitatives mais pas à l'analyse des données d'abondance des espèces car basée sur la distance euclidienne, comme nous l'avons vu au point précédent. De ce fait, nous avons considéré d'autres techniques basées sur d'autres distances.

4.5.1. L'analyse factorielle des correspondances (AFC)

Dans le **Chapitre 2**, le but était de répondre à la question : quelle famille de plante est la plus visitée par chaque caste, en particulier par les mâles ? Pour cela, nous avons utilisé une AFC pour représenter les préférences des trois castes de bourdon pour les différentes familles de plantes (matrice [34 familles × 3 castes]). L'AFC (en anglais CA pour « *Correspondance Analysis* ») permet d'analyser un tableau de contingence en se basant sur la distance du Chi-carré. L'AFC est une variante de l'ACP qui permet de représenter dans l'espace réduit les correspondances entre lignes et colonnes d'un tableau après transformation en profils de probabilités conditionnelles pondérées (Legendre & Legendre 2012). L'AFC préserve dans l'espace réduit la distance euclidienne entre les profils de probabilités conditionnelles pondérées, ce qui équivaut à préserver la distance du Chi-carré entre les lignes et les colonnes du tableau de contingence. De ce fait, les doubles zéros ne sont plus pris en compte. Contrairement à l'ACP qui se base sur les relations linéaires, l'AFC privilégie les relations non-linéaires (gaussiennes). Sur le graphique d'ordination obtenu, plus une famille de plante est proche d'une caste, plus elle est fréquemment visitée par ce caste. Les familles équitablement visitées par les trois castes se retrouvent au centre.

4.5.2. L'analyse en coordonnées principales (PCoA)

Dans les **Chapitres 1 et 3**, nous avons représenté la matrice de dissimilarité de Bray-Curtis en utilisant une PCoA. La PCoA (« *Principal Coordinate Analysis* ») ou

MDS (« *Metric Multidimensional Scaling* ») est une ordination basée sur une matrice de distance laissée au choix de l'utilisateur. Elle permet de situer les points dans un espace de dimension réduite qui respecte les relations de distance choisie entre eux. Si la PCoA est basée sur la distance euclidienne ou sur la distance du Chi-carré, elle donnera respectivement les mêmes résultats qu'une ACP ou une AFC. Dans notre cas, nous avons utilisé le coefficient de Bray-Curtis (transformé par la racine carrée pour éviter les valeurs propres négatives), bien adapté aux abondances d'espèces. La PCoA basée sur ce coefficient est beaucoup moins sensible aux valeurs « extrêmes » que l'ACP ou l'AFC. De plus, les nuages de points obtenus par une PCoA sont souvent mieux dispersés que dans une AFC car elle est moins sensible aux espèces rares et souffre moins de problèmes d'agglomération, qui nécessitent souvent l'élimination des espèces les moins fréquentes (Legendre & Legendre 2012). Contrairement à l'ACP et l'AFC, les coordonnées des objets et des descripteurs résultent de deux analyses différentes : l'une qui ne produit que les coordonnées des objets et l'autre que les coordonnées des descripteurs. Pour pouvoir représenter les objets et les descripteurs sur le même graphique, il suffit de projeter *a posteriori* sur le diagramme les coordonnées des objets ou des descripteurs selon le type d'analyse réalisé. Sur le graphique obtenu, les proximités entre objets et entre descripteurs s'interprètent simplement comme des ressemblances. Dans le cas du Chapitre 1, l'analyse a été réalisée sur les décades (objets) et les espèces (descripteurs) ont ensuite été projetées. Dans le cas du

Chapitre 3, l'analyse a été réalisée sur les localités aux différentes périodes (objets) et les espèces (descripteurs) ont ensuite été projetées.

4.6. Ordination canonique ou sous contrainte

Contrairement aux techniques d'ordination simple effectuées sur une seule matrice [sites × espèces], les techniques d'ordination canonique (ou d'ordination sous contrainte) combinent cette matrice à une (ou plusieurs) matrice(s) [sites × variables environnementales], dont la première est considérée comme une réponse multivariée dépendante des variables explicatives contenues dans les secondes. Ces techniques représentent une approche d'ordination supervisée et reposent sur la modélisation statistique multivariée. Autrement dit, elles combinent une technique d'ordination à une régression multiple. Elles permettent donc d'expliquer la structure observée des données en calibrant et en testant un modèle statistique qui lie les données de la matrice « espèces » à celles de la matrice « environnement », en postulant que la structure de la première est expliquée par la deuxième. Contrairement à une ordination non-supervisée, la matrice de variables explicatives conditionne le poids, l'orthogonalité et la direction des axes. Le graphique qui en résulte est un triplot constitué de deux types d'axes d'ordination: les axes contraints (ou axes canoniques) et les axes non contraints dont les valeurs propres expriment la variation résiduelle non expliquée par les variables explicatives utilisées dans le modèle de régression multiple. L'ordination canonique la plus connue est celle qui combine une régression linéaire à une ACP : l'analyse canonique de redondance (RDA, « *Canonical Redundancy Analysis* »).

L'ordination qui combine une AFC à une régression linéaire est l'analyse canonique des correspondances (CCA, « *Canonical Correspondence Analysis* »). Dans le **Chapitre 3**, nous cherchions à estimer et décrire la part de variabilité des assemblages d'espèces (présentée dans la PCoA) expliquée par les variables de structure et de composition du paysage. Puisque nous nous sommes basés sur une matrice de distance de Bray-Curtis, nous avons utilisé l'analyse canonique basée sur une PCoA : l'analyse canonique en coordonnées principales (CAP pour « *Constrained Analysis of Principal Coordinates* » ou db-RDA pour « *Distance-Based Redundancy Analysis* » ; Legendre & Andersson 1999 ; Andersson & Willis 2003). Par rapport à la RDA (basée sur une ACP) et à la CCA (basée sur une AFC), les avantages de la CAP basée sur une PCoA utilisant le coefficient de Bray-Curtis sont multiples : elle ne prend pas en compte les doubles zéros, elle donne la même contribution aux différences d'abondance des espèces abondantes qu'à celles des espèces rares, elle donne autant de poids aux relevés riches qu'aux relevés pauvres en espèces, elle peut assumer une relation non-linéaire, et elle est plus efficace pour détecter les effets des variables explicatives qui se traduisent par une diminution globale des abondances et du nombre d'espèces (Legendre & Legendre 2012). Afin de tester si la variabilité des assemblages de bourdons expliquée par les variables paysagères est significative, nous avons utilisé un test statistique non-paramétrique par permutations aléatoires sous modèle réduit (1000 permutations) pour l'ensemble du modèle et pour chaque axe contraint (Legendre *et al*. 2011). Ce test permet de dire si les valeurs propres observées des axes

contraints sont plus grandes que celles obtenues aléatoirement, et donc de dire si la relation entre les deux matrices est significative (Legendre & Legendre 1998 ; Legendre *et al*. 2011). Nous avons également testé chaque variable explicative séparément en réalisant une CAP par variable et en effectuant pour chaque CAP un test de permutation.

Dans les études écologiques à l'échelle d'un pays (ou d'un continent), il est fréquent que les données soient influencées par des différences biogéographiques liées à la distribution des espèces (Legendre & Legendre 2012). Il est donc intéressant de retirer l'effet de la localisation spatiale des données avant d'interpréter les relations entre les communautés et leur environnement (Borcard *et al*. 1992). Dans le Chapitre 3, les localités étudiées sont dans des régions biogéographiques distinctes à travers la Belgique, avec des altitudes et des conditions climatiques différentes. Comme l'altitude explique une grande part de la variabilité géographique des gradients environnementaux en Belgique (Dufrene & Legendre 1991), nous avons testé si la part de variabilité des assemblages de bourdons expliquée par les variables paysagères était toujours significative lorsque la variabilité liée à l'altitude était soustraite du modèle. Pour ce faire, nous avons utilisé une analyse canonique partielle (dans notre cas une analyse canonique en coordonnées principales partielle, pCAP). Une pCAP est simplement une CAP dans laquelle on retire préalablement l'effet de variables explicatives (appelées covariables) sans intérêt pour l'étude et dont l'effet pourrait gêner l'analyse des hypothèses.

4.7. Modélisation de distribution d'espèce

Le premier objectif du **Chapitre 4** était d'évaluer si l'occupation du sol et le climat pouvaient influencer la distribution des espèces de bourdons à l'échelle de la Belgique pour les trois périodes de temps 1910-1930, 1970-1989 et 1990-2016. Pour cela, nous avons utilisé les modèles de distribution d'espèce (SDMs pour « *species distribution models* » ; Guisan & Thuiller 2005 ; Elith & Leathwick 2009 ; Franklin 2010 ; Drew *et al.* 2011). Les SDMs permettent de projeter la distribution d'une espèce dans des environnements où les données d'occurrence de l'espèce manquent, en reliant les données d'occurrence disponibles à des prédicteurs environnementaux, appelés « covariables » (Elith *et al.* 2011). La méthode est constituée de quatre étapes : (1) les données d'occurrence (et d'absence) de l'espèce à travers la région d'étude sont géoréférencées et compilées dans une base de données ; (2) les valeurs des prédicteurs environnementaux dans cette région d'étude sont extraites à partir d'une base de données spatiales ; (3) les valeurs des prédicteurs environnementaux sont utilisées pour ajuster un modèle qui estime les similarités entre ces valeurs (variables explicatives) et les occurrences de l'espèce (variable dépendante) ; (4) le modèle est utilisé pour prédire les occurrences de l'espèce en fonction des prédicteurs environnementaux dans des zones ou périodes non échantillonnées.

Nous avons modélisé la distribution des espèces de bourdons en utilisant l'algorithme du logiciel MaxEnt (Phillips *et al*. 2006), qui utilise la méthode d'entropie maximum (« *Maximum Entropy* »). Il s'agit d'un des algorithmes les plus

robustes et les plus utilisés pour les SDMs (Phillips *et al.* 2006; Phillips & Dudík 2008; Elith *et al.* 2011 ; Merow *et al*. 2013). Une explication détaillée de cet algorithme est fournie par Elith *et al.* (2011). Cet algorithme n'utilise que les données de présence d'espèce (« *presence-only model* ») et non les données d'absence (« *presence-absence model* »). Autrement dit, il se base sur un jeu de données regroupant les sites où l'espèce a été observée (présence avérée) et des sites où sa présence est inconnue (« *background* »). L'algorithme repose sur des fonctions de densité de probabilité, c'est-à-dire sur les probabilités relatives des covariables à travers l'ensemble des sites étudiés. Il estime la probabilité de présence de l'espèce conditionnée par l'environnement en minimisant les distances entre la densité de probabilité des covariables dans les sites où l'espèce est présente ($f_1(z)$) et la densité de probabilité des covariables dans l'ensemble des sites du « *background* » ($f(z)$). Autrement dit, la moyenne de chaque covariable dans l'ensemble des sites doit se trouver le plus proche possible de la moyenne dans l'ensemble des sites où l'espèce est présente. Cela revient donc à estimer l'entropie relative de $f_1(z)$ selon $f(z)$ (appelée « *Kullback-Leibler divergence* » ; Elith *et al*. 2011). Comme la réponse des espèces aux variables environnementales est souvent complexe, il est nécessaire d'employer des fonctions non-linéaires dites « ajustées » des covariables plutôt que les covariables elles-mêmes (Austin 2002). Nous avons donc utilisé trois classes de transformation (« *features* ») des covariables disponibles dans MaxEnt (Elith *et al.* 2011): linéaire (variable brute), quadratique

(variable au carré), et produit (« *product* », produits de toutes les combinaisons deux à deux des variables).

Pour estimer la performance des SDMs (c.-à-d. leur fiabilité), nous avons utilisé l'AUC (« *Area Under the Curve* »), qui est l'abréviation de AUROC pour « *Area Under the Receiver Operator Curve* » (Hanley & McNeil 1982; Fielding & Bell 1997; Phillips *et al.* 2006; Elith *et al.* 2006). L'AUC est la surface sous la courbe ROC (« *Receiver Operating Characteristic* » ; Hanley & McNeil 1982). Le ROC est une mesure de la performance d'un classificateur binaire lorsque le seuil de discrimination (*S*) varie. Un classificateur binaire est un modèle de prédiction qui a pour objectif de catégoriser des éléments (ici des sites) en deux groupes (positif et négatif, en théorie présence et absence) sur la base des caractéristiques de ces éléments (ici les prédicteurs environnementaux). La sensibilité (« *sensitivity* ») du modèle est donnée par la proportion de positifs classés positifs (vrais positifs, correspond à 1 – le risque β, ou 1 – l'erreur d'omission) et la spécificité (« *specificity* ») du modèle par la proportion de négatifs classés négatifs (vrais négatifs, correspond à 1 – le risque α). L'anti-spécificité (1-spécificité), ou erreur de commission (« *commission error* »), donne la proportion de négatifs classés positifs (faux positifs) et correspond au risque α. La courbe ROC relie les points *S* avec pour abscisse l'anti-spécificité et pour ordonnée la sensibilité par des lignes droites (méthode non-paramétrique). Elle représente donc l'évolution de la proportion de vrais positifs en fonction de la proportion de faux positifs lorsque le seuil de discrimination varie de 0 à 1. Un AUC de 0,5 correspond à la droite de référence d'une classification

aléatoire (diagonale sur la Figure 11) et signifie que le modèle est aussi performant pour classer les sites qu'une classification aléatoire. Si l'AUC du modèle est supérieur à 0,5, alors le modèle prédit mieux que ce qui serait prédit par chance.

Cependant, cette définition théorique de l'AUC est différente pour les SDMs basés seulement sur les présences comme l'algorithme MaxEnt, car aucune information n'est disponible sur les vraies absences. La spécificité et l'anti-spécificité ne sont donc pas connues. La courbe ROC est donc modifiée de sorte que, au lieu de tracer la sensibilité contre l'anti-spécificité, elle est tracée contre la proportion des sites du « *background* » prédits en positif (présence) pour tous les seuils *S* (« *Fractional Predicted Area* » sur la Figure 11). Ceci implique que l'AUC maximal atteignable est en réalité inférieur à 1. L'AUC maximal est inversement proportionnel à la prévalence de l'espèce, c.-à-d. à la véritable aire de distribution de l'espèce, qui n'est pas connue (Phillips *et al*. 2006 ; Jiménez-Valverde 2012). Plus l'espèce est restreinte et plus l'AUC maximal se rapproche de 1. Au contraire, l'AUC maximal d'une espèce répandue est plus faible. Il n'est donc pas possible de comparer les AUC entre modèles basés sur différentes espèces et différentes périodes (Jiménez-Valverde 2012). Le seul cas où l'AUC peut être comparé est pour la comparaison de modèles effectués sur la même espèce durant la même période (et donc sur le même jeu de données), mais par exemple sur base de variables environnementales différentes. De plus, tout comme l'AUC maximal est inférieur à 1, l'AUC correspondant à une prédiction qui n'est pas meilleure qu'une prédiction aléatoire n'est plus forcément égal à 0,5 (Jiménez-Valverde 2012). Néanmoins,

l'AUC reste une des mesures de la performance de modèles les plus utilisées, probablement car elle fournit une unique mesure de la fiabilité du modèle et elle est indépendante du choix du seuil de discrimination (Phillips *et al*. 2006).

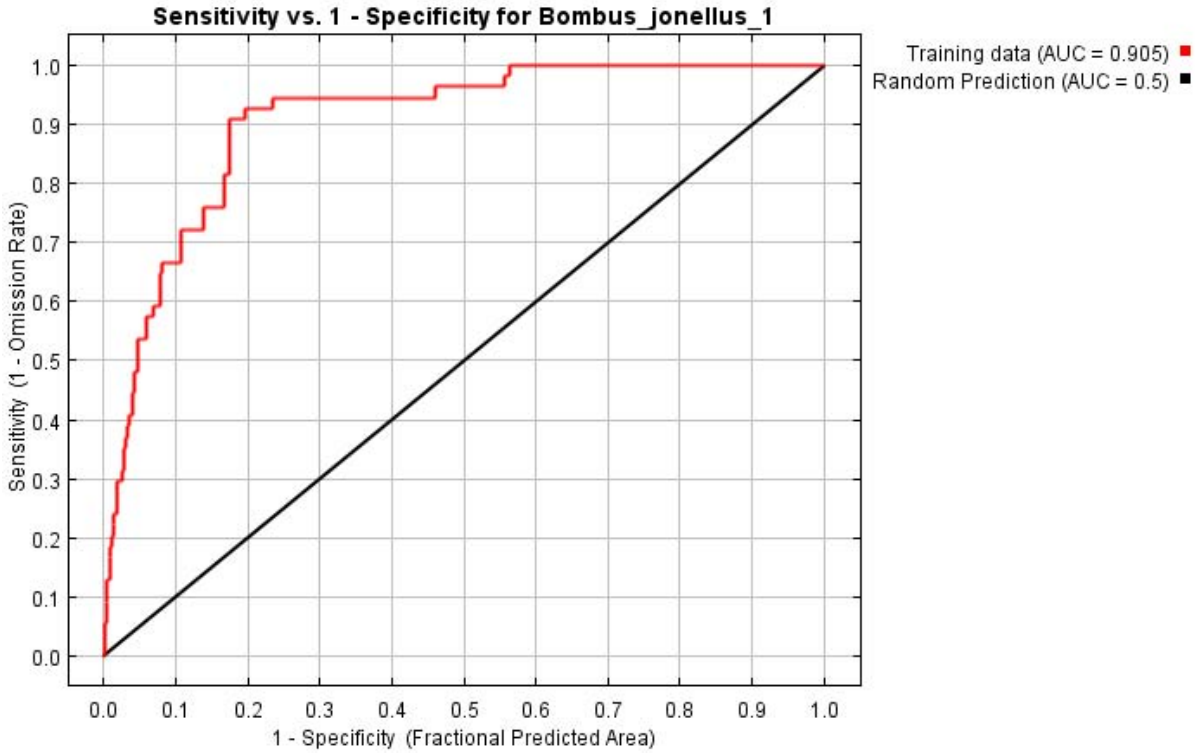

Figure 11. Exemple de courbe ROC et d'AUC pour *B. jonellus* pour la période 1990-2016. La courbe rouge correspond aux prédictions du modèle de distribution de *B. jonellus* et la courbe noire correspond aux prédictions aléatoires. La sensibilité correspond à la proportion de vrais positifs (vrais présences), c.-à-d. 1 - l'erreur d'omission (« *Omission rate* »). La spécificité est définie en utilisant l'aire prédite (*« Fractional Predicted Area* »), c.-à-d. la proportion de tous les sites qui sont prédits comme favorables pour l'espèce, à la place de l'erreur de commission (Anderson *et al.* 2003).

Pour savoir si les prédictions du SDM sont véritablement meilleures que celles d'un modèle aléatoire, et donc si les variables environnementales utilisées influencent significativement la distribution des bourdons, nous avons utilisé la méthode du « modèle nul » (« *null-model methodology* » ; Raes & ter Steege 2007 ; van Proosdij *et al*. 2016). Le modèle nul est un modèle basé sur une distribution

aléatoire des occurrences (Gotelli & McGill 2006). La méthode du modèle nul consiste à comparer l'AUC du SDM obtenu à celui du modèle nul. Nous avons pour cela effectué un test unilatéral basé sur un intervalle de confiance à 95% afin de tester si l'AUC du SDM était significativement supérieur à l'AUC du modèle nul. Un SDM significatif indique que les relations entre les carrés où l'espèce est présente et les valeurs des variables environnementales dans ces carrés sont plus grandes que les relations qui seraient obtenues par chance (Raes & ter Steege 2007). Autrement dit, les variables environnementales choisies expliquent significativement la distribution de l'espèce. Une espèce avec un SDM significatif a donc des exigences en termes d'occupation du sol et de conditions climatiques qui ont pu être capturées par le modèle dans les carrés où elle était présente. L'avantage de la méthode du modèle nul est qu'elle permet d'utiliser toutes les données d'occurrence pour développer et tester un seul SDM, contrairement aux autres méthodes qui découpent aléatoirement le jeu de données en un jeu pour l'entrainement et un jeu pour le test du modèle, ce qui produit plusieurs modèles par espèce (Fielding & Bell 1997).

Quand les sites d'échantillonnage ne sont pas équitablement distribués à travers la région étudiée (ce qui est notre cas), il est possible que la zone échantillonnée n'ait pas capturé toute l'étendue des valeurs des variables environnementales présente dans la région d'étude. Ces biais « environnementaux » qui résultent des biais d'échantillonnage peuvent réduire la performance des modèles (Kadmon *et al.* 2003). Par conséquent, si le modèle nul

est basé sur l'entièreté de la région étudiée (y compris les zones non échantillonnées) mais que l'échantillonnage est biaisé, son AUC a plus de chance d'être significativement différente de celle du SDM (Kadmon *et al.* 2003; Raes & ter Steege 2007). Pour résoudre ce problème, nous avons restreint la distribution aléatoire du modèle nul aux carrés échantillonnés et non à la région entière.

La distribution modélisée issue d'un SDM peut être projetée sous forme de carte de probabilité de présence de l'espèce en fonction des variables environnementales. Cette carte (« *habitat suitability map* ») représente l'espace où les variables environnementales affichent des valeurs favorables à la persistance de l'espèce, avec un score attribué à chaque carré (« *grid-cell* ») de la zone d'étude qui reflète sa « qualité » pour l'espèce. Plus le carré a une valeur élevée, plus les conditions environnementales étudiées sont favorables à la présence de l'espèce. Nous avons calculé la probabilité de présence de l'espèce comme le ratio entre la densité des variables environnementales aux sites d'occurrence et la densité de ces variables à travers le pays entier, afin d'obtenir l'ensemble des conditions représentatives qui suggèrent un habitat convenable pour l'espèce (Elith *et al.*, 2011). Ces cartes de probabilité de présence de chaque espèce sont présentées dans le **Chapitre 4**. Ensuite, nous avons converti ces cartes en cartes binaires de présence-absence en utilisant un seuil qui définit à partir de quelle probabilité de présence une espèce peut être considérée comme effectivement présente. Nous avons utilisé le seuil appelé « *10 Percentile Training Presence Threshold* », qui ne prend pas en compte les erreurs de commission et qui fixe un pourcentage

acceptable d'erreurs d'omission à 10%, communément utilisé dans la littérature (Pearson *et al.* 2004; Martin *et al*. 2013). Nous avons ensuite compté le nombre de carrés de présence pour chaque espèce dans chaque période et avons comparé ces résultats à ceux du Chapitre 1 en utilisant la même méthodologie de rangs. Ces comparaisons sont présentées dans la Discussion générale.

Le second objectif du **Chapitre 4** était d'estimer l'influence respective des variables environnementales sur la distribution des bourdons. D'une manière générale, plus une variable varie dans la région étudiée et atteint des valeurs défavorables pour l'espèce, plus elle détermine la distribution de l'espèce dans cette région (Pearson & Dawson 2003). Pour estimer l'influence de chaque variable environnementale sur la distribution des espèces, nous avons calculé la mesure appelée « *permutation importance* ». Cette mesure est déterminée en permutant aléatoirement les valeurs de la variable entre les points de présence et de « *background* » et en mesurant la diminution de l'AUC qui en résulte. Une grande diminution indique que le modèle dépend fortement de la variable. Cette mesure est normalisée pour donner des pourcentages. Par conséquent, une valeur nulle indique que la variable n'influence pas le SDM. Plus la valeur est grande, plus la variable a de l'influence sur la distribution modélisée de l'espèce.

Afin de voir si l'influence de chaque variable a un effet favorable ou défavorable sur la distribution modélisée des bourdons, nous avons analysé les courbes réponses. Ces courbes montrent comment chaque variable affecte les prédictions du SDM. Nous avons classé chaque courbe en quatre catégories : effet linéaire

négatif (Fig. 12a), effet linéaire positif (Fig. 12b), effet quadratique concave (l'espèce préfère des valeurs intermédiaires de la variable, Fig. 12c), effet quadratique convexe (l'espèce préfère des valeurs extrêmes de la variable, en théorie moins probable biologiquement parlant, Fig. 12d), et effet peu ou pas important (courbe plate, Fig. 12e). Nous avons ensuite évalué l'effet dominant de chaque variable dans chaque période en comptant le nombre d'espèces concernées dans chaque catégorie.

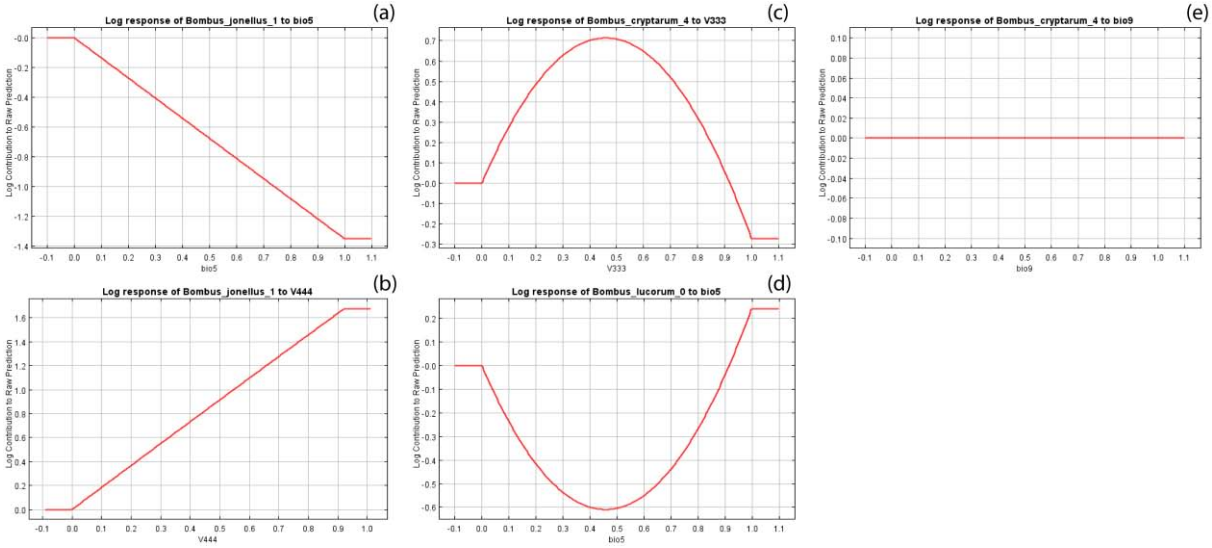

Figure 12. Exemples de courbes réponses avec effet (a) linéaire négatif (effet de la variable « température maximale du mois le plus chaud » sur la distribution modélisée de *B. jonellus* pour la période 1990-2016), (b) linéaire positif (effet de la variable « surface de prairies » sur la distribution modélisée de *B. jonellus* pour la période 1990-2016), (c) quadratique concave (effet de la variable « surface de forêts » sur la distribution modélisée de *B. cryptarum* pour la période 1990-2016), (d) quadratique convexe (effet de la variable « température maximale du mois le plus chaud » sur la distribution modélisée de *B. lucorum* pour la période 1990-2016) et (e) effet peu ou pas important (effet de la variable « température moyenne du trimestre le plus sec » sur la distribution modélisée de *B. cryptarum* pour la période 1990-2016).

4.8. Les modèles linéaires mixtes

Dans le **Chapitre 4**, afin d'évaluer quelles variables environnementales (covariables) influençaient le plus la distribution des bourdons dans chaque période, nous avons testé si la valeur d'importance obtenue précédemment différait entre les types de covariables et entre les périodes. Pour cela, nous avons utilisé un modèle linéaire multiple à effet mixte basé sur une structure Gaussienne et sur la fonction du maximum de vraisemblance (Laird & Ware 1982 ; Lindstrom & Bates 1988). Une des conditions d'un modèle linéaire multiple est que chaque individu (dans notre cas chaque espèce) doit posséder une seule valeur pour toutes les variables explicatives (indépendance des observations ; Searle 1971). Ce type de modèle ne permet donc pas de prendre en compte la variabilité individuelle lorsque plusieurs mesures sont effectuées sur le même individu. Or, les mesures effectuées sur les mêmes individus ont tendance à se ressembler et il y a donc une non-indépendance des observations. Les variables d'un tel modèle sont dites à effet fixe : leurs différents niveaux sont fixés et les effets associés sont des paramètres à estimer qui interviennent dans la moyenne du modèle (Searle 1971). Un modèle linéaire mixte est un modèle linéaire qui permet d'inclure des variables à effet aléatoire en plus des variables à effet fixe (Eisenhart 1947). Un effet aléatoire est l'effet d'une variable dont les niveaux sont échantillonnés aléatoirement dans le modèle (variation aléatoire de l'effet de la variable), ce qui permet de prendre en compte les observations répétées sur les mêmes individus. Ces niveaux sont modélisés en tant qu'observations d'une variable aléatoire normale de moyenne

nulle (la moyenne du modèle est définie par les effets fixes) et de variance à estimer. Chaque variable à effet aléatoire est donc caractérisée par un paramètre de variance qu'il faut estimer en plus de la variance des erreurs du modèle. Autrement dit, les variables à effet fixe interviennent dans la définition de la moyenne du modèle et les variables à effet aléatoire dans la définition de la variance du modèle (Eisenhart 1947).

Dans notre modèle linéaire, la variable réponse est la racine carrée de la valeur d'importance et les variables explicatives sont le type de covariable (occupation du sol, précipitations, températures), la période (P1, P2, P3) et leur interaction. Comme plusieurs valeurs d'importance sont disponibles par espèce, nous avons ajouté dans le modèle la variable « espèce » en facteur aléatoire. La formule de notre modèle s'écrit donc :

$$\sqrt{importance} \sim type\ de\ covariable\ +\ période\ +\ type\ de\ covariable * période\ +\ 1|espèce$$

Afin de tester la significativité des différences entre les types de covariables et entre les périodes, nous avons utilisé des tests post-hoc de comparaisons multiples par paires basés sur les contrastes de Tukey (« *post-hoc pairwise multiple comparison tests with Tukey contrasts* » ; Hothorn *et al.* 2008 ; Bretz *et al*. 2010). Pour les interactions entre les types de covariables et les périodes, nous avons calculé les moyennes des moindres-carrés (« *least-squares means* » ou « *predicted marginal means* ») de chaque combinaison de facteurs et les avons comparées en utilisant la méthode de Tukey (SAS 2012 ; Lenth 2016). Nous avons ensuite réalisé exactement le même modèle mais en remplaçant la variable « type de covariable » par chaque variable environnementale, ce qui donne le modèle :

$$\sqrt{importance} \sim covariable + période + covariable * période + 1|espèce$$

Nous avons ensuité réalisé les mêmes tests post-hoc que précédemment.

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Chapitre 1

*Trends of bumblebee (*Bombus*) populations in Belgium along the last century: drastic shift in community composition correlated to species ecological traits.*

Chapter 1: Trends of bumblebee (*Bombus*) populations in Belgium along the last century: drastic shift in community composition correlated to species ecological traits.

By Sarah Vray, Nicolas Dendoncker, Denis Michez, Marc Dufrêne, Stuart P.M. Roberts, and Pierre Rasmont.

1. Summary

Bumblebees are undergoing a strong decline in Europe fostered by habitat loss and fragmentation, agricultural intensification, and climate change. Long-term records are necessary to estimate precisely population trends and to propose accurate mitigation strategies. Here, we assess the spatial and abundance drift in Belgian bumblebee populations using a specimen-level database of 215 564 specimens from museum collections, scientific monitoring and opportunistic citizen data from 1810 to 2016, subdivided into three time-periods (1910-1930, 1970-1989, and 1990-2016). We also assess if the observed trends are related to ecological species-specific traits. Species richness and diversity decreased overall during the last century in Belgium but some regions stayed relatively rich in species. A strong shift in community composition occurred. Three species remained among the "top five" species between the three time-periods (*i.e*. *B. pascuorum*, *B. lapidarius*, and *B. pratorum*), but several species that were once abundant and widespread drastically declined (*e.g*. *B. muscorum*, *B. ruderarius*, *B. ruderatus*, *B. veteranus*), while a few species increased their distribution and relative abundance (*e.g*. *B. bohemicus, B. hypnorum, B. lucorum, B. terrestris*). Some ecological traits were correlated to a

stronger decline: longer tongue, narrower diet, higher habitat specialization with a preference for open habitats and above-ground nesting (*i.e.* carder species), smaller colony size, and later emergence.

2. Introduction

Bumblebees (*Bombus*) are a diversified species group of common pollinators in temperate, alpine and artic regions. They pollinate a wide diversity of crops and wild plant species (Ollerton *et al.* 2011). Many species are undergoing a strong population decline in Western Europe and North America (Kosior *et al.* 2007; Goulson 2010; Cameron *et al.* 2011; Nieto *et al.* 2014) fostered by, among others, shortage in floral resources, habitat loss and fragmentation, intensive use of agrochemicals and pesticides, mostly resulting from agricultural intensification since the second part of the 20th century (Goulson *et al.* 2008a). More recently, several studies highlighted the contribution of climate change in the bumblebee decline (Kerr *et al*. 2015; Rasmont *et al.* 2015a). Among European bees, the *Bombus* genus includes the highest percentage of species with an extinction risk according to IUCN criteria (Nieto *et al.* 2014), but still presents a very high diversity in some areas (Iserbyt *et al.* 2008, 2015). Among the 68 species in Europe, 45.6% are decreasing, 29.4% are stable, and 13.2% present positive population trends and expansion of their distribution (Nieto *et al.* 2014).

The variability in species responses to threats is predicted to be influenced by species ecological traits (Rasmont & Mersch 1988; Goulson *et al.* 2005; Bommarco *et al.* 2010; Williams *et al.* 2010; De Palma *et al.* 2015). Generally, correlates of

species extinction are narrow geographic extent, slow vital rate, natural rarity, and ecological specialisation (Brook *et al.* 2008). In European bumblebees, the less abundant and most severely declining species tend to be those with a low genetic diversity, a short flight season, a late emergence, a long tongue, a small number of habitat types, and especially associated to flowers with long corolla such as Fabaceae, like *Bombus ruderatus*, *B. humilis* and *B. subterraneus* (Rasmont & Mersch 1988; Goulson *et al.* 2005, 2008b; De Palma *et al.* 2015; Maebe *et al*. 2016). However, some other studies did not find any consistent pattern between dietary breadth and bumblebee species declines at a national scale (Williams 2005; Connop *et al.* 2010). Moreover, most studies analysed bees in general without setting apart bumblebees, which could blur any results specific to bumblebees by lumping traits into categories corresponding to the diversity of all the bee species (*e.g.* Aguirre-Gutiérrez *et al.* 2016). Therefore, taxon-specific multi-trait analyses are needed to identify traits that determine the species most at risk at national level, which can help prevent their decline and to maintain ecosystem functioning by designing adequate conservation strategies.

Here, we perform comparative analyses based on a 100-year record of Belgian bumblebee populations using an original specimen-level database compiled from museum collections, scientific monitoring and opportunistic citizen data from the early nineteenth century to present. Belgium followed a typical pathway of agricultural intensification in Western Europe, and its bumblebee fauna has been studied since the late nineteenth century (Meunier 1888; Ball 1914, 1920). Several early reports based on smaller datasets indicated that numerous bumblebee

species were strongly regressing since 1950 (Rasmont & Mersch 1988; Rasmont *et al.* 1993), and continued to decline after the 1990s unlike other pollinator insects such as hoverflies (Rasmont & Pauly 2010; Carvalheiro *et al.* 2013).

The aim of this study is to qualify and quantify the changes observed in bumblebee populations of Belgium, and to assess the link with species ecological traits. We measure species richness and community composition changes, as well as changes in species abundance and geographic range size over three time periods (1910-1930, 1970-1989, and 1990-2016). We then assess the possible correlations of these changes with species ecological traits. We expect that some species increased their distribution and abundance to the detriment of several other species. We hypothesize that these expanding species are the most generalist ones, in terms of diet and habitat, and the decreasing species the most specialist ones.

3. Material and methods

3.1. Study region

Belgium is a small and densely populated country (11 267 910 habitants in 2016 for 30 528 km^2; Belgian Federal Government 2017) located in NW-Europe. Its temperate oceanic climate is characterized by relatively mild and rainy winters, and relatively cool and wet summers. Between 1990 and 2016, annual mean temperature was 10.2°C, mean minimum temperature of the coldest month was 0.1°C, mean maximum temperature of the warmest month was 22.5°C, and mean annual precipitation was 878 mm (IRM, 2017).

3.2. Bumblebees dataset

We used a dataset consisting of 215 564 bumblebee specimens from 31 species in Belgium which were recorded in the database *Banque de Données Fauniques de Gembloux et Mons* (BDFGM, Rasmont & Pauly 2010), encompassing the period from 1810 to 2016 (Table S1). This database is a compilation of records from museum and university collections (mainly the University of Mons, the University of Liège-Gembloux Agro Bio Tech, and the Royal Belgian Institute of Natural Sciences), scientific monitoring (*e.g*. Leclercq *et al*. 1980; Rasmont 1988) and opportunistic citizen records from NGO initiatives (mainly the Belgian naturalist platforms www.observation.be from Natagora and www.waarnemingen.be from Natuurpunt). The main originality of our dataset comes from the addition to the former database (used for example in Rasmont *et al*. 1993 and Carvalheiro *et al*. 2013) of old Belgian records from the Hymenoptera collection of F. J. Ball (Ball 1914, 1920) stored at the Royal Belgian Institute of Natural Sciences (RBINS) in Brussels, which covers mostly the 1910-1930 period. We identified and encoded more than 60 000 bumblebee specimens from this collection. We assigned conventional geographical coordinates to these old records based on the locality mentioned on the label below the pinned specimen. More precisely, we used the church of the village as location. For each data point, we had the information about (1) the bumblebee species; (2) the recorder; (3) the location; (4) the date; for 60% of them (5) the caste (queen, male, or worker); and for 15% of them (6) the plant on which the specimen was observed. Among the 31 species, three were deleted from the dataset because they were represented by less than 10 records: *Bombus*

cullumanus (5 records, last one in 1918; Rasmont 1982), *B. quadricolor* (2 records in 1943; Peeters *et al.* 1999), and *B. wurflenii* (3 records, last one in 1979; Debaille & Rasmont 1997).

3.3. Species ecological traits

We compiled data on species ecological traits from the BDFGM and other various sources (Table 1). We considered 18 traits: inquilinism (*i.e.* social parasitism; 1 trait), nesting strategies (2 traits), plant preferences and diet breadth (3 traits), habitat specialization and preferences (4 traits), phenology (3 traits), and rarity in Belgium and in Europe (5 traits). As diet breadth assessment should consider similar sampling among species, we first excluded species for which we had less than 30 floral visit records at plant family level (*i.e. B. barbutellus*, *B. confusus*, *B. distinguendus*, *B. humilis*, *B. norvegicus*, *B. pomorum*, and *B. subterraneus*) and plant genera level (*i.e.* the same plus *B. ruderatus*; see Table S2). We then estimated the number of plant families and genera the species would be expected to visit for a standardised number of specimens (here 70, which corresponds to the minimum of specimens observed for a species) with the "vegan" package in R software (Hurlbert 1971; Gotelli & Colwell 2001; Oksanen *et al.* 2017).

Table 1. Ecological traits of Belgian bumblebee species. * in Belgium; ** in Europe; *** in general.

Ecological traits	Proxy variables	Type (units)	Descriptions	References
Cuckoo species***	-	Categorical (yes *vs.* no)	Cuckoo species are inquilines (*i.e.* social parasites) and correspond to *Psithyrus* species, in which queens lay eggs in the nest of other species and do not produce workers.	Løken 1984
Nesting strategy	Nesting preference***	Categorical (renter *vs.* carder)	Renter species are nesting in existing cavities (either above or below the ground), whereas carder species are nesting in above-ground nests using shredded plant material.	Sladen 1912; von Hagen & Aichhorn 2014
	Maximum colony size***	Continuous	Maximal number of individuals inside the colony.	
Host plants and diet breadth	Tongue length***	Categorical (short, medium, long)	Short-tongue workers have tongue shorter than 8mm, medium tongues are between 8 and 9mm, and long tongue are longer than 9mm.	Goulson & Darvill 2004; BDFGM
	No. of plant families*	Continuous	Expected (rarefied) number of visited plant families in a subsample of 70 specimens.	
	No. of plant genera*	Continuous	Expected (rarefied) number of visited plant genera in a subsample of 70 specimens.	
Habitat specialization and preferences	Forest habitat***	Categorical (yes *vs.* no)	Species living in forest habitats.	Pittioni & Schmidt 1942; Reinig 1972; Rasmont 1988
	Edge habitat***	Categorical (yes *vs.* no)	Species living in edge habitats between forest and open lands.	
	Open habitat***	Categorical (yes *vs.* no)	Species living in open habitats (*e.g.* grasslands).	
	Number of EU priority habitats the bumblebees are associated with**	Continuous (1 to 10) The higher the number, the more generalist the species	The 10 habitat types are coastal & halophytic habitats; coastal & continental dunes; freshwater habitats; temperate heath and scrub; sclerophyllous scrub; natural & semi-natural grasslands; raised bogs, mires and fens; rocky habitats & caves; forests & woodlands, anthropogenic habitats.	
Phenology	Flight season*	Continuous (days)	Mean duration of the flight season (*i.e.* between the first and the last occurrence) in P1 (1910-1930), considering only years representing more than 3% of all the species occurrence and with more than 90 days between the first and the last observation.	BDFGM
	Month of queens emergence*	Continuous (1 to 12)	Month of the first decile of queens appearance in P1 (1910-1930).	
	Month of males emergence*	Continuous (1 to 12)	Month of the first decile of males appearance in P1 (1910-1930).	
Rarity	European actual AOO**	Continuous (km^2)	The European area of occupancy (AOO) = no. of occupied cells × area of a cell (IUCN)	Nieto *et al.* 2014; Rasmont *et al.* 2015; BDFGM
	European actual EOO**	Continuous (km^2)	The European Extent of occurrence (EOO) is a parameter that measures the spatial spread of the areas currently occupied by the species (IUCN)	
	European trend**	Categorical (decreasing, stable, increasing)	European current population trend (IUCN)	
	Past relative range size*	Continuous	Relative number of 5km grid-cells occupied by the species in P1 (1910-1930).	
	Past rank-abundance*	Continuous	Species rank-abundance in P1 (1910-1930).	

3.4. Temporal subdivision of the dataset

As sampling effort and amount of data differed greatly between years (Fig. 1), we needed to aggregate the data into time-periods. We first divided the dataset into decades (10 year periods) and we performed Principal Coordinate Analysis (PCoA, "vegan" package of R software; Oksanen *et al.* 2017), also called Multidimensional Scaling (MDS), in order to identify groups of decades which best represent the dataset. This distance-based statistical method allows choosing adapted distance measurement for quantitative species data. Here, we chose the square root (to avoid negative eigenvalues) of the Bray-Curtis dissimilarity coefficient, which does not take into account double-zeros and gives the same contribution to differences in abundant species than in rare species (Legendre & Legendre 2012). We performed these analyses on the absolute and relative numbers of 5 and 10km grid-cells (corresponding to the UTM squares) for each species and each decade. All these analyses revealed a distinct separation between data before 1930 and after 1970 (Fig. 2). Taking this into account and in order to keep the comparability with some previous studies (*e.g*. Carvalheiro *et al.* 2013), we decided to subdivide the dataset into three time-periods: 1910-1930 (P1, 60 498 records), 1970-1989 (P2, 23 854 records), and 1990-2016 (P3, 89 364 records), which represent a total of 173 716 records. These similar length time-periods correspond to the three peaks in the numbers of samples (*i.e.* one sample is one 5x5km grid-cell sampled by one recorder during one day; Fig. 1). P1 broadly corresponds to the time before the use of agricultural moto-mechanization and chemical fertilizers, occurring mainly from

1950 to 1970 (Mazoyer & Roudart 2006), which profoundly changed the structure of agricultural landscapes and the availability of floral resources for bumblebees (Rasmont & Mersch 1988). The year 1970 broadly corresponds to the beginning of the application of the Mansholt Plan, leading to fast intensification of agriculture (*e.g.* polycultures transformed into monocultures through land consolidation, livestock management with higher productivity), and to the felling of most orchards (Christians 1998). The year 1990 corresponds to the establishment of agri-environmental schemes in Europe (AES), which allowed the integration of environmental concerns into the Common Agricultural Policy (CAP) and became mandatory for EU Member States in 1992 (Kleijn & Sutherland 2003; Batáry *et al.* 2015).

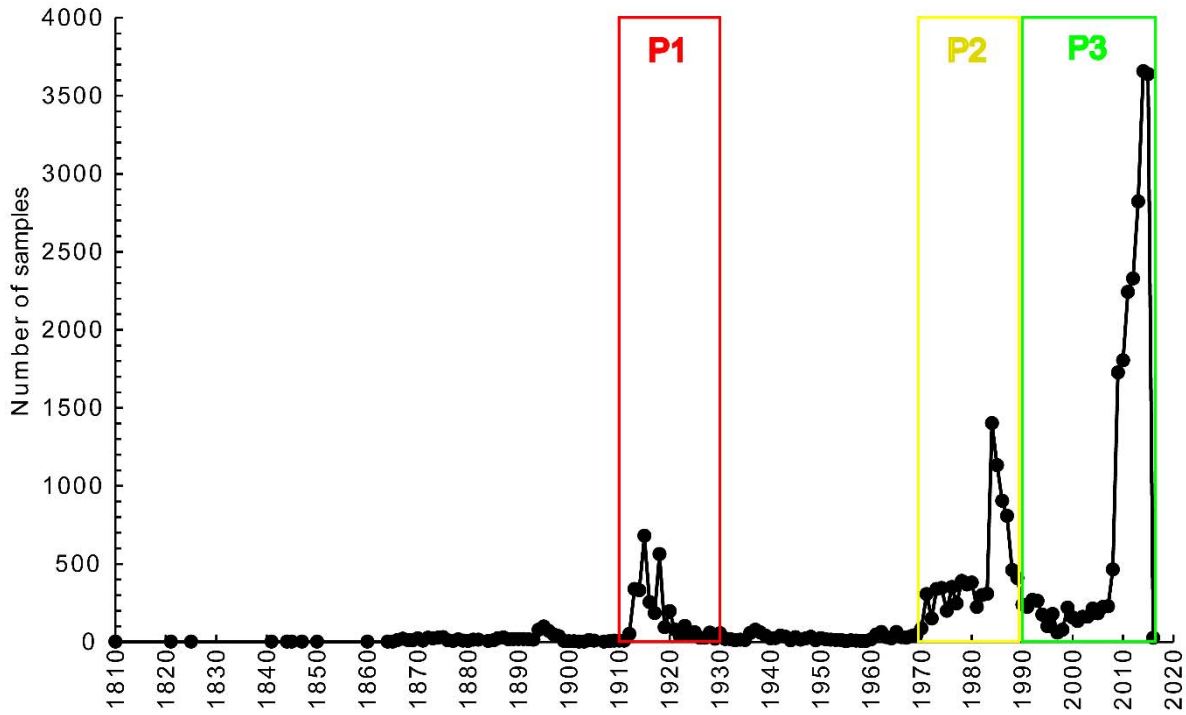

Figure 1. Temporal distribution of the number of samples (one sample is one 5x5km grid-cell sampled by one recorder during one day).

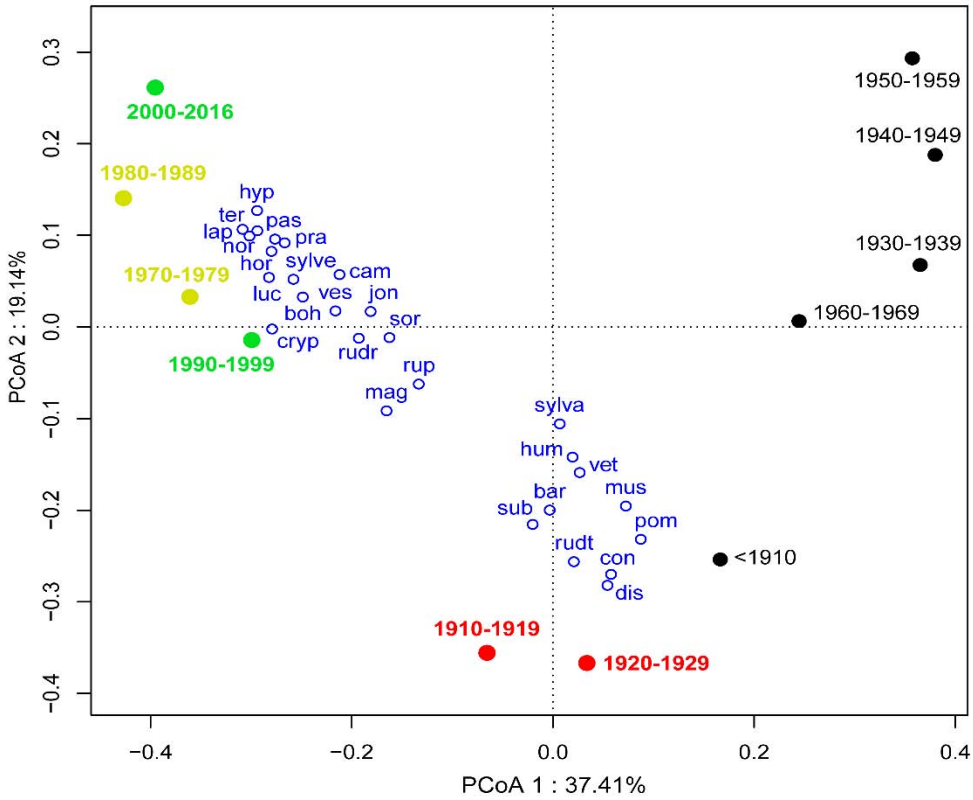

Figure 2. Principal Coordinate Analysis graph of the Bray–Curtis dissimilarity matrix of the raw number of 10 km grid-cells between decades and species, with *a posteriori* projection of species (in blue) as weighted averages of their contributions to the decades. The two first components account for 56.55% of the total variance. Decades are gathered into three main groups: one before 1930 (in red the two decades kept in P1), one between 1930 and 1969 (not considered due to lack of data), and one after 1970, which we subdivided into two time-periods: P2 (1970-1989, in yellow) and P3 (1990-2016, in green). bar = *B. barbutellus*, boh = *B. bohemicus*, cam = B. campestris, con = *B. confusus*, cry = *B. cryptarum*, dis = *B. distinguendus*, hor = *B. hortorum*, hum = *B. humilis*, hyp = *B. hypnorum*, jon = *B. jonellus*, lap = *B. lapidarius*, luc = *B. lucorum*, mag = *B. magnus*, mus = *B. muscorum*, nor = *B. norvegicus*, pas = *B. pascuorum*, pom = *B. pomorum*, pra = *B. pratorum*, rudr = *B. ruderarius*, rudt = *B. ruderatus*, rup = *B. rupestris*, sor = *B. soroeensis*, sub = *B. subterraneus*, syla = *B. sylvarum*, syle = *B. sylvestris*, ter = *B. terrestris*, ves = *B. vestalis*, vet = *B. veteranus*. Analyses on absolute numbers of 5 km grid-cells and relative numbers of 5 and 10km grid-cells show the same patterns and are not presented.

Finally, we tested how the data aggregation did affect our results by considering a second temporal subdivision with two time-periods (*a*: 1900-1949 and *b*: 1950-1992). These periods were the same than the ones used by Rasmont *et al.* (1993) on a reduced Belgian dataset.

3.5. Analyses of variation in species richness and diversity indices

Richness change estimates can depend on the spatial scale at which they are considered (Keil *et al*. 2010). Such scale-related variation in estimates provide valuable information to help understand the patterns of change, and for mitigation strategy (Whittaker *et al*. 2001). For example, range expansions affect richness values of small scale cells, and hence have an effect on the mean change value at finer spatial scales (Gotelli & Colwell 2001), but no effect at country level (if the species was present in both periods and simply changed its distribution pattern). Therefore, we computed species richness and diversity indices at the scale of the whole country as well as for each 10x10 km grid-cell (corresponding to the UTM squares) with at least 30 specimens recorded in each of the three time-periods (Fig. 3a). We chose the 10 km spatial scale after testing different grid-cell sizes. This size was the best compromise between the amount of data per grid-cell and the spatial resolution of the oldest data (*i.e.* 5 km grid-cells, Fig. 3b). We calculated several indicators of species richness and diversity: the species richness, the Shannon's diversity index, the 1-Simpson index (*i.e.* the probability that two individuals drawn at random are from different species), and the Berger-Parker's index (*i.e.* the proportional abundance of the most dominant species; Berger & Parker, 1970). Additionally, we assessed expected (*i.e.* rarefied) species richness in random subsamples of 100 specimens using the Hurlbert's index (Hurlbert 1971) for each 10 km grid-cell containing at least 100 specimens (Fig. 3a). We then estimated the changes in expected species richness by calculating their difference between the time-periods two by two. These analyses were performed in R with packages

"vegan" (Oksanen *et al.* 2017) and "BiodiversityR" (Kindt & Coe 2005). We then mapped these indicators for each time-period and the difference in expected species richness between time-periods two-by-two using ArcGIS 10 software (ESRI 2011).

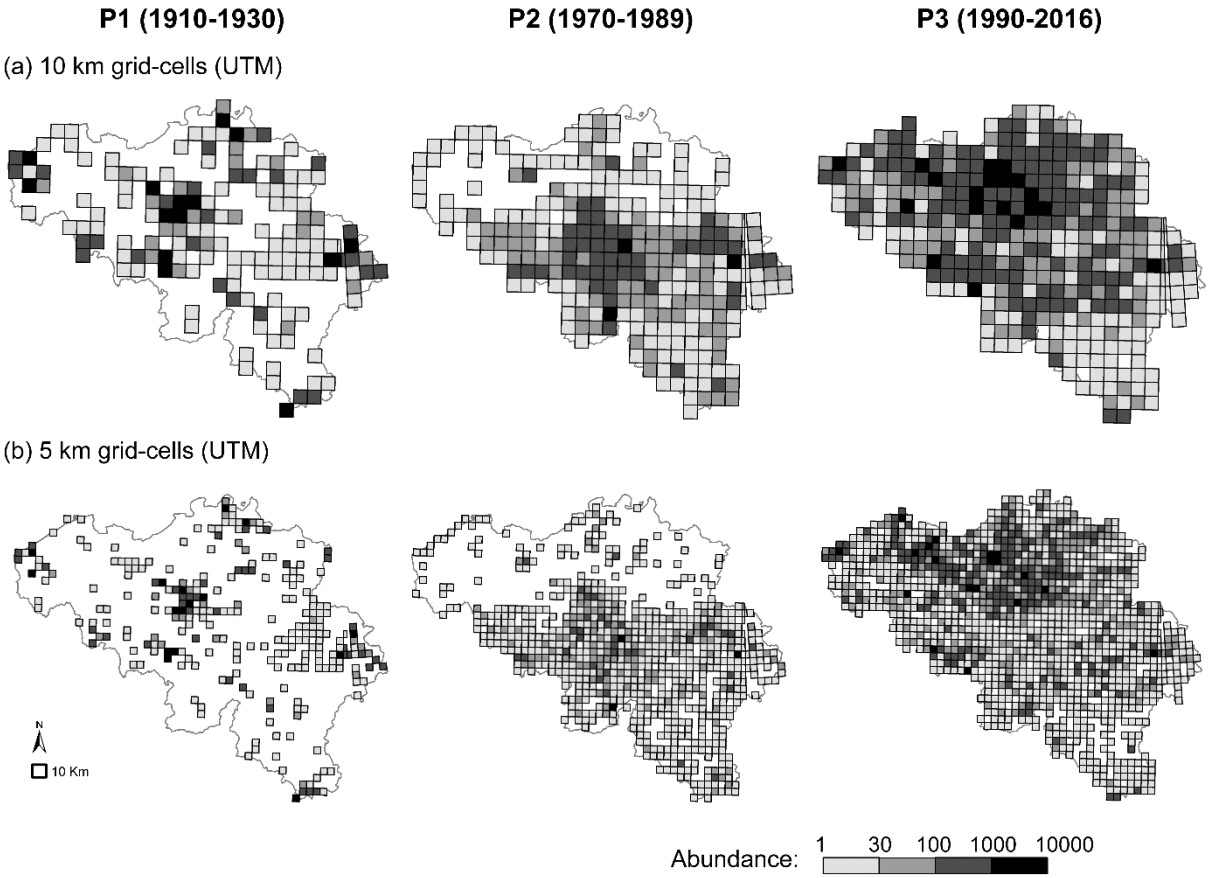

Figure 3. Spatial distribution of the number of specimens (*i.e.* abundance) in grid-cells of (a) 10x10 km (used for analyses of variation in species richness and diversity indices) and (b) 5x5 km (used for analyses of variation in species geographic range size) in each time-period.

3.6. Analyses of variation in abundance

In order to assess for each species the variation of its abundance (*i.e.* number of specimens) between the three time-periods at the country scale, we used the Stroot & Depiereux (1989) method, as in Rasmont *et al.* (1993). This methodology is based on the chi-square index and estimates for each species the difference between its

relative abundance between two time-periods on the one hand, and the same proportion for the whole group on the other hand, which enables to take into account the global evolution of sampling during the time-periods considered. In order to assess the relative dominance of each species in each time-period and limit the influence of sampling bias, we estimated their rank-abundance based on their relative abundance (*i.e.* the ratio between the number of specimens of the species and the number of specimens of all species in the same time-period). We then calculated the difference (delta-rank) between each period.

As workers do not participate in reproduction and as their number in a colony varies greatly between species (and are even absent in inquiline species), we performed the same analyses only on the queens and males dataset in order to have the information about the effective population size of each species. Moreover, workers are often very difficult to determine in some groups (*e.g.* the *Bombus* subgenus, including *B. cryptarum*, *B. lucorum*, *B. magnus*, and *B. terrestris*), leading to frequent mistakes, and many of them were not determined for the *Bombus* subgenus species in P1. However, in order to keep the comparability with previous studies, we decided to keep the analyses including the workers records in geographic range and species traits analyses.

3.7. Analyses of variation in geographic range size

We performed range analyses at the spatial scale of 5x5 km grid-cells (corresponding to the UTM squares), which was the finest scale we could assess considering the spatial accuracy of the oldest records. We estimated the range size

of each species by counting the number of 5 km grid-cells where each species was recorded in each time period. We mapped each species range using ArcGIS 10 software (ESRI 2011). In order to control for different sampling efforts in the comparisons between time-periods, we calculated the proportion of grid-cells for each species in each time-period (*i.e.* the ratio between the number of grid-cells where the species was observed and the number of grid-cells for all species). We then calculated the ratio of the relative numbers of grid-cells between time-periods. Like for abundance analyses, we assessed the species population trends based on their changes in range size between time-periods by using the Stroot & Depiereux (1989) method.

3.8. Relation between species ecological traits and population trends

We assessed the relation between species traits and population trends, based on the delta-ranks of the abundances and on the ratios of the relative range size between time-periods. For the continuous variables, we calculated Spearman rank-correlation coefficients. For the categorical variables, we performed parametric tests for the delta-ranks (two sample t-test for variables with two categories and ANOVA followed by Tukey contrasts for variables with more than two categories, with R packages “stats” and “multcomp”; Hothorn *et al.* 2008) and non-parametric tests for the ratios of the relative range size (Wilcoxon and Kruskal-Wallis rank sum tests, with R package “stats”).

4. Results

4.1. Variation in species richness and diversity

At the country scale, a total of 28 species was recorded before 1930 (excluding *B. cullumanus*, *B. quadricolor* and *B. wurflenii*). The species richness fell to 26 species between 1930 and 1990, and to 24 species in the recent period despite the increase in sampling for the whole country (Table 2). The expected species richness in a subsample of 100 specimens decreased by almost 9 species between P1 and P3. Similarly, Shannon's and Simpson's indexes decreased along the three time-periods. The proportion of the community represented by the most abundant species (*i.e.* Berger-Parker's index) remained relatively constant between the first two periods and increased in P3, showing that the dominant species became even more dominant.

Table 2. Species richness, expected richness (*i.e.* Hurlbert's index) in a subsample of 100 specimens, Shannon's index, 1-Simpson's index, and Berger-Parker's index, at the Belgian and the 10 km grid-cell scales (mean value ± standard deviation). Only the grid-cells containing at least 100 specimens are considered for expected richness, and 30 specimens for other indicators.

	P1 (1910-1930)	**P2 (1970-1989)**	**P3 (1990-2016)**
Belgian scale			
Species richness	28	26	24
Expected species richness	18.84	11.64	10.01
Shannon's index	2.401	2.058	1.737
1-Simpson's index	0.841	0.823	0.744
Berger-Parker's index	0.329	0.317	0.419
10 km scale (mean ± sd)			
Species richness	14.11 (± 6.85)	9.56 (± 2.49)	8.21 (± 2.54)
Expected species richness	11.51 (± 3.58)	9.27 (± 1.27)	7.22 (± 1.68)
Shannon's index	1.631 (± 0.545)	1.637 (± 0.254)	1.404 (± 0.311)
1-Simpson's index	0.681 (± 0.187)	0.729 (± 0.082)	0.663 (± 0.123)
Berger-Parker's index	0.464 (± 0.187)	0.415 (± 0.104)	0.479 (± 0.142)

Among the sampled 10x10km grid-cells with at least 30 specimens (Fig. 3a), some show higher diversity than others depending on the time-period (Fig. 4). The sampled grid-cells showing the highest expected species richness before 1930 are around Brussels city and in southern-eastern Belgium after 1970. Among the sampled grid-cells presenting at least 100 specimens (Fig. 3a), the overall trend is a decrease in expected species richness, with the exception of some grid-cells which show an increase in expected number of species between P1 and P2 or P3 (Fig. 5). Despite the conserved high number of specimens during the three time-periods (Fig. 3a), the region around Brussels shows the highest decrease in expected species richness between P1 and P3 among the sampled regions (Fig. 5).

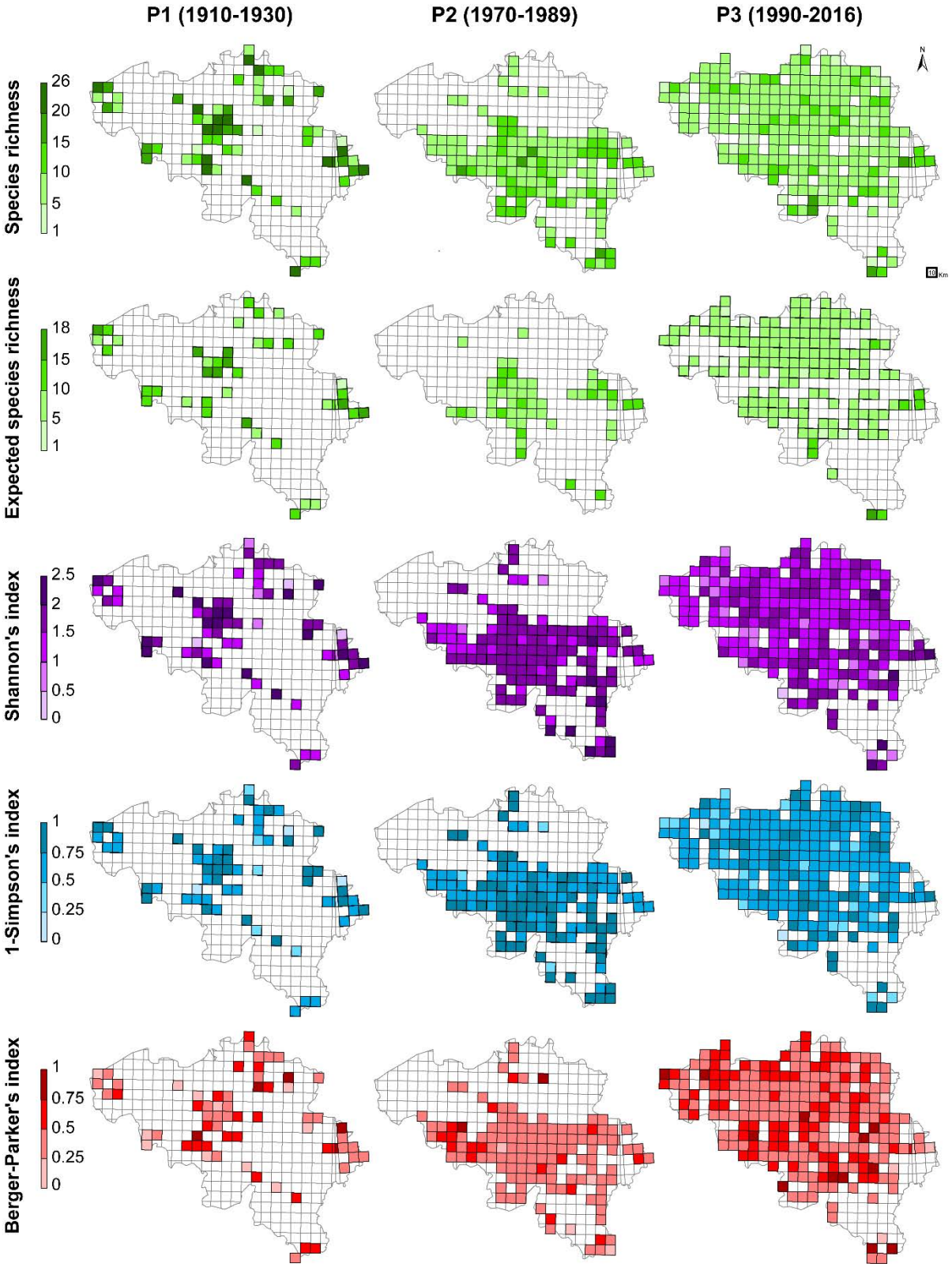

Figure 4. Species richness, expected species richness in a subsample of 100 specimens, Shannon's index, 1-Simpson's index, and Berger-Parker's index in each time-period. Only the grid-cells containing at least 100 specimens are considered for expected richness, and 30 specimens for other indicators. See Fig. 3a for information about sampling effort.

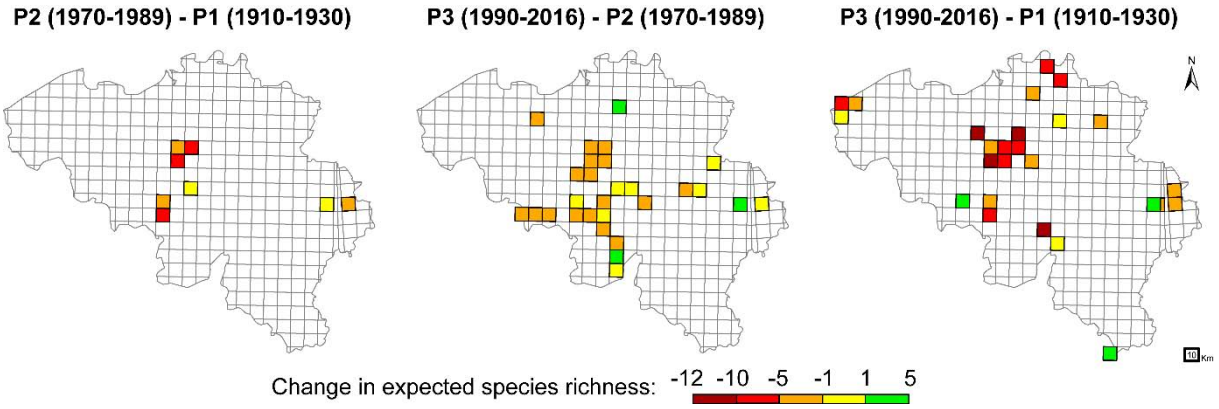

Figure 5. Change in expected species richness in a subsample of 100 specimens between each time-period two-by-two in the 10x10 km grid-cells with a least 100 specimens. Colored grid-cells are where at least 100 specimens were sampled in both time-periods. See Fig. 3a for information about sampling effort.

4.2. Variation in abundance

Population trends assessment following the Stroot & Depiereux (1989) method show 20 declining, 1 stable, and 7 increasing species between P1 and P2 (Table 3). These trends change to 19 regressing, 1 stable, and 8 increasing species between P1 and P3. Between P2 and P3, 16 species were in regression, against 3 stable and 7 increasing species. Ten species present the same trends in the three time-periods comparisons (Table 3), with 9 always decreasing (*B. barbutellus, B. distinguendus, B. hortorum, B. humilis, B. muscorum, B. ruderarius, B. subterraneus, B. sylvarum*, and *B. veteranus*) and only *B. hypnorum* always increasing. Among species with non-constant trends, some were increasing or stable between P1 and P2 but decreasing between P2 and P3 (*B. bohemicus, B. cryptarum, B. lucorum, B. norvegicus, B. pratorum, B. sylvestris, B. terrestris*) and others show the opposite trend (*B. campestris, B. jonellus, B. lapidarius, B. magnus, B. pascuorum, B. rupestris, B. soroeensis, B. vestalis*).

Two species disappeared from Belgium between P1 and P2 (*B. confusus* and *B. pomorum*, last observation in 1957 and 1947, respectively) and two between P2 and P3 *(B. distinguendus* and *B. subterraneus*, last observation in 1971 and 1982, respectively*)*. Among them, three were already rare in the past (*B. subterraneus*, *B. pomorum*, and *B. confusus*). *B. distinguendus* was on the contrary relatively abundant at the 11th position of rank-abundance before 1930, and fell to the 25th position in P2 before disappearing in P3 (Table 4). The other species which were also formerly abundant and declined drastically between P1 and P3 are, by order of decline intensity (*i.e.* delta-ranks), *B. ruderatus*, *B. veteranus, B. muscorum*, *B. ruderarius*, *B. humilis*, and *B. barbutellus*. At the opposite, several species kept a quite stable rank-abundance across time-periods (Table 4). Some of them remained among the most abundant species (*B. pascuorum*, *B. lapidarius*, *B. pratorum*, *B. terrestris*), with even a global tendency to increase (Table 3). Some others remained relatively rare (*B. rupestris*, *B. vestalis*, and *B. sylvarum*), with a decreasing trend between P1 and P2 and between P1 and P3, but with various trends between P2 and P3 (Table 3). Furthermore, some species increased their relative abundance such as, by order of increase intensity, *B. bohemicus*, *B. lucorum*, and *B. hypnorum,* which are among the ten most abundant species today (Table 4). *Bombus pascuorum* remained the most dominant species in the three time-periods (Fig. 6). The other dominant species before 1930 were *B. lapidarius*, *B. hortorum*, *B. pratorum*, and *B. veteranus* (Fig. 6). The latter drastically declined from the 5th position to the 21st position, whereas the others remained among the most

abundant, but with a decreasing trend for some of them, such as *B. hortorum* (Tables 3 and 4).

Table 3. Species abundance trends estimated with the Stroot & Depiereux (1989) method between P1 (1910-1930) and P2 (1970-1989), P2 and P3 (1990-2016), and P1 and P3. "-" and "+" represent negative and positive significant trends at the p-value of * 0.05 (Chi^2 = 3.8), ** 0.01 (Chi^2 = 6.6), and *** 0.001 (Chi^2 = 10.8). Results for species with [a] could be biased by the misidentification of workers. Species are ordered by their abundance in P1. Black cells are disappeared species.

Species	Observed			Expected		Chi^2	Trend 1-2	Observed			Expected		Chi^2	Trend 2-3	Observed			Expected		Chi^2	Trend 1-3
	P1	P2	Total	P1	P2			P2	P3	Total	P2	P3			P1	P3	Total	P1	P3		
pascuorum	19913	7561	27474	19705	7769	8	- **	7561	37411	44972	9475	35497	490	+ ***	19913	37411	57324	23141	34183	755	+ ***
lapidarius	10260	1954	12214	8760	3454	908	- ***	1954	21684	23638	4980	18658	2330	+ ***	10260	21684	31944	12896	19048	903	+ ***
hortorum	5479	1119	6598	4732	1866	417	- ***	1119	3430	4549	958	3591	34	- ***	5479	3430	8909	3596	5313	1652	- ***
pratorum	3942	4843	8785	6301	2484	3122	+ ***	4843	8268	13111	2762	10349	1985	- ***	3942	8268	12210	4929	7281	331	+ ***
veteranus	3618	19	3637	2608	1029	1382	- ***	19	22	41	9	32	16	- ***	3618	22	3640	1469	2171	5268	- ***
ruderatus	2798	3	2801	2009	792	1096	- ***	3	7	10	2	8	0	=	2798	7	2805	1132	1673	4109	- ***
ruderarius	1414	281	1695	1216	479	114	- ***	281	170	451	95	356	461	- ***	1414	170	1584	639	945	1573	- ***
muscorum	1398	7	1405	1008	397	535	- ***	7	5	12	3	9	10	- **	1398	5	1403	566	837	2048	- ***
terrestris [a]	1267	2127	3394	2434	960	1979	+ ***	2127	6005	8132	1713	6419	127	- ***	1267	6005	7272	2936	4336	1591	+ ***
campestris	1215	79	1294	928	366	314	- ***	79	519	598	126	472	22	+ ***	1215	519	1734	700	1034	635	- ***
distinguendus	1192	3	1195	857	338	463	- ***	3	0	3	1	2	11	- ***	1192	0	1192	481	711	1761	- ***
hypnorum	920	1076	1996	1432	564	646	+ ***	1076	7154	8230	1734	6496	316	+ ***	920	7154	8074	3259	4815	2816	+ ***
sylvestris	914	377	1291	926	365	1	=	377	375	752	158	594	382	- ***	914	375	1289	520	769	499	- ***
humilis	794	13	807	579	228	283	- ***	13	25	38	8	30	4	- *	794	25	819	331	488	1089	- ***
vestalis	729	45	774	555	219	193	- ***	45	179	224	47	177	0	=	729	179	908	367	541	601	- ***
rupestris	602	15	617	443	174	203	- ***	15	182	197	42	155	21	+ ***	602	182	784	316	468	432	- ***
barbutellus	577	14	591	424	167	196	- ***	14	10	24	5	19	20	- ***	577	10	587	237	350	818	- ***
lucorum [a]	567	2743	3310	2374	936	4864	+ ***	2743	2055	4798	1011	3787	3760	- ***	567	2055	2622	1058	1564	383	+ ***
sylvarum	519	30	549	394	155	141	- ***	30	69	99	21	78	5	- *	519	69	588	237	351	560	- ***
cryptarum [a]	517	1141	1658	1189	469	1343	+ ***	1141	312	1453	306	1147	2885	- ***	517	312	829	335	494	167	- ***
magnus [a]	478	42	520	373	147	105	- ***	42	349	391	82	309	25	+ ***	478	349	827	334	493	104	- ***
soroeensis	369	68	437	313	124	35	- ***	68	226	294	62	232	1	=	369	226	595	240	355	116	- ***
subterraneus	302	12	314	225	89	93	- ***	12	0	12	3	9	45	- ***	302	0	302	122	180	446	- ***
jonellus	249	29	278	199	79	44	- ***	29	401	430	91	339	53	+ ***	249	401	650	262	388	1	=
bohemicus	198	225	423	303	120	129	+ ***	225	476	701	148	553	51	- ***	198	476	674	272	402	34	+ ***
pomorum	170	0	170	122	48	67	- ***	0	0	0	/	/	/	/	170	0	170	69	101	251	- ***
confusus	90	0	90	65	25	35	- ***	0	0	0	/	/	/	/	90	0	90	36	54	133	- ***
norvegicus	7	28	35	25	10	46	+ ***	28	30	58	12	46	26	- ***	7	30	37	15	22	7	+ **
Total	60498	23854	84352	60498	23854			23854	89364	113218	23854	89364			60498	89364	149862	60498	89364		

Table 4. Abundance (*i.e.* number of specimens), proportional abundance (specific abundance / total abundance), and rank-abundance in the three time-periods. Delta-ranks are the difference between ranks of each time-period. P1 = 1910-1930; P2 = 1970-1989; P3 = 1990-2016. Results for species with [(a)] could be biased by the misidentification of workers. Species are ordered by their abundance in P1. Black cells are disappeared species.

	Abundance			Proportion (%)			Rank-abundance			Delta-rank		
Species	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1-P2	P2-P3	P1-P3
pascuorum	19913	7561	37411	32.92	31.70	41.86	1	1	1	0	0	0
lapidarius	10260	1954	21684	16.96	8.19	24.26	2	5	2	-3	0	3
hortorum	5479	1119	3430	9.06	4.69	3.84	3	7	6	-4	-3	1
pratorum	3942	4843	8268	6.52	20.30	9.25	4	2	3	2	1	-1
veteranus	3618	19	22	5.98	0.08	0.02	5	19	21	-14	-16	-2
ruderatus	2798	3	7	4.62	0.01	0.01	6	26	23	-20	-17	3
ruderarius	1414	281	170	2.34	1.18	0.19	7	10	17	-3	-10	-7
muscorum	1398	7	5	2.31	0.03	0.01	8	24	24	-16	-16	0
terrestris[(a)]	1267	2127	6005	2.09	8.92	6.72	9	4	5	5	4	-1
campestris	1215	79	519	2.01	0.33	0.58	10	12	8	-2	2	4
distinguendus	1192	3	0	1.97	0.01	0.00	11	25	/	-14	/	/
hypnorum	920	1076	7154	1.52	4.51	8.01	12	8	4	4	8	4
sylvestris	914	377	375	1.51	1.58	0.42	13	9	11	4	2	-2
humilis	794	13	25	1.31	0.05	0.03	14	22	20	-8	-6	2
vestalis	729	45	179	1.20	0.19	0.20	15	14	16	1	-1	-2
rupestris	602	15	182	1.00	0.06	0.20	16	20	15	-4	1	5
barbutellus	577	14	10	0.95	0.06	0.01	17	21	22	-4	-5	-1
lucorum[(a)]	567	2743	2055	0.94	11.50	2.30	18	3	7	15	11	-4
sylvarum	519	30	69	0.86	0.13	0.08	19	16	18	3	1	-2
cryptarum[(a)]	517	1141	312	0.85	4.78	0.35	20	6	13	14	7	-7
magnus[(a)]	478	42	349	0.79	0.18	0.39	21	15	12	6	9	3
soroeensis	369	68	226	0.61	0.29	0.25	22	13	14	9	8	-1
subterraneus	302	12	0	0.50	0.05	0.00	23	23	/	0	/	/
jonellus	249	29	401	0.41	0.12	0.45	24	17	10	7	14	7
bohemicus	198	225	476	0.33	0.94	0.53	25	11	9	14	16	2
pomorum	170	0	0	0.28	0.00	0.00	26	/	/	/	/	/
confusus	90	0	0	0.15	0.00	0.00	27	/	/	/	/	/
norvegicus	7	28	30	0.01	0.12	0.03	28	18	19	10	9	-1
Total	60498	23854	89364									

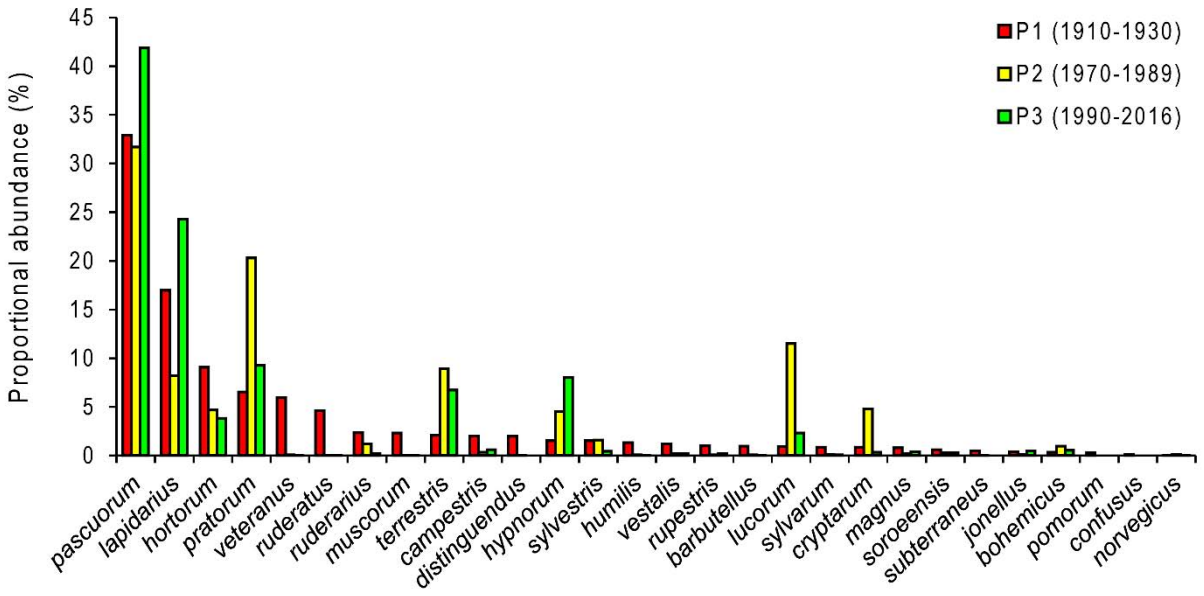

Figure 6. Species proportional abundance (species abundance / total abundance) in each time-period, ordered by species ranks in P1 (1910-1930).

Analyses based only on queens and males records show similar patterns in trends (Table S3) and delta-ranks (Table S4), except for trends between P2 and P3. As abundances are lower than in the dataset including workers (Table 3), more species are considered to have a stable trend (*i.e.* a non-significant difference between the two time-periods). Moreover, removal of workers from the dataset implies higher proportional abundance in workerless inquiline species (*i.e. B. barbutellus, B. bohemicus, B. campestris, B. norvegicus, B. rupestris, B. sylvestris,* and *B. vestalis*), and consequently more positive trends, than in the analyses including workers (Tables 3 and 4).

Population trends assessment following the same methodology and the same time-periods (*a*: 1900-1949 and *b*: 1950-1992) than in Rasmont *et al.* (1993) shows the same results as in their study (Table S5), with only two exceptions: *B. pascuorum* and *B. sylvestris* had an increasing trend in Rasmont *et al.* (1993) but do no longer show a significant trend in our results with the completed database.

Moreover, the identification and encoding of the four species from the *Bombus* sub-genus allow assessing their trends, which were not available in Rasmont *et al.* (1993). Species trends between time-periods *a* and *b* are the same as between time-periods P1 (1910-1930) and P2 (1970-1989), except for *B. pascuorum*, which is stable between *a* and *b* (Table S5) but decreasing between P1 and P2 (Table 3).

4.3. Variation in geographic range size

Concerning the trends observed in geographic range size (Table 5), 18 species were in regression, 5 were stable, and 5 were expanding between P1 and P2. These trends change to 21 decreasing, 3 stable and 4 expanding species between P1 and P3. Between P2 and P3, only 12 species were declining, against 8 stable and 6 expanding. Eight species present the same trend in the three time-periods comparisons (Table 5). Six were always decreasing (*B. barbutellus* (Fig. S2a)*, B. cryptarum* (Fig. S3a), *B. ruderarius* (Fig. S6c), *B. subterraneus* (Fig. S7c), *B. sylvarum* (Fig. S7d), and *B. veteranus* (Fig. S8d)), one remained stable (*B. norvegicus*, Fig. S5c) and one was expanding (*B. hypnorum*, Fig. S4a). Among species with non-constant trends, some were increasing or stable between P1 and P2 but decreasing between P2 and P3 (*B. bohemicus* (Fig. S2b)*, B. hortorum* (Fig. S3c), *B. lucorum* (Fig. S4d)*, B. pratorum* (Fig. S6b)*, B. sylvestris* (Fig. S8a), and *B. terrestris* (Fig. S8b)) and others show a regression between P1 and P2 followed by an expansion between P2 and P3 (*B. campestris* (Fig. S2c)*, B. jonellus* (Fig. S4b)*, B. magnus* (Fig. S5a), and *B. rupestris* (Fig. S7a)). The proportions of grid-cells indicate that the four more widespread species before 1930 were, by descending order, *B. pascuorum* (Fig.

S5d), *B. hortorum* (Fig. S3c), *B. lapidarius* (Fig. S4c), and *B. pratorum* (Fig. S6b, Table 6). These species remain among the most widespread ones in the recent period and some of them even increased their relative range (Table 6). The most geographically restricted species in P1 were *B. norvegicus* (Fig. S5c), *B. pomorum* (Fig. S6a), *B. confusus* (Fig. S2d), and *B. soroeensis* (Fig. S7b). Among them, two went extinct (*B. pomorum* and *B. confusus*) in P2, *B. norvegicus* remained stable, and *B. soroeensis* suffered a decrease in its range (Table 6). Species that presented the strongest range retraction between P1 and P3 are, by order of retraction intensity (Table 6), *B. distinguendus* (Fig. S3b), *B. subterraneus* (Fig. S7c), *B. muscorum* (Fig. S5b), *B. barbutellus* (Fig. S2a), *B. ruderatus* (Fig. S6d), *B. veteranus* (Fig. S8d), *B. humilis* (Fig. S3d), and *B. sylvarum* (Fig. S7d). On the contrary, species in expansion between P1 and P3 were, by order of expansion intensity (Table 6), *B. hypnorum* (Fig. S4a), *B. lapidarius* (Fig. S4c), *B. pratorum* (Fig. S6b), and *B. pascuorum* (Fig. S5d).

Table 5. Trends based on species range size (number of 5km grid-cells) estimated with the Stroot & Depiereux (1989) method between P1 (1910-1930) and P2 (1970-1989), P2 and P3 (1990-2016), and P1 and P3. Trends with "()" are for species with an expected estimator < 5 in the second time-period. "-" and "+" represent negative and positive significant trends at the p-value of * 0.05 (Chi^2 = 3.8), ** 0.01 (Chi^2 = 6.6), and *** 0.001 (Chi^2 = 10.8). Results for species with [a] could be biased by the misidentification of workers. Species are ordered by their number of grid-cells in P1. Black cells are disappeared species.

Species	Observed			Expected		Chi^2	Trend 1-2	Observed			Expected		Chi^2	Trend 2-3	Observed			Expected		Chi^2	Trend 1-3
	P1	P2	Total	P1	P2			P2	P3	Total	P2	P3			P1	P3	Total	P1	P3		
pascuorum	138	573	711	194	517	22.31	+ ***	573	965	1538	579	959	0.1	=	138	965	1103	204	899	26.12	+ ***
hortorum	106	316	422	115	307	1.01	=	316	438	754	284	470	5.83	- *	106	438	544	101	443	0.36	=
lapidarius	104	297	401	109	292	0.38	=	297	824	1121	422	699	59.42	+ ***	104	824	928	172	756	32.62	+ ***
pratorum	90	473	563	154	409	36.32	+ ***	473	683	1156	435	721	5.26	- *	90	683	773	143	630	24.01	+ ***
distinguendus	73	2	75	20	55	185.33	- ***	2	0	2	1	1	3.31	(=)	73	0	73	13	60	321.93	- ***
terrestris [a]	70	323	393	107	286	17.83	+ ***	323	392	715	269	446	17.25	- ***	70	392	462	85	377	3.41	=
ruderarius	70	113	183	50	133	11.06	- ***	113	54	167	63	104	64.09	- ***	70	54	124	23	101	118.63	- ***
campestris	66	42	108	29	79	62.2	- ***	42	167	209	79	130	27.43	+ ***	66	167	233	43	190	14.98	- ***
ruderatus	63	3	66	18	48	154.46	- ***	3	6	9	3	6	0.07	=	63	6	69	13	56	242.83	- ***
barbutellus	60	11	71	19	52	117.07	- ***	11	4	15	6	9	8.14	- **	60	4	64	12	52	240.62	- ***
lucorum [a]	58	238	296	81	215	8.86	+ **	238	118	356	134	222	129.35	- ***	58	118	176	33	143	24.46	- ***
rupestris	57	12	69	19	50	106.34	- ***	12	67	79	30	49	16.98	+ ***	57	67	124	23	101	62.16	- ***
veteranus	53	17	70	19	51	82.66	- ***	17	8	25	9	16	9.81	- **	53	8	61	11	50	189.41	- ***
muscorum	52	5	57	16	41	117.36	- ***	5	2	7	3	4	3.4	(=)	52	2	54	10	44	216.99	- ***
sylvarum	48	18	66	18	48	68.62	- ***	18	13	31	12	19	5.5	- *	48	13	61	11	50	146.74	- ***
cryptarum [a]	47	78	125	34	91	6.68	- **	78	44	122	46	76	35.91	- ***	47	44	91	17	74	66.42	- ***
sylvestris	46	149	195	53	142	1.35	=	149	115	264	99	165	39.71	- ***	46	115	161	30	131	10.87	- ***
hypnorum	44	263	307	84	223	26.02	+ ***	263	573	836	315	521	13.64	+ ***	44	573	617	114	503	52.78	+ ***
magnus [a]	44	6	50	14	36	92.82	- ***	6	36	42	16	26	9.77	+ **	44	36	80	15	65	70.79	- ***
humilis	38	9	47	13	34	67.91	- ***	9	7	16	6	10	2.36	=	38	7	45	8	37	129.94	- ***
vestalis	37	28	65	18	47	28.74	- ***	28	70	98	37	61	3.44	=	37	70	107	20	87	18.4	- ***
subterraneus	37	8	45	12	33	68.39	- ***	8	0	8	3	5	13.25	- ***	37	0	37	7	30	163.17	- ***
bohemicus	34	91	125	34	91	0	=	91	67	158	59	99	26.78	- ***	34	67	101	19	82	15.44	- ***
jonellus	33	6	39	11	28	64.55	- ***	6	54	60	23	37	19.54	+ ***	33	54	87	16	71	21.84	- ***
soroeensis	24	26	50	14	36	10.79	- **	26	41	67	25	42	0.04	=	24	41	65	12	53	14.67	- ***
confusus	20	0	20	5	15	53.26	- ***	0	0	0	/	/	/	/	20	0	20	4	16	88.2	- ***
pomorum	14	0	14	4	10	37.28	- ***	0	0	0	/	/	/	/	14	0	14	3	11	61.74	- ***
norvegicus	5	19	24	7	17	0.51	=	19	18	37	14	23	2.96	=	5	18	23	4	19	0.16	=
Total	261	695	956					695	1151	1846					261	1151	1412				

Table 6. Absolute number and proportion (relative number in percent) of 5km grid-cells where each species was observed. The ratio is calculated based on the proportions. A ratio > 1 or < 1 means that the proportion of grid-cells where the species was recorded respectively increased or decreased between the two time-periods. Results for species with [(a)] could be biased by the misidentification of workers. Species are ordered by their number of grid-cells in P1. Black cells are disappeared species.

	Number of grid-cells			Proportion of grid-cells			Ratio		
Species	P1	P2	P3	P1	P2	P3	P2/P1	P3/P2	P3/P1
pascuorum	138	573	965	52.87	82.45	83.84	1.56	1.02	1.59
hortorum	106	316	438	40.61	45.47	38.05	1.12	0.84	0.94
lapidarius	104	297	824	39.85	42.73	71.59	1.07	1.68	1.80
pratorum	90	473	683	34.48	68.06	59.34	1.97	0.87	1.72
distinguendus	73	2	0	27.97	0.29	0.00	0.01	0.00	0.00
terrestris[(a)]	70	323	392	26.82	46.47	34.06	1.73	0.73	1.27
ruderarius	70	113	54	26.82	16.26	4.69	0.61	0.29	0.17
campestris	66	42	167	25.29	6.04	14.51	0.24	2.40	0.57
ruderatus	63	3	6	24.14	0.43	0.52	0.02	1.21	0.02
barbutellus	60	11	4	22.99	1.58	0.35	0.07	0.22	0.02
lucorum[(a)]	58	238	118	22.22	34.24	10.25	1.54	0.30	0.46
rupestris	57	12	67	21.84	1.73	5.82	0.08	3.37	0.27
veteranus	53	17	8	20.31	2.45	0.70	0.12	0.28	0.03
muscorum	52	5	2	19.92	0.72	0.17	0.04	0.24	0.01
sylvarum	48	18	13	18.39	2.59	1.13	0.14	0.44	0.06
cryptarum[(a)]	47	78	44	18.01	11.22	3.82	0.62	0.34	0.21
sylvestris	46	149	115	17.62	21.44	9.99	1.22	0.47	0.57
hypnorum	44	263	573	16.86	37.84	49.78	2.24	1.32	2.95
magnus[(a)]	44	6	36	16.86	0.86	3.13	0.05	3.62	0.19
humilis	38	9	7	14.56	1.29	0.61	0.09	0.47	0.04
vestalis	37	28	70	14.18	4.03	6.08	0.28	1.51	0.43
subterraneus	37	8	0	14.18	1.15	0.00	0.08	0.00	0.00
bohemicus	34	91	67	13.03	13.09	5.82	1.01	0.44	0.45
jonellus	33	6	54	12.64	0.86	4.69	0.07	5.43	0.37
soroeensis	24	26	41	9.20	3.74	3.56	0.41	0.95	0.39
confusus	20	0	0	7.66	0.00	0.00	0.00	/	0.00
pomorum	14	0	0	5.36	0.00	0.00	0.00	/	0.00
norvegicus	5	19	18	1.92	2.73	1.56	1.43	0.57	0.82
All *Bombus* spp.	261	695	1151						

4.4. Species ecological traits and population trends

4.4.1. Inquinilism and nesting strategy

We find no significant difference in delta-rank and ratios of relative range size between the inquilines species and the species living in colonies. Delta-ranks between P1 and P3 are significantly different between the renter and the carder species (Welch Two Sample t-test, p-value = 0.01). Mean delta-ranks in the 15 renter species is positive (3.82) and higher than mean in the 6 carder species which is negative (-7.83). This indicates that renter species tend to increase (except for *B. ruderatus*) and carder species to decrease (except for *B. pascuorum*) in their abundance between P1 and P3. The maximal colony size is positively correlated with the ratios of range size proportions between P1 and P2, and between P1 and P3 (Table 7). This is also correlated to the delta-rank between P1 and P2 and between P1 and P3. This suggests that species with bigger colonies tend to increase more or decline less in their relative abundance and their relative range size than species with smaller colonies.

4.4.2. Host plants and diet breadth

Three plant families account for almost 60% of the 33 382 bumblebee observations on a plant: Asteraceae (26%), Fabaceae (23%), and Lamiaceae (10%). The following ones are Ericaceae (7%), Rosaceae (7%), Brassicaceae (6%), and Boraginaceae (4%). The “top five” visited genera are *Trifolium* spp. (16%), *Cirsium* spp. (6%), *Carduus* spp. (5%), *Brassica* spp. (5%), and *Centaurea* spp. (5%; Table

S2). The number of families and genera are highly correlated with ratios between relative range size of P1 and P2, and of P1 and P3 (Table 7). This suggests that the range of species with a more restricted diet is more regressing. More generally, species observed on fewer plant genera are less abundant and less widespread in all time-periods.

Delta-rank between P1 and P2 is significantly higher for species with short tongue length (mean of 4.88) than for species with medium and long tongues (ANOVA, df = 2, $p < 0.001$, followed by Tukey Contrasts: short-medium and short-long: $p < 0.01$, means of -6.33 and -9.5 respectively). We observe the same for delta-ranks between P1 and P3 (ANOVA, df = 2, $p < 0.001$, followed by Tukey Contrasts: short-medium $p < 0.001$ and short-long $p < 0.05$, means of 5.38 for short, -7.83 for medium, and -10 for long tongues). This suggests that species with longer tongue are more declining in their relative abundances than species with short tongue. We observe similar differences for the changes in relative range size between P1 and P2 (Kruskal-Wallis chi-squared = 6.14, df = 2, p-value < 0.05, medians: 0.81, 0.12 and 0.017 for short, medium and long tongues respectively), and between P1 and P3 (Kruskal-Wallis chi-squared = 9.70, df = 2, p-value < 0.01, medians: 0.45, 0.04 and 0 for short, medium and long tongues respectively).

4.4.3. Habitat specialization and preferences

The 9 woodland species have a significantly higher delta-rank between P1 and P2 (Welch Two Sample t-test, p-value < 0.0001, mean = 8.78) and between P1 and P3 (Welch Two Sample t-test, p-value = 0.0001, mean = 8.44) than the 19 species not

found in woodland (means of -4.53 and -3.80, respectively). Woodland species tend to increase in abundance while the others tend to decrease. We find the same for the relative changes in range size between P1 and P2 (Wilcoxon rank sum test, p-value < 0.01) and between P1 and P3 (Wilcoxon rank sum test, p-value < 0.05). Woodland species tend to increase their range more than others, which tend to be more restricted (except for *B. pascuorum*, *B. lapidarius*, *B. terrestris* and *B. hortorum*). Complementarily, we obtain the opposite for the 8 species living in open habitats, which have a significantly smaller delta-rank between P1 and P2 (Welch Two Sample t-test, p-value < 0.05, mean = -8.29) and between P1 and P3 (Welch Two Sample t-test, p-value < 0.05, mean = -9.20) than the 20 species not found in open lands (means of 3.16 and 3.42 respectively). We observe no difference in the distinction between species living and not living in edge habitats. The number of EU habitats is positively correlated with ratios between range size in P1 and P2, and in P1 and P3 (Table 7), suggesting that species associated to fewer habitats are more declining in their relative range size. Additionally, we notice that species with a more restricted number of habitats also have longer tongues and a more restricted diet (Table 7). Similarly to results about diet breadth, species living in a lower number of habitat types are less abundant in the three time-periods and less widespread in P2 and P3. Finally, species with higher number of habitat types present larger European area of occupancy.

4.4.4. Phenology

Flight season duration in P1 is not correlated to delta-ranks nor ratios in relative range size between each time-period two-by-two. However, it is negatively correlated to the species rank and positively correlated to the relative range size in P1 (Table 7). This means that species that were more abundant and more widespread had a longer flight period in P1, which could be a consequence of sampling bias. The month of first queens observation in P1 is negatively correlated to the ratios of relative range sizes between P1 and P2, and between P1 and P3, suggesting that species emerging earlier in P1 expanded more (or declined less) their relative range than species emerging later in the season. This variable is also positively correlated to the species rank and negatively correlated to the species relative range size in the three time-periods, indicating that species emerging later are less abundant and less widespread than others. Moreover, flight period length and first month of queens emergence are, respectively, positively and negatively correlated to the number of habitat types and of plant families and genera, suggesting that species with a longer flight period and emerging earlier are less specialized in their habitat and have a wider diet breadth.

4.4.5. Rarity

The delta-ranks between P1 and P2 and between P1 and P3 are positively correlated to the species rank-abundance in P1 (Table 7). This indicates that species with a higher rank-abundance (*i.e.* less abundant) in P1 have a higher delta-rank, so a tendency to increase between P1 and P2 or P1 and P3. Conversely, some

species that were among the most abundant in P1 were the most declining between P1 and P2. Similarly, delta-ranks between P1 and P2 are negatively correlated to the proportion of 5km grid-cells in P1, indicating that the more widespread the species in the past, the higher the decline in abundance between P1 and P2. In other words, the species rarity in the past does not necessarily imply a tendency to decline (except for the disappeared species).

However, delta-ranks are significantly different according to population trends at the European scale. The seven stable species at the European scale tend to show higher delta-ranks between P1 and P2 (mean of 6.29, ANOVA, df = 2, $p < 0.01$, followed by Tukey Contrasts: $p < 0.01$) and between P1 and P3 (mean of 7.29, ANOVA, df = 2, $p < 0.01$, followed by Tukey Contrasts: $p < 0.01$) than the 10 declining ones (means of -6.7 and -7.63, respectively), but the six increasing species do not show any difference. This suggests that species decreasing at the European scale also have a higher tendency to decline in Belgium. We observe the same with the changes in relative range size between P1 and P2, P1 and P3, and P2 and P3 (Kruskal-Wallis chi-squared of 14.59, 18.98, 9.08 and $p < 0.0001$, $p < 0.0001$, $p < 0.05$, respectively).

Table 7. Spearman rank-correlation coefficients. Only significant correlations with a $p < 0.05$ are shown, with bold numbers indicating a $p < 0.01$, from red (high negative correlation) to green (high positive correlation).

	ratio5km P2.P1	ratio5km P3.P1	ratio5km P3.P2	rank P1.P2	rank P1.P3	rank P2.P3	Rank P1	Rank P2	Rank P3	no 5km P1	no 5km P2	no 5km P3	max colony size	no plant fam	no plant gen	no habitat types	flight season P1	1st month Q P1	1st month M P1	EU EOO	EU AOO
ratio5km P2.P1		**0.88**		**0.52**				**-0.87**	**-0.73**		**0.95**	**0.83**	0.52	**0.86**	**0.77**	**0.68**		**-0.51**			
ratio5km P3.P1	**0.88**		**0.55**	0.45	0.45			**-0.83**	**-0.89**		**0.88**	**0.95**	0.56	**0.78**	**0.72**	**0.73**		**-0.53**			
ratio5km P3.P2		**0.55**				**0.71**			-0.44			**0.55**									
rank P1.P2	**0.52**	0.45			**0.92**		**0.63**	**-0.54**	-0.42	-0.48			**0.63**								
rank P1.P3		0.45		**0.92**			**0.69**		-0.47	-0.49			**0.61**								
rank P2.P3			**0.71**																		
Rank P1				**0.63**	**0.69**					**-0.87**	**-0.50**	**-0.50**			-0.48	-0.43	**-0.60**	0.43	**0.51**		
Rank P2	**-0.87**	**-0.83**		**-0.54**					**0.88**		**-0.94**	**-0.89**	-0.54	**-0.81**	**-0.68**	**-0.73**	-0.41	**0.57**			
Rank P3	**-0.73**	**-0.89**	-0.44	-0.42	-0.47			**0.88**			**-0.82**	**-0.95**	-0.51	**-0.79**	**-0.59**	**-0.71**		**0.59**			
no 5km P1				-0.48	-0.49		**-0.87**				**0.50**	**0.48**			0.45		**0.56**	-0.46	-0.48		
no 5km P2	**0.95**	**0.88**					**-0.50**	**-0.94**	**-0.82**	**0.50**		**0.91**		**0.84**	**0.79**	**0.76**	0.43	**-0.52**			
no 5km P3	**0.83**	**0.95**	**0.55**				**-0.50**	**-0.89**	**-0.95**	**0.48**	**0.91**			**0.77**	**0.74**	**0.80**	0.40	**-0.54**			0.57
max colony size	0.52	0.56		**0.63**	**0.61**			-0.54	-0.51											-0.75	
no plant fam	**0.86**	**0.78**						**-0.81**	**-0.79**		**0.84**	**0.77**			**0.78**	0.51	0.53	**-0.62**			
no plant gen	**0.77**	**0.72**					-0.48	**-0.68**	**-0.59**	0.45	**0.79**	**0.74**		**0.78**		0.56	**0.60**	-0.50			
no habitat types	**0.68**	**0.73**					-0.43	**-0.73**	**-0.71**		**0.76**	**0.80**		0.51	0.56		0.42	-0.47			**0.77**
flight season P1							**-0.60**	-0.41		**0.56**	0.43	0.40		0.53	**0.60**	0.42		**-0.71**			
1st month Q P1	**-0.51**	**-0.53**					0.43	**0.57**	**0.59**	-0.46	**-0.52**	**-0.54**		**-0.62**	-0.50	-0.47	**-0.71**				
1st month M P1							**0.51**			-0.48											
EU EOO													-0.75								**0.76**
EU AOO												0.57				**0.77**				**0.76**	

5. Discussion

5.1. Changes in Belgian bumblebee communities and populations

We show that bumblebees are experiencing a strong decline in their species richness, abundance and distribution since the last century in Belgium, especially between 1930 and 1990 (Tables 3 and 4). Species richness decreases from 28 to 24 species, with the total disappearance of *B. confusus*, *B. distinguendus*, *B. pomorum*, and *B. subterraneus*. Furthermore, the expected species richness in a 100 specimens sample decreases continually across the time-periods, from 22 before 1910 to only 10 species after 1990 (Table 2). This decline in richness and diversity is generalized in the country but its intensity depends on the regions, with the exception of some regions that remain relatively rich such as the south-east of Belgium (Fig. 4 and 5). Previous Belgian studies based on abundance trends estimated that, among the 28 species, 14 to 19 species were in regression between the time-periods 1900-1949 and 1950-1987 (Rasmont & Mersch 1988) or 1950-1992 (Rasmont *et al.* 1993). Applying the same methodology than Rasmont *et al.* 1993 to our abundance data, we find 20 regressing, one stable, and seven increasing species between 1910-1930 and 1970-1989, 19 regressing, one stable and eight increasing species between 1910-1930 and 1990-2016, and 16 regressing, three stable, and seven increasing species between 1970-1989 and 1990-2016 (Table 3). Like in Sweden (Bommarco *et al.* 2012), this decrease in species richness goes along with a decrease in evenness and shifts in communities' composition (Tables 4 and 6). While the dominant species (*i.e. B. pascuorum*) remains the same in the three time-periods

and increases from 33% in 1910-1930 to 42% in 1990-2016, relative abundance and relative range size of several species change significantly. Many species once abundant and widespread dramatically decrease between 1930 and 1990 (*B. barbutellus, B. distinguendus*, *B. humilis, B. muscorum, B. ruderarius*, *B. ruderatus, B. subterraneus*, *B. vestalis*, *B. veteranus*), while some others increase in their relative abundance (*B. bohemicus*, *B. hypnorum, B. lucorum, B. norvegicus, B. pratorum, B. terrestris*, table 4) or their relative range (*B. hypnorum*, *B. lapidarius*, *B. pascuorum*, *B. pratorum*, table 6). Species trends based on their abundances (Table 3) differ sometimes from trends based on their range size (Table 5). Between 1930 and 1990, only four species present an increasing trend in both abundance and range size (*B. hypnorum, B. lapidarius, B. pascuorum*, and *B. pratorum*), against 18 species with a declining trend (*B. barbutellus, B. campestris, B. confusus, B. cryptarum, B. distinguendus, B. humilis, B. magnus, B. muscorum, B. pomorum, B. ruderarius, B. ruderatus, B. rupestris, B. soroeensis, B. subterraneus, B. sylvarum, B. sylvestris, B. vestalis*, and *B. veteranus*). However, some species with a decreasing or a stable trend in their range size have an increasing trend in their abundances (*B. bohemicus*, *B. lucorum, B. norvegicus, B. terrestris*). On the contrary, *B. hortorum* has a stable range size between 1910-1930 and 1990-2016 but presents a continuous decrease in its relative abundance, meaning that even if it is still among the six most widespread species (Fig. S3c), it is less abundant than in the past (Table 3). However, its range size shows a regressing trend between 1970-1989 and 1990-2016. A decline in abundance followed by a range contraction could indicate a potential extinction debt, *i.e*. the future extinction of species due to events (*e.g*.

habitat destruction) that occurred in the past (Tilman *et al*. 1994; Kuussaari *et al*. 2009). This time-delayed but deterministic extinction can also affect dominant species (Tilman *et al*. 1994). The phenomenon of extinction debt has already been suggested for pollinators in south-eastern Sweden (Bommarco *et al*. 2014) and the Netherlands (Aguirre-Gutiérrez *et al*. 2015), where historical modifications of landscape better explain current species distribution and the recent changes in species richness than current landscape. Therefore, even if several species are still persisting today, sometimes in very few local populations (*e.g. B. barbutellus, B. humilis, B. muscorum, B. ruderatus, B. sylvarum, B. veteranus*), they could still become extinct due to past drivers that weakened their populations, even if these impacts have ceased or regressed today.

Similar trends are observed in other countries, such as the UK (Edwards & Williams 2004; Williams 2005; Fitzpatrick *et al.* 2007), Poland (Kosior *et al.* 2008), Hungary (Sárospataki *et al.* 2005), and Denmark (Dupont *et al.* 2011). At the European scale, four species observed in Belgium are categorized as "vulnerable" by the IUCN (Nieto *et al*. 2014; Rasmont *et al.* 2015b): *B. confusus*, *B. distinguendus* (which is "near threatened" in Sweden and "critically endangered" in Denmark; Dupont *et al.* 2011; Bommarco *et al.* 2012), *B. pomorum*, and *B. muscorum*. The first three disappeared in Belgium and *B. muscorum* decreased drastically from 1398 observations in 1910-1930 to only 5 observations in 1990-2016. The other species present in Belgium are categorized as "least concerned" at the European scale, but their European population trends are relatively similar to those observed in Belgium. The eight species with a decreasing trend in Europe also have a decreasing

trend in Belgium (*B. barbutellus, B. humilis, B. ruderarius, B. ruderatus, B. soroeensis, B. sylvarum, B. veteranus*) or even disappeared (*B. subterraneus*). However, among the six species with an increasing trend in Europe, only three present a clear increasing trend in Belgium across all time-periods (*B. hypnorum, B. lapidarius, B. pascuorum*). *B. hypnorum* tends also to increase in Denmark (Dupont *et al.* 2011) and Hungary (Sárospataki *et al.* 2005), has completely colonized the UK and, with *B. pascuorum*, has now reached Iceland (Goulson & Williams 2001; Prŷs-Jones *et al.* 2016). *B. pratorum* and *B. terrestris* have a tendency to increase since 1930 but a decreasing trend between 1970 and 1990 (Tables 3 and 5), the latter also slightly decreasing in Denmark (Dupont *et al.* 2011) and Britain (Williams 2005). *B. vestalis* shows a clear declining trend (Tables 3 and 5), whereas it is expanding in Scandinavia from the south (Andersson 1992; Pekkarinen & Teräs 1993). Among the seven species with a stable trend in Europe, only *B. norvegicus* present a stable range size in Belgium, whereas four species tend to decrease (*B. bohemicus, B. hortorum, B. lucorum*, *B. sylvestris*) and two to increase (*B. campestris* and *B. jonellus*) in both range size and abundances between 1970 and 1990 (Tables 3 and 5). *B. hortorum* tends also to slightly decrease in Denmark (Dupont *et al.* 2011), Britain (Williams 2005; Fitzpatrick *et al.* 2007), and Sweden (Bommarco *et al.* 2012).

Bumblebees are not the only taxonomic group strongly declining in Belgium. Other pollinator groups are also regressing since 1950, especially butterflies and other bee species (Carvalheiro *et al*. 2013). The butterfly diversity loss in Flanders (north Belgium) is among the worst in Europe (Maes & Van Dyck 2001). Among the

68 butterfly species living in this region, 20 disappeared between 2001 and 2010, and 25 were categorized from "near threatened" to "critically endangered" according to the IUCN Red List criteria (Maes *et al*. 2012). Similar results were found in Wallonia (south Belgium): 18 out of the 101 evaluated species disappeared between 1985 and 2007, and 41 were classified from "near threatened" to "critically endangered" (Fichefet *et al*. 2008). A global pollinator crisis seems therefore to affect Belgium. This could directly threaten the ecosystem service of pollination in this country, which represented a value of 251.6 million euros in 2010 according to Jacquemin *et al*. (2017).

5.2. Relation between species ecological traits and population changes

As previous studies (Goulson & Darvill 2004; Goulson *et al.* 2005; Fitzpatrick *et al.* 2007; Williams *et al.* 2009; Dupont *et al.* 2011; De Palma *et al.* 2015), our results show that long-tongued species with higher specialization in their habitat and their diet are the most threatened. As Kleijn & Raemakers (2008) and Williams *et al.* 2007, we find positive correlations between species diet breadth and abundance. In general, there appears to be a negative relationship between diet breadth and tongue length (Goulson *et al.* 2005, 2008b). Species with long tongue tend to get the great majority of their pollen from Fabaceae (Rasmont 1988; Rasmont *et al.* 2005; Goulson *et al.* 2008b), and they are all declining (*B. hortorum, B. humilis, B. muscorum, B. ruderarius, B. ruderatus, B. sylvarum,* and *B. veteranus*) or even vanished (*B. confusus*, *B. distinguendus*, *B. pomorum*, and *B. subterraneus*, table 3). The long-tongued species specialized on Fabaceae tend to be associated with open

lands, more precisely unimproved grasslands (*e.g.* calcareous grasslands and hay meadows; Rasmont & Mersch 1988, Goulson 2010). We find indeed that species living in open habitats are more prone to decline than species preferring forests, as in previous studies (Rasmont & Mersch 1988).

Like in the UK (Goulson *et al.* 2005; Fitzpatrick *et al.* 2007; Williams *et al.* 2009; Dupont *et al.* 2011), our results show a correlation between decline and queens emergence time, with the declining species tending to emerge later. Species with later emergence time tend to have queens more specialized on Fabaceae (Goulson 2010) and males more observed on thistles (Vray *et al.* 2017), which bloom later in the season. Moreover, species which develop their colony later tend to produce males when summers' heat waves occur (Iserbyt & Rasmont 2012; Rasmont & Iserbyt 2012), which are very damaging for male bumblebees (Martinet *et al.* 2015). In addition, we show that other correlates of species decline are a smaller colony size and a tendency to nest above the ground (*i.e.* carder species). Species which emerge later tend to have smaller colonies, and therefore smaller productivity of males and new queens (Goulson 2010; von Hagen & Aichhorn 2014). These could have smaller effective population size and would therefore be more vulnerable to environmental perturbations (Williams & Osborne 2009).

Our results do not show any significant difference in decline between cuckoo bumblebees (*i.e.* inquiline species) and free-living bumblebees based on delta-ranks and ratios of range size. However, trends between 1930 and 1990 show that five of the seven inquiline species are declining in their abundances (*B. barbutellus, B. campestris, B. rupestris, B. sylvestris, B. vestalis*), and that, except for *B. norvegicus*,

all of them are regressing in their range size. Cuckoo species depend on their host species for their reproduction and should therefore be vulnerable to changes in their host abundance, making them more prone to decline. Three cuckoo species follow the same trends than their host species: one declining (*B. barbutellus* hosted by *B. hortorum*), one increasing (*B. norvegicus* hosted by *B. hypnorum*), and one increasing in abundances but decreasing in range size (*B. bohemicus* hosted by *B. lucorum*; Løken 1984). Similarly, *B. campestris* is decreasing and is hosted by two declining species (*B. humilis* and *B. pomorum*), but also by two abundant species (*B. pascuorum* and *B. pratorum*; Løken 1984). Like in other studies (*e.g.* Kosior *et al.* 2008), we show that several species do not follow the same trends than their hosts. Three species tend to decrease since 1930 whereas their host species remain among the most abundant and widespread (*B. rupestris* hosted by *B. lapidarius*, *B. sylvestris* hosted by *B. pratorum*, and *B. vestalis* hosted by *B. terrestris*; Løken 1984). This could suggest that other causes than their host population trends could act on cuckoo species vulnerability. Indeed, these species tend to have smaller climatic niche breadth than their hosts (Rasmont *et al.* 2015a) and their males are mainly foraging on thistles targeted by national regulations (Vray *et al.* 2017).

5.3. Causes of the bumblebees' decline in Belgium

As mentioned earlier, the overall decline of bumblebees could be explained by a reduction in floral resources, habitat loss and fragmentation caused by agricultural intensification (Williams 1986; Rasmont & Mersch 1988; Goulson *et al.* 2005; Kosior *et al.* 2007; Williams & Osborne 2009), coupled with climate change

(Rasmont *et al.* 2015a). The development of agricultural mechanisation and chemical fertilizers in the 1950s, as well as the Mansholt Plan in the 1970s, led to the transformation of a traditional countryside of small mixed crops shaped by hedges and trees toward large monocultures, intensive pastures and hayfields in early mowing (Christians 1998; Mazoyer & Roudart 2006). These modifications greatly simplified agricultural landscapes and reduced nest sites availability for bumblebees, which nest in tufts of grass above the ground (*i.e*. carder species) or in old rodents' burrows (*i.e*. renter species), more abundant in hedges (Alford 1975; Goulson 2010; Goulson *et al.* 2010). Moreover, the mainstreaming of nitrogenous chemical fertilisers led to the replacement of leguminous (Fabaceae) crops, known to be very important for bumblebee's diet, by other crops (*e.g*. sugar beet, corn) that do not constitute a food resource for these species (Rasmont 2007). In Belgium, the area covered by forage leguminous crops decreased from 164 000 hectares before 1910 to 45 000 in 1950, 13 000 in 1970, 2 300 in 1990 and 3 000 hectares in 2014 (Rasmont & Mersch 1988; Belgian Federal Government 2017). This drastic reduction of leguminous crops, and especially clover (*i.e. Trifolium* spp.) crops, could be sufficient to explain the regression of most bumblebee species (Rasmont & Mersch 1988; Goulson & Darvill 2004; Goulson *et al.* 2005; Rasmont *et al.* 2005; Carvell *et al.* 2006). Indeed, Fabaceae represent the second most visited plant family by bumblebees in our database (23% of the 33 382 bumblebee observations), with the most visited genera being *Trifolium* spp. (Table S2). Asteraceae is the first plant family and account for 26% of all observations. More precisely, the two most visited genera are *Cirsium* spp. and *Carduus* spp., which

account for more than 11% of all the bumblebee observations. The importance of these thistle species in the bumblebee diet has been demonstrated by Vray *et al.* (2017), who argue that legislations requiring the destruction of these plants, such as the one existing in Belgium since 1987, could greatly threaten bumblebees and probably other wild bees (A. Pauly pers. comm., 2017).

5.4. Agri-environmental schemes and conservation measure recommendations

Starting in the 1990s, conservation measures have been developed to counter the pollinators' decline and avoid a major disruption in pollination service through *e.g.* agri-environmental schemes (Kleijn & Sutherland 2003; Scheper *et al.* 2013; Batáry *et al.* 2015), by for example mitigating the current reduction in floral resources (Pywell *et al.* 2006; Carvell *et al.* 2007; Terzo & Rasmont 2007; Batáry *et al.* 2010). Our analyses show a reduced number of species declining between 1970-1989 and 1990-2016, compared to the trends between 1910-1930 and 1970-1989 or 1910-1930 and 1990-2016 (Tables 3 and 5). Seven species switch from a decreasing trend between 1910-1930 and 1970-1989 to a stable (*B. ruderatus, B. soroeensis, B. vestalis*) or even an increasing trend (*B. campestris, B. jonellus, B. magnus, B. rupestris*) between 1970-1989 and 1990-2016 (Tables 3 and 5). Even if these results could come from higher and more widespread sampling with the development of citizen sciences in the recent time-period (Dufrêne *et al.* 1992; Isaac *et al.* 2014; Maes *et al.* 2015), conservation measures more and more widespread across the country could also partly explain this phenomenon (Terzo

& Rasmont 2007; Carvalheiro *et al*. 2013). Indeed, measures such as sowing bee-friendly plants on arable field margins appear to be favourable to bumblebees and bees in general (Kells *et al.* 2001; Croxton *et al.* 2002; Carvell *et al.* 2004, 2007; Pywell *et al.* 2006; Terzo & Rasmont 2007). The composition of flower mixes sown should take into account the various preferences of each caste and a continuous bloom until late in the season, with a high proportion of Fabaceae (especially *Trifolium* spp.) for queens and workers and Asteraceae (especially *Carduus* spp., *Cirsium* spp., and *Centaurea* spp.) for males (Carvell *et al.* 2007; Terzo & Rasmont 2007; Vray *et al.* 2017). As already stated, legislations requiring the destruction of thistles (*i.e. Carduus* and *Cirsium* spp.) should be reviewed (Vray *et al.* 2017). Moreover, roadsides and meadows in late mowing, as well as pastures with extensive cattle-grazing could provide important foraging habitats for bumblebees during the most part of their flight period (Carvell 2002; Osgathorpe *et al.* 2011). Mowing of the sown areas, roadsides or meadows should not occur before the end of the cycle of bumblebee colonies (*i.e.* September). In addition to the improvement of floral resources availability, nesting sites could be provided by withered grass and tussocks found in hedges, banks and field margins in late mowing (Svensson *et al.* 2000; Croxton *et al.* 2002; Kells & Goulson 2003).

5.5. Caveat and recommendations for future research

The greatest difficulty in comparing species assemblages between several time periods is to manage sampling bias (Carvalheiro *et al*. 2013; Van Strien *et al.* 2013; Isaac & Pocock 2015; Maes *et al.* 2015). As sampling was performed by different

recorders across space and time and mostly without any standardized protocol, several biases could blur the results and trends observed. Sampling effort was very different between time-periods, from an intense sampling restricted to selected places in 1910-1930 to more extensive and opportunistic volunteer-collected data spread over more visits and more places across the country in 1990-2016 (Fig. 1 and 3). Therefore, we always considered relative abundance and relative number of grid-cells of each species in each time-period, as well as the rank methodology, in order to level these differences in sampling effort between time-periods. However, some biases could still remain, such as those coming from the uneven sampling over space. Sampling does not always cover the same regions in each time-period (Fig. 3). Species abundant in the regions sampled in the earlier time-periods but not in the most recent may therefore appear to decrease, and *vice versa*. For instance, *B. jonellus* (Fig. S4b) and *B. magnus* (Fig. S5a) are more abundant in the north-east of the country, which was more sampled in P3 than in P1 and especially in P2 (Fig. 3), and present decreasing trends between P1 and P2 but increasing trends between P2 and P3 (Tables 3 and 5). Moreover, the higher number of observations of rare species in the recent time-period could come from the greater attraction of volunteer recorders for rare species and for places especially rich in biodiversity (Isaac & Pocock 2015).

Recommendations for future research would be, first, to better manage sampling bias in the trends assessment (Van Strien *et al.* 2013; Maes *et al.* 2015), and second, to estimate the reciprocal roles of the various factors of decline in the observed trends. Very few studies investigate the several drivers of bumblebee

decline in the same analysis (but see Goulson *et al.* 2015; Aguirre-Gutiérrez *et al.* 2016), while they are highly likely to act in synergy (Brook *et al.* 2008; Tylianakis *et al.* 2008; Schweiger *et al.* 2010; Potts *et al.* 2010; Oliver & Morecroft 2014; Schleuning *et al.* 2016).

6. Conclusion

Based on an original data set of 173 716 specimens, we found a huge proportion of species declining in both abundances and range size during the last century in Belgium. The four dominant species remain the same across time-periods and even increase their dominance, leading to community homogenization in most Belgian regions. However, the other species that were once very abundant and widespread are drastically declining, while some others are increasing, leading to drastic shifts in community composition. Species with a declining trend tend to have longer tongue, narrower diet, higher habitat specialization with a preference for open habitats and above-ground nesting, and tend to emerge later in the season and to produce smaller colonies. The flora and landscape homogenization, as well as the lack of Fabaceae and plants flowering in late season such as thistles, may be among the most important causes explaining the observed trends.

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9. Supporting information

Table S1. Temporal structure of the dataset. One sample is one 5x5km grid-cell sampled by one recorder during one day. "<1910" is the period from 1810 to 1909. RBINS is the Royal Belgian Institute of Natural Sciences (Brussels). Spatial distributions of the 5x5 km and 10x10 km grid-cells are visible on fig. 3.

<table>
<tr><td></td><td><1910</td><td>P1
(1910-1930)</td><td>1931-1969</td><td>P2
(1970-1989)</td><td>P3
(1990-2016)</td></tr>
<tr><td>Number of samples</td><td>829</td><td>3227</td><td>1039</td><td>9108</td><td>21972</td></tr>
<tr><td>Number of recorders</td><td>14</td><td>40</td><td>13</td><td>587</td><td>1647</td></tr>
<tr><td>Number of sampling days</td><td>673</td><td>1261</td><td>826</td><td>2789</td><td>3425</td></tr>
<tr><td>Number of specimens</td><td>1629</td><td>60498</td><td>3409</td><td>23854</td><td>89364</td></tr>
<tr><td>Number of 5x5km grid-cells</td><td>135</td><td>261</td><td>250</td><td>695</td><td>1151</td></tr>
<tr><td>Number of 10x10km grid-cells</td><td>96</td><td>156</td><td>159</td><td>273</td><td>360</td></tr>
<tr><td rowspan="3">Main origin of the data</td><td colspan="3" rowspan="2">Ball's collection (RBINS), including De Moffart's and Tosquinet's collections</td><td colspan="2">University of Liège-Gembloux Agro Bio Tech</td></tr>
<tr><td>Rasmont (1988)</td><td rowspan="2">University of Mons; NGOs (Natuurpunt, Natagora)</td></tr>
<tr><td colspan="2"></td><td colspan="2">Leclercq et al. 1980</td></tr>
</table>

Table S2. Number of specimens, observed and expected number of plant families and of plant genera (in a subsample of 70 specimens), and families and genera most recorded per species. Species with red numbers have less than 70 specimens and therefore do not have expected plant numbers.

	Plant families				Plant genera			
Species	Specimens	Number	Expected number (70)	Most recorded	Specimens	Number	Expected number (70)	Most recorded
B. barbutellus	29	3		Asteraceae	15	6		*Trifolium, Cirsium*
B. bohemicus	432	15	7.91	Asteraceae	430	31	14.15	*Centaurea, Cirsium*
B. campestris	260	14	8.07	Asteraceae	154	32	20.31	*Cirsium, Centaurea*
B. confusus	2	2		Asteraceae, Dipsacaceae	2	2		*Cirsium, Knautia*
B. cryptarum	623	17	9.73	Ericaceae	619	34	15.50	*Brassica, Calluna*
B. distinguendus	18	4		Asteraceae	18	5		*Carduus*
B. hortorum	2113	41	12.87	Fabaceae	2052	118	20.60	*Trifolium*
B. humilis	22	5		Lamiaceae	22	8		*Trifolium, Echium*
B. hypnorum	1110	50	19.21	Rosaceae	1109	136	32.39	*Rubus*
B. jonellus	124	10	8.30	Ericaceae	124	15	12.47	*Erica, Calluna*
B. lapidarius	6355	47	12.28	Asteraceae	4942	184	27.66	*Centaurea, Trifolium*
B. lucorum	2724	39	11.20	Ericaceae	2720	107	19.81	*Calluna, Brassica*
B. magnus	133	6	4.48	Ericaceae	133	11	8.61	*Calluna, Erica*
B. muscorum	163	6	5.05	Asteraceae	151	15	9.35	*Carduus*
B. norvegicus	15	4		Asteraceae	15	6		*Cirsium, Centaurea*
B. pascuorum	10209	65	14.50	Fabaceae	9902	238	31.12	*Trifolium*
B. pomorum	6	3		Fabaceae	6	5		*Lotus*
B. pratorum	2631	60	18.20	Rosaceae	2609	177	33.92	*Rubus, Ribes*
B. ruderarius	183	10	8.24	Fabaceae	179	32	22.37	*Trifolium*
B. ruderatus	242	6	2.65	Asteraceae	8	7		*Cirsium*
B. rupestris	96	7	6.64	Asteraceae	93	16	14.32	*Trifolium, Origanum, Cirsium*
B. soroeensis	172	9	7.99	Asteraceae	116	20	16.21	*Cirsium, Centaurea*
B. subterraneus	1	1		Asteraceae	1	1		*Centaurea*
B. sylvarum	70	10	10.00	Lamiaceae	70	23	23.00	*Trifolium, Origanum*
B. sylvestris	219	20	12.74	Asteraceae	215	38	21.05	*Centaurea*
B. terrestris	3340	51	18.39	Fabaceae	3209	170	31.15	*Brassica, Trifolium*
B. vestalis	102	11	3.86	Lamiaceae	102	27	22.56	*Origanum*
B. veteranus	1544	5	1.62	Asteraceae	1542	13	2.05	*Carduus*

Table S3. Species abundance trends estimated with the Stroot & Depiereux (1989) method based on queens and males data only. Trends with "()" are for species with an expected estimator < 5 in the second time-period. "+", "-", "=" indicate increasing, decreasing and stable trends, respectively. Species are ordered by their abundance in P1. Black cells are disappeared species.

	Observed			Expected			Trend	Observed			Expected			Trend	Observed			Expected			Trend
Species	P1	P2	Total	P1	P2	Chi²	1-2	P2	P3	Total	P2	P3	Chi²	2-3	P1	P3	Total	P1	P3	Chi²	1-3
pascuorum	9103	4505	13608	9189	4419	2.47	=	4505	5191	9696	4614	5082	4.89	+	9103	5191	14294	9344	4950	17.93	+
lapidarius	4542	1039	5581	3769	1812	488.78	-	1039	2903	3942	1876	2066	712.14	+	4542	2903	7445	4867	2578	62.57	+
hortorum	3166	877	4043	2730	1313	214.38	-	877	530	1407	670	737	122.68	-	3166	530	3696	2416	1280	672.2	-
pratorum	1833	3478	5311	3586	1725	2639.37	+	3478	1714	5192	2471	2721	783.75	-	1833	1714	3547	2319	1228	293.73	+
ruderatus	1705	3	1708	1153	555	812.57	-	3	7	10	5	5	1.24	=	1705	7	1712	1119	593	885.67	-
veteranus	1520	9	1529	1032	497	708.93	-	9	9	18	9	9	0.04	=	1520	9	1529	999	530	782.71	-
terrestris	1243	1721	2964	2001	963	885.02	+	1721	3463	5184	2467	2717	430.13	+	1243	3463	4706	3076	1630	3154.8	+
campestris	1211	65	1276	862	414	436.25	-	65	198	263	125	138	55.15	+	1211	198	1409	921	488	263.56	-
sylvestris	914	336	1250	844	406	17.84	-	336	290	626	298	328	9.31	-	914	290	1204	787	417	59.13	-
vestalis	729	43	772	521	251	254.84	-	43	139	182	87	95	41.88	+	729	139	868	567	301	132.89	-
ruderarius	613	138	751	507	244	68.08	-	138	51	189	90	99	49.01	-	613	51	664	434	230	213.03	-
rupestris	597	15	612	413	199	251.58	-	15	123	138	66	72	74.58	+	597	123	720	471	249	97.93	-
barbutellus	573	13	586	396	190	244.64	-	13	10	23	11	12	0.74	=	573	10	583	381	202	279.02	-
lucorum	561	1800	2361	1594	767	2062.13	+	1800	781	2581	1228	1353	508	-	561	781	1342	877	465	329.22	+
cryptarum	509	556	1065	719	346	189.09	+	556	118	674	321	353	329.31	-	509	118	627	410	217	69.24	-
muscorum	483	7	490	331	159	215.38	-	7	4	11	5	6	1.14	=	483	4	487	318	169	245.9	-
magnus	475	7	482	325	157	211.54	-	7	180	187	89	98	144.1	+	475	180	655	428	227	14.79	-
hypnorum	470	546	1016	686	330	209.52	+	546	670	1216	579	637	3.51	=	470	670	1140	745	395	293.49	+
sylvarum	386	23	409	276	133	134.48	-	23	33	56	27	29	0.95	=	386	33	419	274	145	132.49	-
distinguendus	340	3	343	232	111	156.19	-	3	0	3	1	2	3.3	(=)	340	0	340	222	118	180.12	-
humilis	285	6	291	196	95	122.74	-	6	8	14	7	7	0.13	=	285	8	293	192	101	131.71	-
bohemicus	198	203	401	271	130	60.23	+	203	442	645	307	338	67.13	+	198	442	640	418	222	335.17	+
soroeensis	198	16	214	145	69	60.98	-	16	54	70	33	37	17.16	+	198	54	252	165	87	19.4	-
subterraneus	194	11	205	138	67	68.7	-	11	0	11	5	6	12.12	-	194	0	194	127	67	102.78	-
jonellus	122	13	135	91	44	32.13	-	13	88	101	48	53	48.79	+	122	88	210	137	73	4.91	+
pomorum	115	0	115	78	37	55.31	-	0	0	0	/	/	/	/	115	0	115	75	40	60.92	-
confusus	56	0	56	38	18	26.93	-	0	0	0	/	/	/	/	56	0	56	37	19	29.67	-
norvegicus	7	28	35	24	11	36.05	+	28	25	53	25	28	0.58	=	7	25	32	21	11	26.74	+
Total	32148	15461	47609					15461	17031	32492					32148	17031	49179				

Table S4. Species abundance, proportional abundance (species abundance / total abundance), and rank-abundance for the three time-periods, based on queens and males data only. Delta-rank are the difference between ranks of each time-period. P1 = 1910-1930; P2 = 1970-1989; P3 = 1990-2016. Species are ordered by their abundance in P1. Black cells are disappeared species.

	Abundance			Proportion (%)			Rank-abundance			Delta-rank		
Species	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1-P2	P2-P3	P1-P3
pascuorum	9103	4505	5191	28.32	29.14	30.48	1	1	1	0	0	0
lapidarius	4542	1039	2903	14.13	6.72	17.05	2	5	3	-3	2	-1
hortorum	3166	877	530	9.85	5.67	3.11	3	6	7	-3	-1	-4
pratorum	1833	3478	1714	5.7	22.5	10.06	4	2	4	2	-2	0
ruderatus	1705	3	7	5.3	0.02	0.04	5	26	23	-21	3	-18
veteranus	1520	9	9	4.73	0.06	0.05	6	21	21	-15	0	-15
terrestris	1243	1721	3463	3.87	11.13	20.33	7	4	2	3	2	5
campestris	1211	65	198	3.77	0.42	1.16	8	12	10	-4	2	-2
sylvestris	914	336	290	2.84	2.17	1.7	9	9	9	0	0	0
vestalis	729	43	139	2.27	0.28	0.82	10	13	12	-3	1	-2
ruderarius	613	138	51	1.91	0.89	0.3	11	11	17	0	-6	-6
rupestris	597	15	123	1.86	0.1	0.72	12	17	13	-5	4	-1
barbutellus	573	13	10	1.78	0.08	0.06	13	18	20	-5	-2	-7
lucorum	561	1800	781	1.75	11.64	4.59	14	3	5	11	-2	9
cryptarum	509	556	118	1.58	3.6	0.69	15	7	14	8	-7	1
muscorum	483	7	4	1.5	0.05	0.02	16	23	24	-7	-1	-8
magnus	475	7	180	1.48	0.05	1.06	17	22	11	-5	11	6
hypnorum	470	546	670	1.46	3.53	3.93	18	8	6	10	2	12
sylvarum	386	23	33	1.2	0.15	0.19	19	15	18	4	-3	1
distinguendus	340	3	0	1.06	0.02	0	20	25	/	-5	/	/
humilis	285	6	8	0.89	0.04	0.05	21	24	22	-3	2	-1
bohemicus	198	203	442	0.62	1.31	2.6	22	10	8	12	2	14
soroeensis	198	16	54	0.62	0.1	0.32	23	16	16	7	0	7
subterraneus	194	11	0	0.6	0.07	0	24	20	/	4	/	/
jonellus	122	13	88	0.38	0.08	0.52	25	19	15	6	4	10
pomorum	115	0	0	0.36	0	0	26	/	/	/	/	/
confusus	56	0	0	0.17	0	0	27	/	/	/	/	/
norvegicus	7	28	25	0.02	0.18	0.15	28	14	19	14	-5	9
Total	32148	15461	17031									

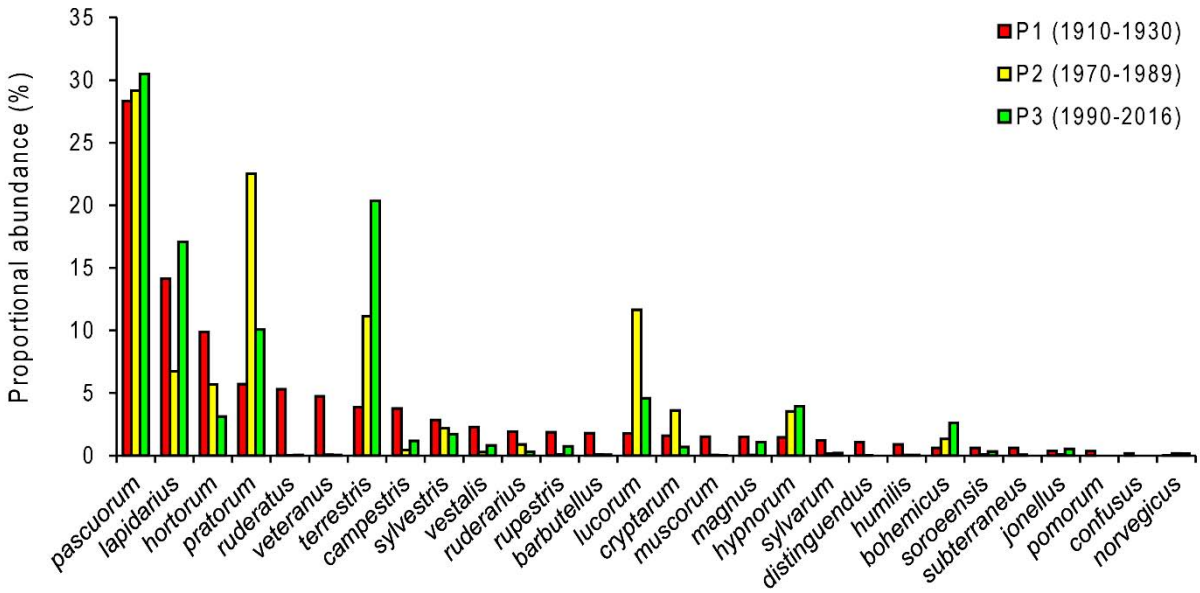

Figure S1. Species proportional abundance (species abundance / total abundance) in each time-period, ordered by species ranks in P1 (1910-1930), based on queens and males data only.

Table S5. Species abundance trends estimated with the Stroot & Depiereux (1989) method compared to the trends estimated by Rasmont *et al.* 1993 with the same method between time-periods *a* (1900-1949) and *b* (1950-1992). "-" and "+" are negative and positive significant trends at the p-value of * 0.05 (Chi^2 = 3.8), ** 0.01 (Chi^2 = 6.6), and *** 0.001 (Chi^2 = 10.8). Trends with "()" are for species with an expected estimator < 5 in the second time-period. Results for species with [(a)] could be biased by the misidentification of workers. "n. c." = not calculated. Species are ordered by their abundance in *a*. Black cells are disappeared species.

Species	Observed			Expected		Chi^2	Trend *a-b*	Trend in Rasmont *et al.* 1993
	a	*b*	Total	*a*	*b*			
pascuorum	20020	9243	29263	20058	9205	0.23	=	+ **
lapidarius	10288	2526	12814	8783	4031	819.39	- ***	- ***
hortorum	5519	1322	6841	4689	2152	466.85	- ***	- ***
pratorum	4108	5709	9817	6729	3088	3245.82	+ ***	+ ***
veteranus	3708	25	3733	2559	1174	1640.86	- ***	- ***
ruderatus	2851	8	2859	1960	899	1288.72	- ***	- ***
ruderarius	1456	313	1769	1213	556	155.37	- ***	- ***
muscorum	1423	14	1437	985	452	619.21	- ***	- ***
campestris	1338	119	1457	999	458	366.46	- ***	- ***
terrestris[(a)]	1305	2519	3824	2621	1203	2101.1	+ ***	n. c.
distinguendus	1210	4	1214	832	382	545.48	- ***	- ***
hypnorum	1053	1347	2400	1645	755	677.48	+ ***	+ ***
sylvestris	924	465	1389	952	437	2.64	=	+ ***
humilis	841	17	858	588	270	345.69	- ***	- ***
vestalis	767	84	851	583	268	183.88	- ***	- ***
rupestris	689	26	715	490	225	256.63	- ***	- ***
lucorum[(a)]	608	2904	3512	2407	1105	4275.59	+ ***	n. c.
barbutellus	604	21	625	428	197	228.8	- ***	- ***
sylvarum	579	33	612	419	193	192.81	- ***	- ***
cryptarum[(a)]	517	1154	1671	1145	526	1096.03	+ ***	n. c.
magnus[(a)]	478	43	521	357	164	130.08	- ***	n. c.
soroeensis	385	80	465	319	146	43.8	- ***	- ***
subterraneus	316	15	331	227	104	111.28	- ***	- ***
jonellus	287	32	319	219	100	67.9	- ***	- ***
bohemicus	206	284	490	336	154	159.65	+ ***	+ ***
pomorum	179	0	179	123	56	82.14	- ***	- ***
confusus	92	1	93	64	29	39.81	- ***	- ***
norvegicus	7	32	39	27	12	46.31	+ ***	(+)
Total	61758	28340	90098	61758	28340			

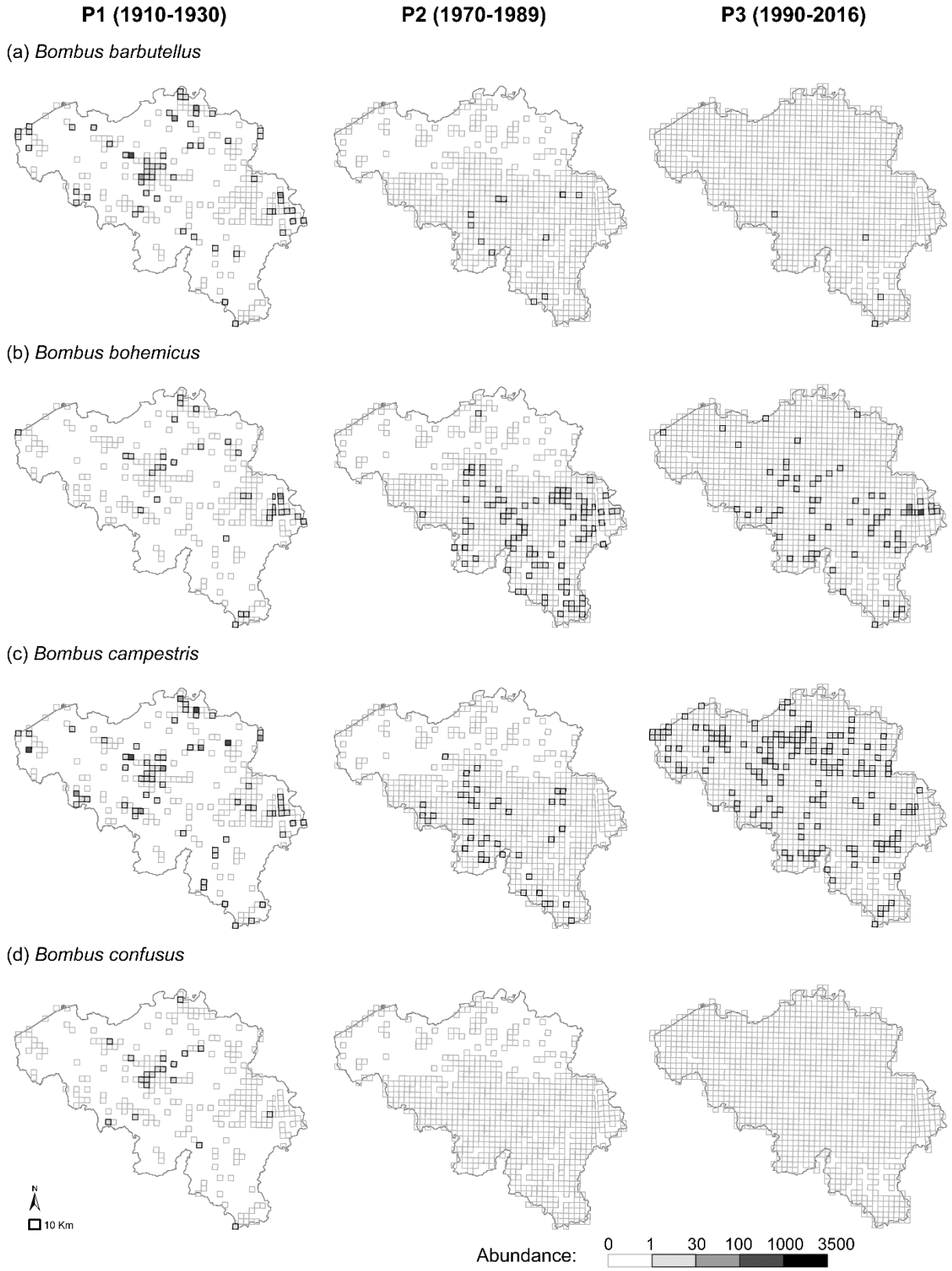

Figure S2. Distribution maps of (a) *B. barbutellus,* (b) *B. bohemicus,* (c) *B. campestris*, and (d) *B. confusus* in each time-period. Squares framed in grey are the 5x5 km grid-cells (UTM) sampled during the time-period. Squares framed in black are the grid-cells where the species was recorded.

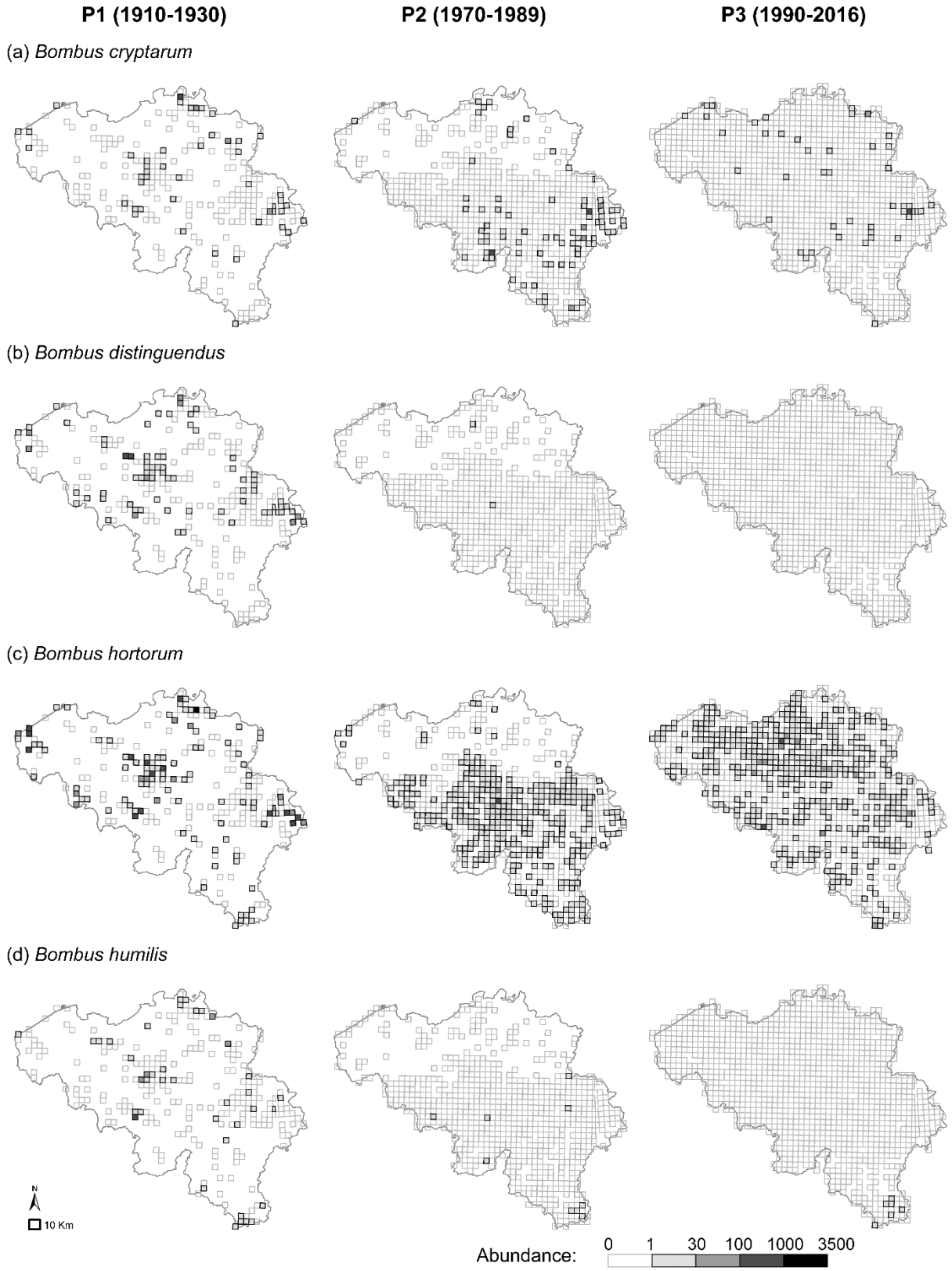

Figure S3. Distribution maps of (a) *B. cryptarum,* (b) *B. distinguendus,* (c) *B. hortorum*, and (d) *B. humilis* in each time-period. Squares framed in grey are the 5x5 km grid-cells (UTM) sampled during the time-period. Squares framed in black are the grid-cells where the species was recorded.

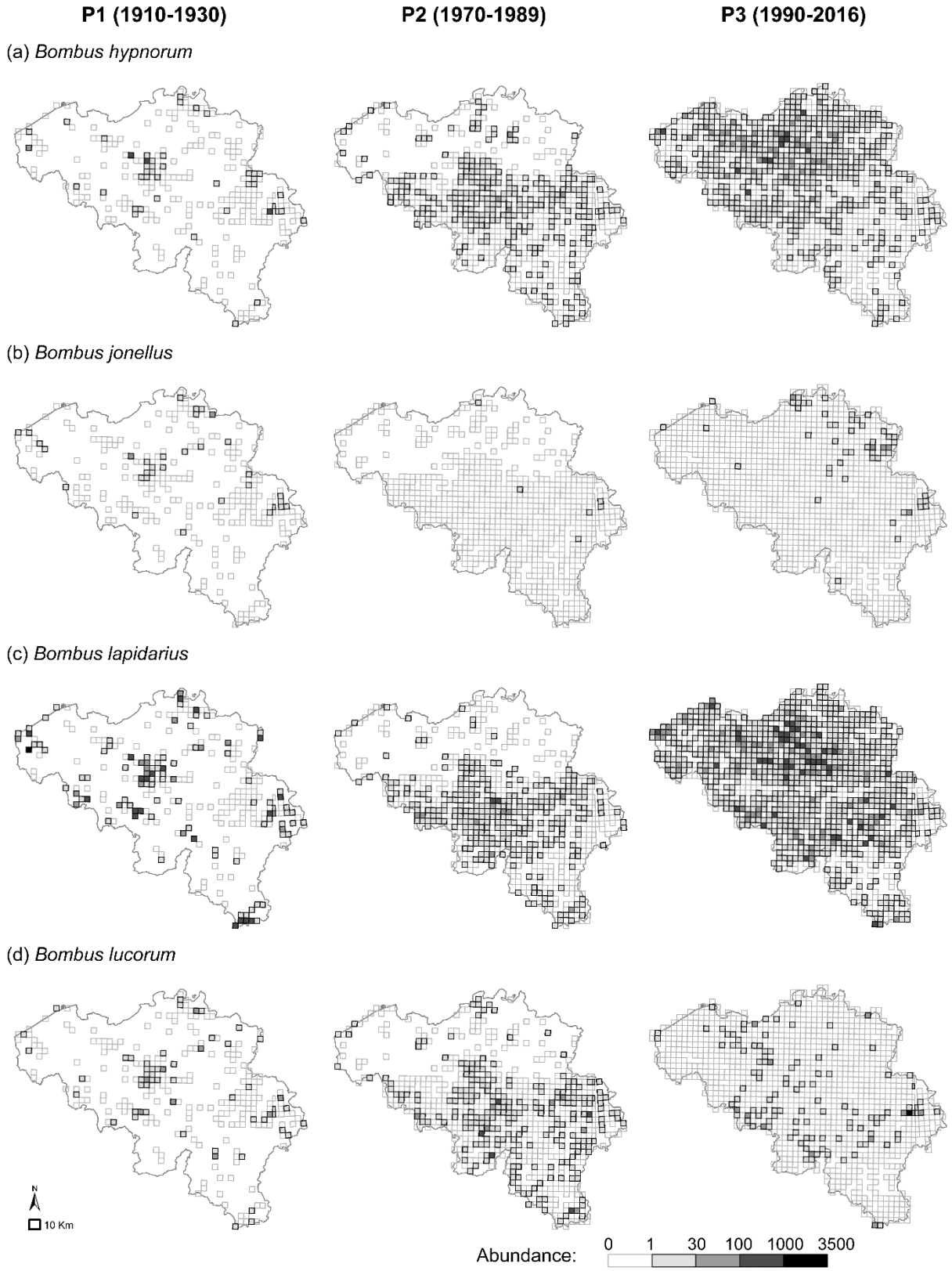

Figure S4. Distribution maps of (a) *B. hypnorum,* (b) *B. jonellus,* (c) *B. lapidarius*, and (d) *B. lucorum* in each time-period. Squares framed in grey are the 5x5 km grid-cells (UTM) sampled during the time-period. Squares framed in black are the grid-cells where the species was recorded.

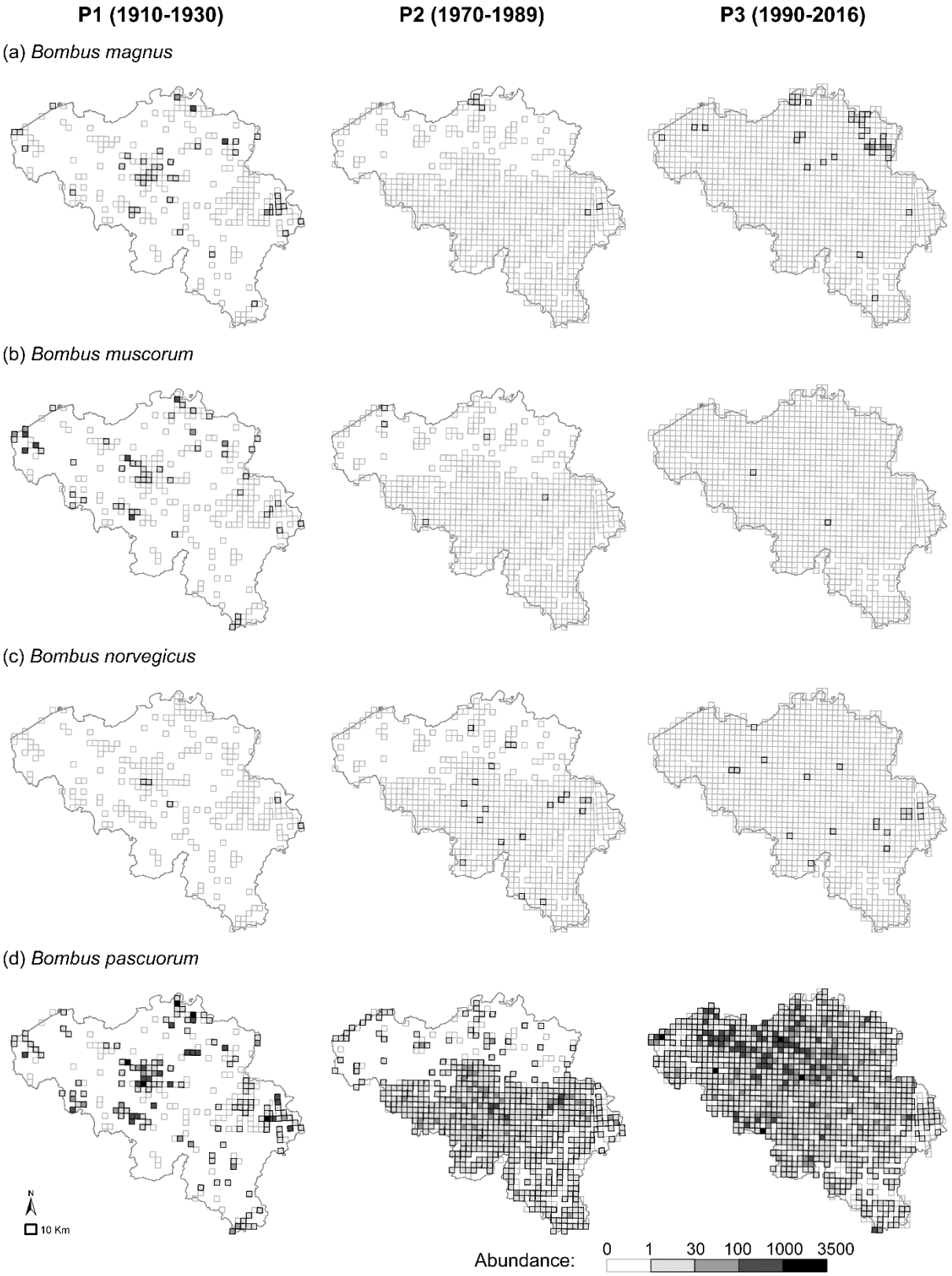

Figure S5. Distribution maps of (a) *B. magnus,* (b) *B. muscorum,* (c) *B. norvegicus*, and (d) *B. pascuorum* in each time-period. Squares framed in grey are the 5x5 km grid-cells (UTM) sampled during the time-period. Squares framed in black are the grid-cells where the species was recorded.

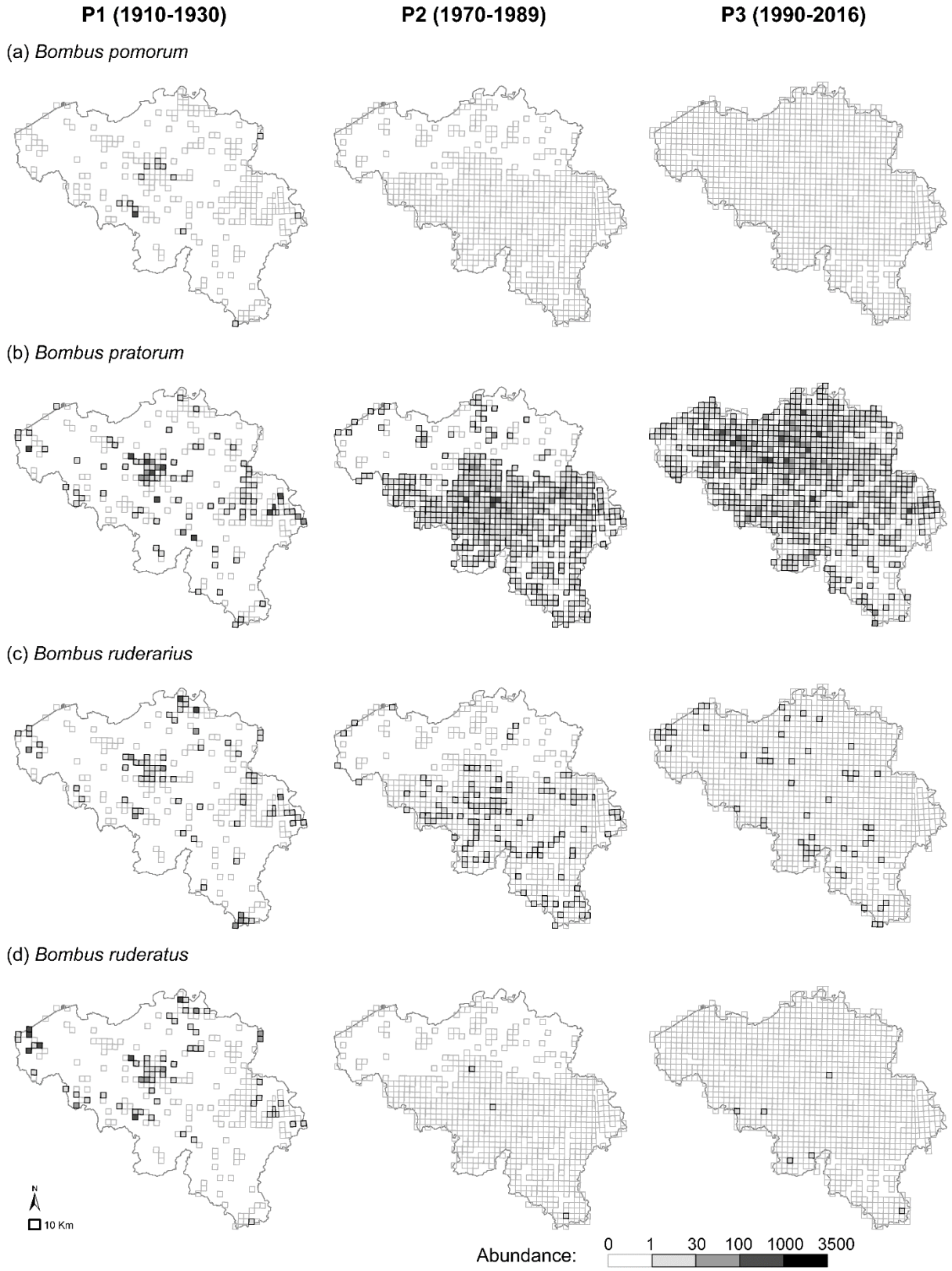

Figure S6. Distribution maps of (a) *B. pomorum,* (b) *B. pratorum,* (c) *B. ruderarius*, and (d) *B. ruderatus* in each time-period. Squares framed in grey are the 5x5 km grid-cells (UTM) sampled during the time-period. Squares framed in black are the grid-cells where the species was recorded.

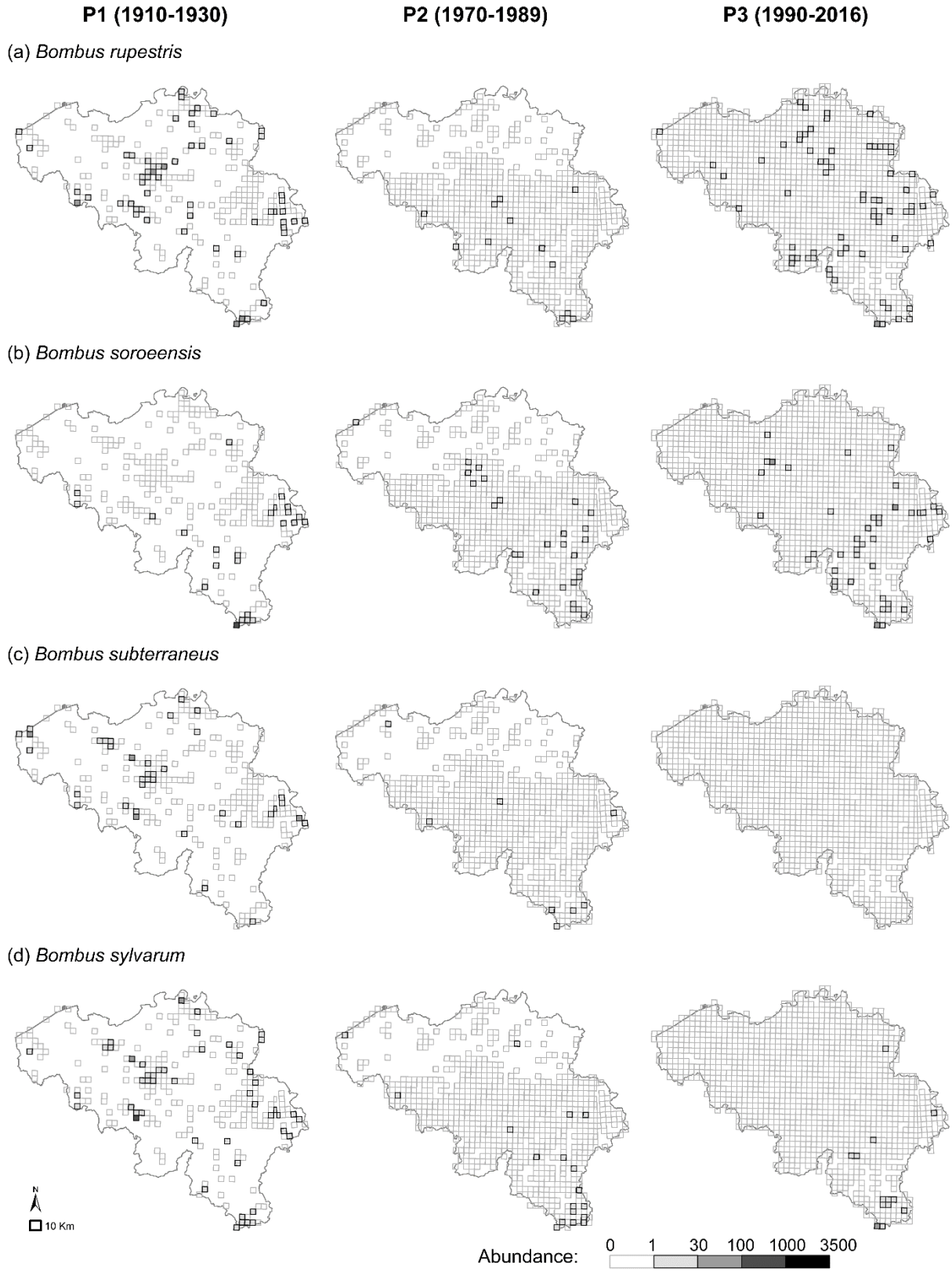

Figure S7. Distribution maps of (a) *B. rupestris,* (b) *B. soroeensis,* (c) *B. subterraneus*, and (d) *B. sylvarum* in each time-period. Squares framed in grey are the 5x5 km grid-cells (UTM) sampled during the time-period. Squares framed in black are the grid-cells where the species was recorded.

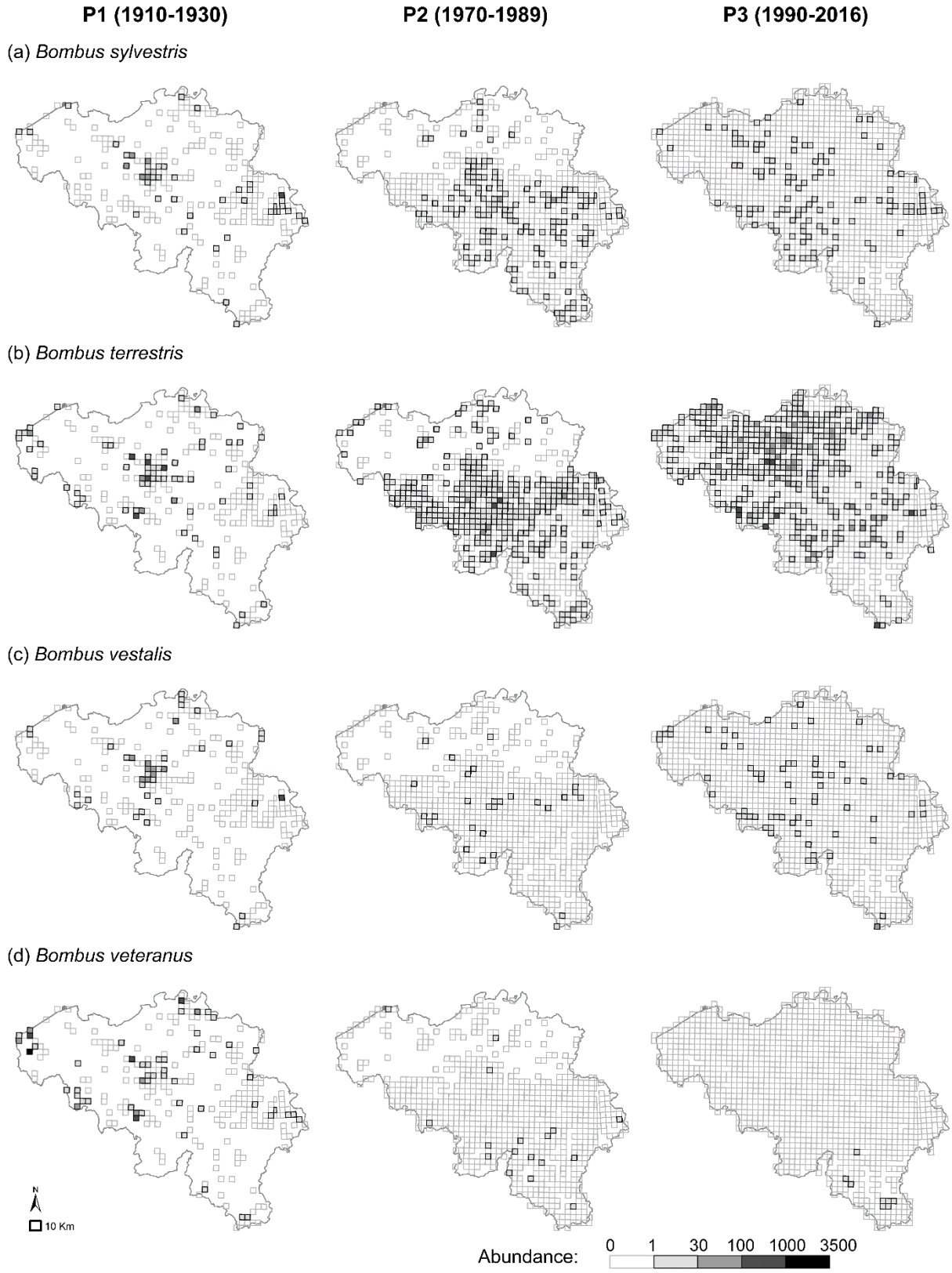

Figure S8. Distribution maps of (a) *B. sylvestris,* (b) *B. terrestris,* (c) *B. vestalis*, and (d) *B. veteranus* in each time-period. Squares framed in grey are the 5x5 km grid-cells (UTM) sampled during the time-period. Squares framed in black are the grid-cells where the species was recorded.

Chapitre 2

Endangered by laws: potential consequences of regulations against thistles on bumblebee conservation.

Chapter 2: "Endangered by laws: potential consequences of regulations against thistles on bumblebee conservation"

From Vray S., Lecocq T., Roberts S.P.M., Rasmont P. 2017. Endangered by laws: potential consequences of regulations against thistles on bumblebee conservation. Annales de la Société entomologique de France (N.S.) 53:33–41. See Annex I.

1. Background

In the previous chapter, we saw that the diet of bumblebees was correlated with their declining trend: species foraging on a smaller number of families and types of plants tend to decline more than the others. We also showed that the Asteraceae family was the most frequently visited, accounting for 26% of the 33,382 observations of bumblebees. Yet, several European countries, including Belgium, impose the mandatory destruction of several species of late-blooming Asteraceae, *i.e*. thistles. In this chapter, we explore in more detail the importance of Asteraceae, Cardueae, Cardueae genera, and finally the species concerned by the regulations for the different bumblebee castes, with a focal point on males. Our analysis includes the four countries where such legislation is in effect: Belgium, France, the Netherlands and the United Kingdom.

2. Summary

Many Bumblebee (*Bombus*) species are undergoing a strong decline in Europe due to, amongst other things, a decrease of food resources. While leguminous plants (Fabaceae) are considered to be one of the main pollen sources of bumblebees, thistles (Asteraceae tribe Cardueae) have been suggested to be important for male diet. Yet, several European countries apply strict regulations against thistles since they are considered to be one of the principal weeds in agricultural landscapes. Such regulations could impact bumblebee conservation through disruption of male diet and ecology. Here, we assess the importance of male-dependence on thistles for bumblebee species based on field observations across countries where a legal regulation against thistles is in effect. We ultimately aim to evaluate the potential consequences of these regulations on bumblebee conservation. Our results confirm that most floral visit observations of males occur on thistles (mainly *Cirsium* spp. and *Carduus* spp.) and some species are almost exclusively observed on them. Thistle removal is thus most likely a threat for bumblebees. Therefore, we advocate repealing the thistle removal acts to make way for alternative thistle regulations reconciling biodiversity conservation and agricultural requirements.

3. Introduction

Almost 90% of all flowering plant species are pollinated by animals (Ollerton *et al.* 2011). In cold and temperate regions of the Northern Hemisphere, the social species group of bumblebees (*Bombus* spp.) is the main pollinating agent (Heinrich 1979). However, many species are experiencing a strong population decline and range contraction fostered by landscape modifications and fragmentation, intensive use of agrochemicals, pathogen infections, competition with alien species, climate change, reduction in floral resources, and interactions between all these factors (Goulson *et al.* 2005). Conservation actions have been developed to ensure bumblebee survival and to avoid a major disruption of the pollination service. One strategy aims to mitigate the current qualitative and quantitative reduction in floral resources (e.g. Carvell *et al.* 2007; Kleijn & Raemakers 2008; Scheper *et al.* 2014) because bumblebees require a long term flowering and a large diversity in plant species (Persson & Smith 2013; Rundlöf *et al.* 2014). Determining the most important plants for bumblebee diet and ecology is therefore essential to plan efficient conservation management.

Several studies based on field observations have pointed out that leguminous plants (Fabaceae) are among the main pollen sources for bumblebees, especially for scarcer species (Rasmont & Mersch 1988; Goulson *et al.* 2005, 2008). However, most of these assessments consider only the worker caste. Since workers are often the most abundant caste during field studies, floral preferences of other castes could be blurred. Yet, other caste requirements are thought to be different (Goulson 2010). For instance, some studies have suggested the importance of thistles

(Asteraceae tribe Cardueae; Tree of Life Web Project 2009) for male diet at regional scales (e.g. Croxton *et al.* 2002; Pywell *et al.* 2005; Carvell *et al.* 2006). Yet, several European countries apply strict legal regulations against thistles, aimed at curbing flowering, plant development, and seed dispersal since they are considered to be noxious weeds in the agricultural landscape (Table 1). Under the assumption of the importance of thistles for bumblebees, such regulations could impact bumblebee conservation through disruption of male diet and ecology.

Here, we assess the importance of thistles for male bumblebees across countries with regulations against thistles by using field observations. We ultimately aim to evaluate the potential consequences of regulations against thistles on bumblebee conservation.

Table 1. National regulations requiring the control of thistles in the European Union

Countries	Period	Species concerned	Regulation's name / source
Belgium	1987 to present	*Carduus crispus* *Cirsium arvense* *Cirsium palustre* *Cirsium vulgare*	"*Arrêté royal du 19 novembre 1987 relatif à la lutte contre les organismes nuisibles aux végétaux et aux produits végétaux.*" (Crémer et al. 2008)
France	1994 to present	*Cirsium arvense*	*"Article L383 du Code Civil"*; and subsequent local legislations (i.e. " *arrêtés préfectoraux/municipaux"*)
Netherlands (only provinces Friesland, Utrecht, Noord-Holland, Zeeland, Zuid-Holland, and Noord-Brabant)	1950 to present	Varies per province, usually : *Cirsium arvense* *Cirsium palustre* *Cirsium vulgare*	Local legislations (i.e. "*Distelverordening (bestrijdingsplicht)*" and "*Algemene Plaatselijke Verordening (APV)*")
United Kingdom	1959 to present	*Cirsium arvense* *Cirsium vulgare*	"Weeds Act 1959"

4. Material and methods

4.1. Plant systematics and definition of "Thistles"

We define thistles as all species included in Cardueae (synonym: Cynareae); a monophyletic worldwide tribe in the Asteraceae family. It contains 2400 species in 73 genera (Barres *et al.* 2013). For historic reasons, we follow the broad interpretation of Scrophulariaceae s.l. as used in *Flora Europaea* Vol. 3 (Tutin *et al.* 1972).

4.2. Database and description of the dataset

We used opportunistic data consisting of 88 974 field observations of bumblebee (only observations of individuals feeding on flowers) which are recorded in the database *Banque de Données Fauniques de Gembloux et Mons* (Rasmont & Iserbyt 2013; Rasmont *et al.* 2015). For each observation, we looked at: (i) the plant species visited; (ii) the location; (iii) the date; (iv) the visiting bumblebee species; and (v) the caste. All observations were performed in countries with a regulation against thistles (Table 1), with a proportion of 61% in France, 28% in Belgium, 11% in UK and 0.05% in the Netherlands. These four countries represent together the study area used for the analyses. Observations were not fully distributed evenly within the study area but were gathered in specific regions (see the spatial distribution map in Figure 1). Observations were carried out from 1878 to 2015 and more than three-quarters (78%) were in summer (11% in June, 49% in July, and 18% in August).

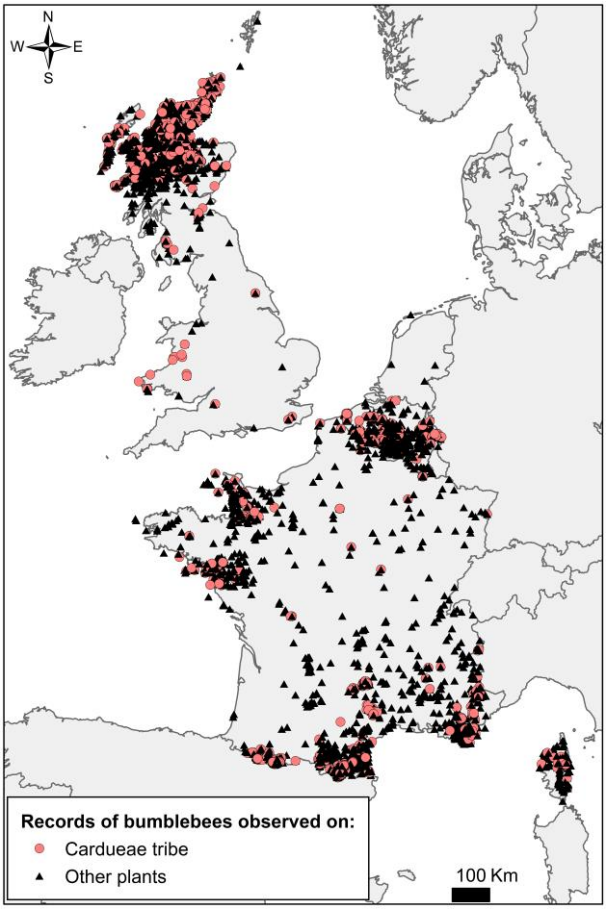

Figure 1. Spatial distribution of the bumblebee observations feeding on flower in the study area, consisting of the four countries affected by thistle regulations (Belgium, France, the Netherlands and UK). Each dot/triangle is one record. Created using ArcGIS 10 software (ESRI, Redlands, CA, USA, www.esri.com).

4.3. Assessing the importance of thistles for bumblebees

We assessed the importance of thistles at *Bombus* generic and specific levels.

At the generic level, we compared for each caste the number of observations on flower at three plant taxonomic levels: between (i) plant families (n = 88 974 observations), (ii) Asteraceae tribes (n = 15 746 observations) and (iii) Cardueae genera (n = 14 110 observations). Visit preferences between castes to different plant families were characterised using Correspondence Analysis (CA; 'FactoMineR' package) on the contingency table [34 plant families × 3 castes], only

considering the plant families for which we had at least 50 observations (all castes combined). Moreover, since our data does not follow a normal distribution (Shapiro-Wilk test), differences in floral preferences between plant taxonomic groups were examined with nonparametric tests (two-sample Wilcoxon sign-rank tests) for each caste. All statistical analysis were done using R version 3.3.1 (R Development Core Team 2016).

At the *Bombus* species level (20 567 queen observations, 15 007 male observations and 38 892 worker observations), we compared for each caste the relative number of observations on the Cardueae tribe.

Thereafter, we assessed more specifically the consequences of national regulations against thistles by comparing the number of individuals from each *Bombus* species feeding on the four thistle species affected by national regulations (n= 3016 observations): *Carduus crispus* L., *Cirsium arvense* (L.) (Fig. 2a) Scop., *Cirsium palustre* (L.) Scop. (Fig. 2b), and *Cirsium vulgare* (Savi) Ten. (Table 1).

Figure 2. (a) *Bombus rupestris* male on *Cirsium arvense*, and (b) *Bombus sylvestris* male on *Cirsium palustre* (Photos: J.-M. Michalowski).

5. Results

5.1. Bumblebees' floral preferences and importance of thistles

Our CA shows differences in floral visit preferences between castes (Fig. 3). The total inertia contained in the contingency table [34 plant families × 3 castes] is entirely captured by two dimensions (respectively 79.93% for Axis 1 and 20.07% for Axis 2). The first dimension (Axis 1) is mainly defined by three plant families: Solanaceae (39.79%), Asteraceae (16.89%), and Brassicaceae (15.48%), and by two castes: queens (68.45%) and males (21.98%). The second dimension (Axis 2) is mainly explained by Fabaceae (28.59%), Asteraceae (26.81%) and Scrophulariaceae (11.36%), and for the castes by males (57.86%) and workers (38.14%). The global pattern presented by the CA shows that, among the five most visited plant families, Asteraceae are highly associated with male observations (Fig. 3).

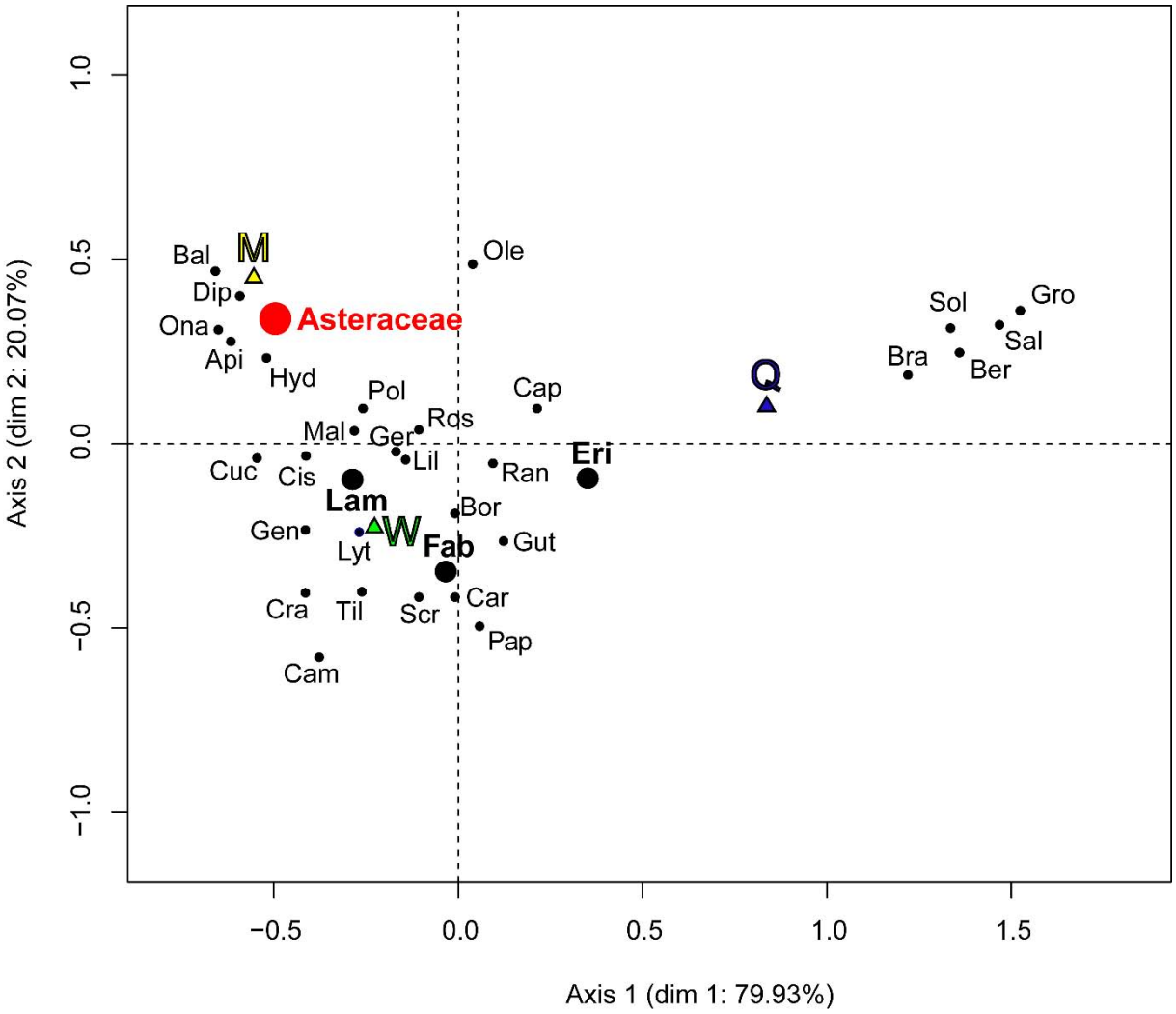

Figure 3. Correspondence analysis factor map obtained from the contingency table of the three castes (triangles) and the 34 plant families for which we have more than 50 observations (points). Bigger points are the four plant families with more than 9000 observations, Asteraceae being the most visited family with 15 015 observations. M = males (14 874 obs.); Q = queens (20 326 obs.); W = workers (38 579 obs.); Api = Apiaceae (1493 obs.); Bal = Balsaminaceae (52 obs.); Ber = Berberidaceae (74 obs.); Bor = Boraginaceae (2642 obs.); Bra = Brassicaceae (2163 obs.); Cam = Campanulaceae (747 obs.); Cap = Caprifoliaceae (74 obs.); Car = Caryophyllaceae (114 obs.); Cis = Cistaceae (184 obs.); Cra = Crassulaceae (113 obs.); Cuc = Cucurbitaceae (320 obs.); Dip = Dipsacaceae (1223 obs.); **Eri = Ericaceae (9570 obs.)**; **Fab = Fabaceae (12 556 obs.)**; Gen = Gentianaceae (436 obs.); Ger = Geraniaceae (227 obs.); Gro = Grossulariaceae (485 obs.); Gut = Guttiferae (176 obs.); Hyd = Hydrophyllaceae (443 obs.); **Lam = Lamiaceae (9624 obs.)**; Lil = Liliaceae (618 obs.); Lyt = Lythraceae (142 obs.); Mal = Malvaceae (121 obs.); Ole = Oleaceae (64 obs.); Ona = Onagraceae (1533 obs.); Pap = Papaveraceae (149 obs.); Pol = Polygonaceae (52 obs.); Ran = Ranunculaceae (2430 obs.); Ros = Rosaceae (2386 obs.); Sal = Salicaceae (359 obs.); Scr = Scrophulariaceae (3434 obs.); Sol = Solanaceae (4643 obs.); Til = Tiliaceae (117 obs.).

Focus on percentages of observations (Fig. 4a) and Wilcoxon sign-rank tests confirm that males were significantly more observed on Asteraceae than on other families (41% of the 15 007 male observations; Wilcoxon sign-rank test, W = 1324400, p-value < 0.01). Queens were mainly observed on Solanaceae, Ericaceae, and Fabaceae families (respectively 20, 19 and 14% of the 20 567 queen observations; only 7% on Asteraceae) and workers on Fabaceae, Asteraceae and Lamiaceae families (respectively 22, 19 and 14% of the 38 892 worker observations). The surprisingly high percentage of queens recorded on Solanaceae is explained by observations of *Bombus terrestris* queens on *Salpichroa origanifolia* (Lam.) Baill. during September and October (4082 of the 4123 queens observations on Solanaceae). Among observations on Asteraceae, the Cardueae tribe was significantly more visited than other tribes by males (90% of the 5947 males observations, Wilcoxon sign-rank test, W = 84052, p-value < 0.001) and by workers (92% of the 7413 workers observations, Wilcoxon sign-rank test, W = 70105, p-value < 0.001), and recorded 79% of the 1446 queens (Fig. 4b). Among observations on Cardueae, 61% of the 1112 queens were observed on *Cirsium* spp., 22% on *Centaurea* spp. and 13% on *Carduus* spp. The 4722 male observations and the 6738 worker observations are quite equitably distributed among the three genera (respectively 36% and 35% for *Carduus*, 30% and 32% for *Centaurea*, and 29% both for *Cirsium*; Fig. 4c).

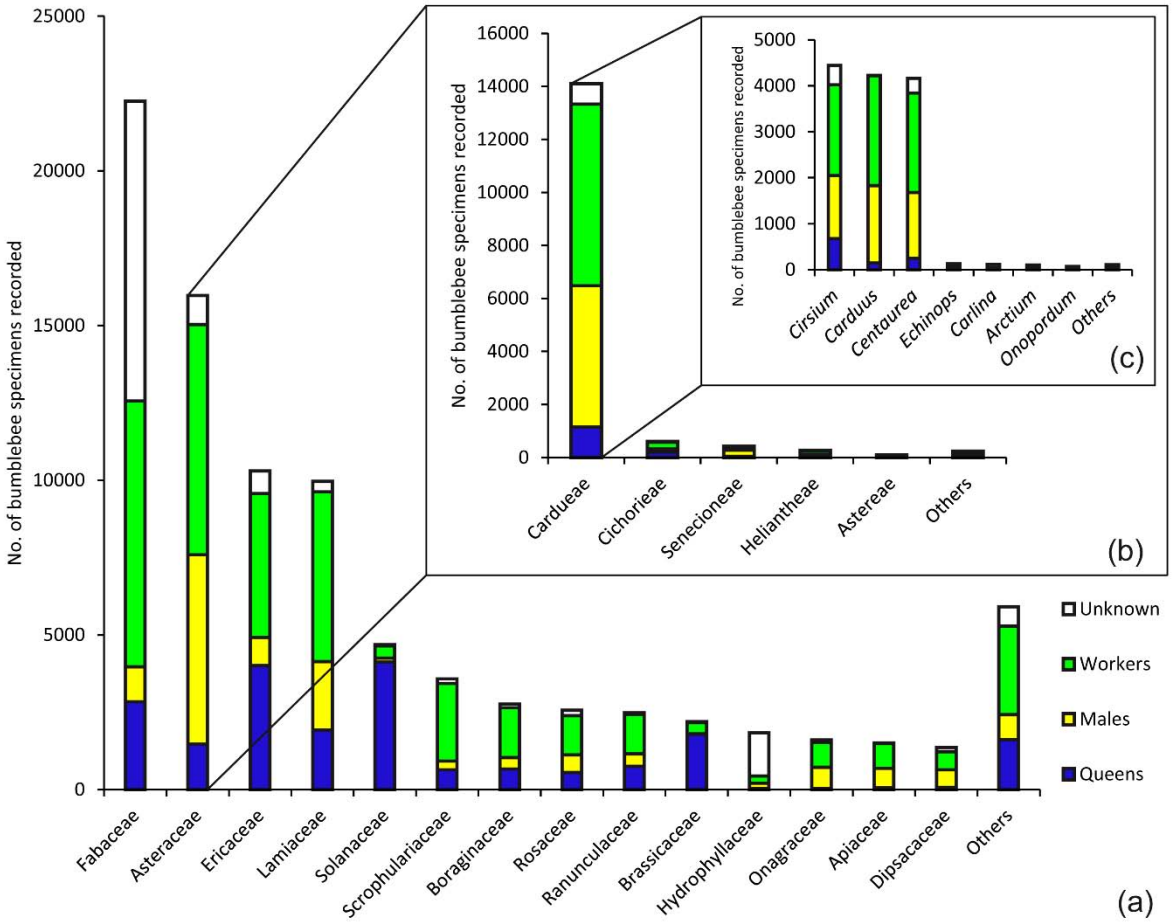

Figure 4. Number of bumblebee specimens recorded on different (a) plant families (all bumblebee species combined; n = 88 974 specimens), (b) tribes of the Asteraceae family (all bumblebee species combined; n = 15 746 specimens), and (c) genera of the Cardueae tribe (all bumblebee species combined; n = 13 353 specimens). "Others" are (a) 79 plant families with less than 800 observations each totalling 5912 observations, (b) 10 tribes of Asteraceae with less than 70 observations each totalling 240 observations, and (c) eight genera of Cardueae with less than 40 observations each totalling 100 observations.

5.2. Bumblebee species most observed on thistles

Regarding the bumblebee specific richness, a total of 43 species was recorded on Cardueae (of the 45 species, all plant species included). For several species, more than half of recorded visits were to this tribe (Fig. 5a, Table 2). The largest numbers, in descending order, were: *Bombus veteranus* (95% of the observations), *B.*

quadricolor (61%), *B. mucidus* (59%), *B. mesomelas* (56%) and *B. norvegicus* (52%; Fig. 5a).

Moreover, comparative analyses between castes show that many species have one or two castes which are very often observed on Cardueae. *Bombus veteranus* males and workers were almost exclusively recorded on this tribe (respectively 98% and 96% of the observations). Males of *B. mucidus* (87%), *B. mesomelas* (86%), *B. confusus* (85%), *B. mendax* (81%), *B. lapidarius* (73%), *B. quadricolor* (70%) and *B. sylvarum* (70%) were also commonly seen on Cardueae (Fig. 5a).

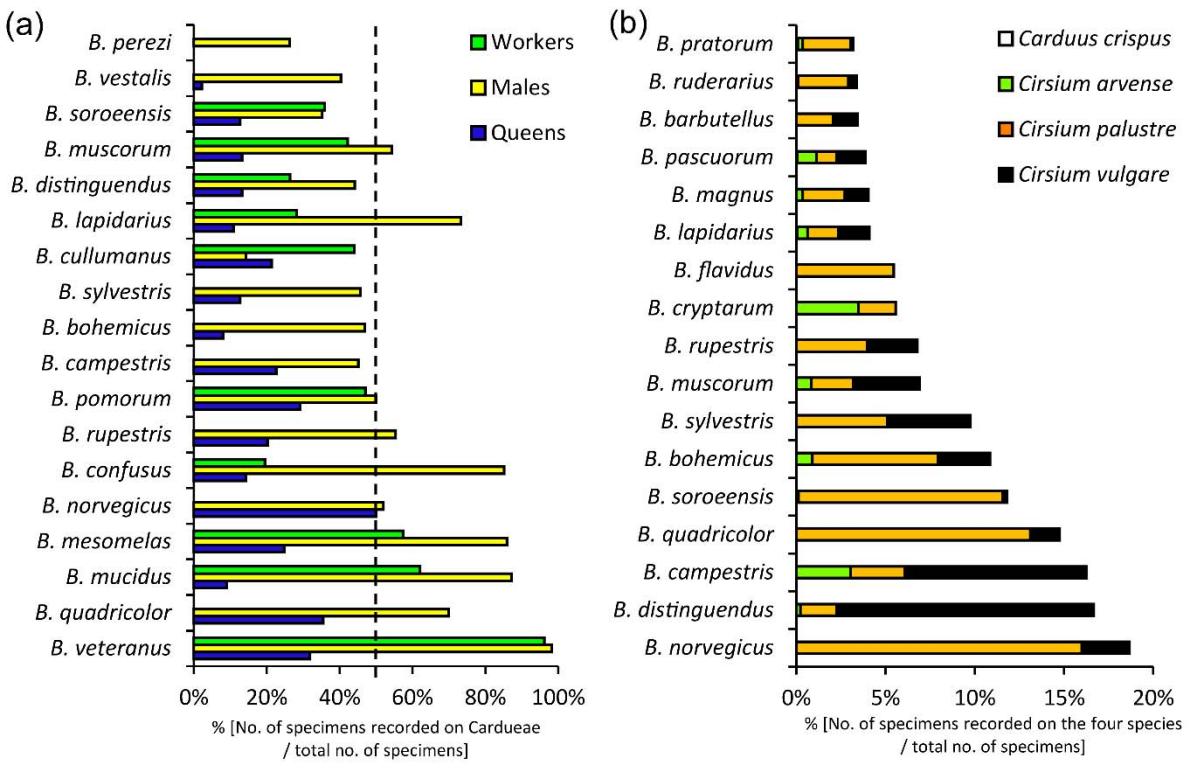

Figure 5. Proportion of bumblebee specimens observed on (a) Cardueae and (b) the four thistle species concerned by the regulations (number on Cardueae = 14 113 specimens; number on the four species = 3016 specimens; total number = 88 974 specimens). Only species with a percentage higher than (a) 25% and (b) 3% (for all castes combined) are presented.

Table 2. Total number of specimens recorded on all plant species and number of specimens recorded on a plant species of the Cardueae tribe. Q = queen; M = male; W = worker; U = unknown. *Bombus* sp. are *Bombus* unspecified specimens in the database.

Bombus species	Total number of specimens					Number of specimens observed on a Cardueae				
	Q	M	W	U	Total	Q	M	W	U	Total
All *Bombus* species	20567	15007	38892	14509	88974	1145	5334	6851	783	14113
Bombus sp.	38	53	185	152	428	2	9	72	9	92
B. alpinus (L.)	4	3	11	3	21	-	-	-	-	-
B. argillaceus (Scopoli)	12	23	89	1	125	-	2	25	-	27
B. barbutellus (Kirby)	26	118	-	2	146	4	25	-	-	29
B. bohemicus Seild	249	1164	-	25	1438	20	546	-	8	574
B. brodmannicus Vogt	36	-	29	2	67	-	-	1	-	1
B. campestris (Panzer)	31	164	-	2	197	7	74	-	-	81
B. confusus Schenk	21	81	92	-	194	3	69	18	-	90
B. cryptarum (Fabricius)	528	98	235	1	862	2	56	16	-	74
B. cullumanus (Kirby)	14	7	34	-	55	3	1	15	-	19
B. distinguendus Morawitz	226	77	265	1564	2132	30	34	70	556	690
B. flavidus Eversmann	5	122	-	1	128	4	45	-	1	50
B. gerstaeckeri Morawitz	239	55	112	3	409	-	-	-	-	-
B. hortorum (L.)	1051	661	1573	1357	4642	31	103	144	30	308
B. humilis Illiger	306	148	1300	1	1755	10	77	263	-	350
B. hypnorum (L.)	90	88	274	100	552	4	21	18	4	47
B. jonellus (Kirby)	766	104	319	334	1523	16	16	13	2	47
B. lapidarius (L.)	1056	1237	3204	477	5974	115	907	905	24	1951
B. lucorum (L.)	1939	1722	3270	284	7215	31	219	215	17	482
B. magnus Vogt	431	72	263	76	842	19	-	20	13	52
B. mendax Gerstaecker	44	42	359	1	446	4	34	22	-	60
B. mesomelas Gerstaecker	121	121	1058	17	1317	30	104	608	2	744
B. mocsaryi Kriechbaumer	4	-	13	-	17	2	-	13	-	15
B. monticola Smith	179	566	2357	213	3315	2	72	58	-	132
B. mucidus Gerstaecker	33	47	182	2	264	3	41	113	-	157
B. muscorum (L.)	204	81	239	70	594	27	44	101	20	192
B. norvegicus (SparreSchneider)	2	73	-	-	75	1	38	-	-	39
B. pascuorum (Scopoli)	2695	984	4611	2343	10633	320	266	468	33	1087
B. perezi (Schulthess-Rechberg)	3	114	-	-	117	-	30	-	-	30
B. pereziellus (Skorikov)	19	6	32	-	57	-	-	1	-	1
B. pomorum (Panzer)	41	16	119	-	176	12	8	56	-	76
B. pratorum (L.)	886	718	1432	216	3252	7	105	131	17	260
B. pyrenaeus Pérez	33	162	833	12	1040	4	82	114	1	201
B. quadricolor (Lepeletier)	31	90	-	1	122	11	63	-	-	74
B. ruderarius (Müller)	450	628	1608	24	2710	22	297	303	7	629
B. ruderatus (Fabricius)	309	513	981	2	1805	4	36	33	-	73
B. rupestris (Fabricius)	79	166	-	6	251	16	92	-	1	109
B. sichelii Radoszkowski	40	75	940	14	1069	7	14	196	2	219
B. soroeensis (Fabricius)	729	445	3671	65	4910	93	157	1321	4	1575
B. subterraneus (L.)	94	155	385	1	635	4	60	50	-	114
B. sylvarum (L.)	220	146	2400	20	2786	25	102	522	8	657
B. sylvestris (Lepeletier)	118	306	-	27	451	15	140	-	4	159
B. terrestris (L.)	6876	2447	4628	7052	21003	212	448	235	18	913
B. vestalis (Fourcroy)	45	109	-	20	174	1	44	-	2	47
B. veteranus (Fabricius)	69	848	705	-	1622	22	833	678	-	1533
B. wurflenii Radoszkowski	175	151	1074	28	1428	-	20	32	1	53

5.3. Importance of the four thistle species included in the regulations

Thirty-six bumblebee species were observed on the four Cardueae species included in the regulations (see Table S1 in Supporting Information), with the two most visited being *Cirsium palustre* and *C. vulgare*. The most frequently observed bumblebee species on these four plants were *B. norvegicus*, *B. distinguendus*, *B. campestris*, *B. quadricolor*, *B. soroeensis*, *B. bohemicus* and *B. sylvestris* (respectively 19, 17, 16, 15, 12, 11, and 10% of the observations; Fig. 5b). 16% of all the *B. norvegicus* specimens, 13% of all the *B. quadricolor* specimens and 11% of all the *B. soroeensis* specimens were observed on the single species *Cirsium palustre*, while 14% of all the *B. distinguendus* specimens and 10% of all the *B. campestris* were observed on *Cirsium vulgare*.

6. Discussion

6.1. Importance of thistles for male bumblebees

We confirm over a larger geographic extent previous results (e.g. Carvell 2002; Carvell *et al.* 2006; Goulson *et al.* 2008) highlighting the large number of male bumblebees floral visits on Cardueae (mainly *Carduus* spp., *Centaurea* spp. and *Cirsium* spp). This strongly suggests an important role of Cardueae as a nectar resource during late summer when males perform their energy-consuming courtship behaviour (e.g. Croxton *et al.* 2002; Pywell *et al.* 2005; Goulson 2010). The lower number of workers and queens foraging on Cardueae could be explained by their need to collect pollen in addition to nectar for the colony development,

unlike males which only feed on nectar. Yet, Asteraceae pollen is rarely found in queens and workers pollen loads (Goulson 2010), probably because it may represent a non-optimal diet for bumblebee colonies. This has recently been shown by the relative unsuitability of *Cirsium* pollen for the colony development of *B. terrestris* (Vanderplanck *et al.* 2016). It would be of great interest to perform a comparison between time-periods of the importance of thistles in the foraging choice of bumblebees. However, our dataset does not allow us to perform such a detailed study, because of the very different sampling efforts along time.

The importance of Cardueae has also been pointed out for other insect pollinators (Vanbergen *et al.* 2014; Ricou *et al.* 2014). For instance, in the greenways and sown wildflower strips of Southern Sweden, *Cirsium* spp. and *Centaurea* spp. were the most visited plant species by eight bumblebee species, but also by 18 butterfly species (Haaland & Gyllin 2009). Cardueae are especially important in areas with intensive agriculture, where pollinators in general have a narrower spectrum and abundance of flowering plants (e.g. *Cirsium* species are a major foraging source where other wildflower species are absent; Haaland & Gyllin 2009). Beside flower visiting insects, many phytophagous species are associated with Cardueae (Freese 1994) while thistle seeds provide food resource for some beetles, birds and rodents (McCallum & Kelly 1990).

6.2. Potential consequences of regulations against thistles on bumblebee conservation

The four thistle species concerned by regulations against thistles in Belgium, France, the Netherlands and UK (i.e. *Carduus cripus*, *Cirsium arvense*, *Cirsium palustre* and *Cirsium vulgare,* Table 1) are commonly visited by male bumblebees (Fig. 5b and Table S1 in Supplementary Information). The regulations could thus (i) greatly reduce the availability of food resources for, especially, male bumblebees during mating period and (ii), consequently, decrease the offspring production reinforcing the bumblebee decline through the population size decrease and the "extinction vortex" (Gilpin & Soulé 1986; Zayed & Packer 2005). Such a phenomenon is most likely in "Threatened" or "Near Threatened" (Cederberg *et al.* 2013) species commonly visiting thistle flowers (e.g. *B. confusus, B. distinguendus* , *B. mucidus*, *B. veteranus*; Rasmont *et al.* 2015). Moreover, thistle removal acts may affect several other plant species. Indeed, the correct identification of the species that are legislated against is not always easy for farmers and landowners. For the general public, "thistle" refers to all flowering plants characterized by leaves with sharp prickles on the margins. This usually implies that all thistles and thistle-like plants are wiped out leading to other Cardueae species decline (e.g. in Belgium, *Cirsium dissectum*, *Cirsium tuberosum*, *Carduus nutans* and *Cirsium eriophorum*; Crémer *et al.* 2008). Furthermore, many measures taken to eradicate thistles (e.g. repeated mowing, fertilize and lime to ensure the maintenance of an herbaceous cover, or herbicides) also affect non-Cardueae plants (e.g. Turner *et al.* 2007;

Crémer *et al.* 2008; Andreasen & Andresen 2011), including Fabaceae, the main food resources for bumblebee females (Fig. 4a).

6.3. Guidelines for policy-makers and conservation organizations

Because of the importance of thistles for bumblebee males (and also for other species ensuring the pollination ecosystem service) there is a premium on repealing the thistle removal acts but also on limiting Cardueae suppression in regions where thistle removal is not mandatory but is still carried out by most farmers (Bond & Grundy 2001; Turner *et al.* 2007; Melander *et al.* 2012). Moreover, bumblebees can act as umbrella species for the other organisms associated with thistle and thistle-like plants (butterflies, other bees and hoverflies).

Despite the obvious benefit of minimising the removal of Cardueae for many pollinators, agricultural policy requires the control of thistles in order to maintain farmland productivity. Indeed, some thistles may weaken pasture and crops due to their allelopathy (i.e. releasing soluble inhibitors that discourage the growth of pasture grasses and legumes; Wardle *et al.* 1993), their highly effective seed dispersal and their clonal spreading ability (leading to large area monopolization; Kelemen *et al.* 2014). In grasslands, thistle control can be achieved by prevention techniques that maintain a large floral diversity (e.g. avoiding over-fertilizing, over- or under-grazing, and bare soils) coupled with more specific methods (e.g. alternating mowing and grazing). In crops, thistle control without the use of pesticides is possible by using crop rotation and stubble techniques (Andreasen & Andresen 2011; Nicholls & Altieri 2013). When such actions in croplands and

grasslands are not possible, maintaining tolerable densities of thistles in fields could help to support pollinator populations (Nicholls & Altieri 2013). Moreover, thistle removal in other areas should not be promoted because they are very rarely a problem outside farmlands. Therefore, gardens can act as refuges for bees and bumblebees in urban environments and intensively managed farmlands, as already suggested by previous studies (e.g. Ahrné *et al.* 2009; Samnegård *et al.* 2011).

7. Conclusion

In summary, thistle removal regulations are obsolete because they are based on an old agricultural context, and methods of weed control have evolved since their establishment (Andreasen & Andresen 2011). These regulations should therefore be re-evaluated, taking into account the increasing consideration of biodiversity in agricultural landscapes (Nicholls & Altieri 2013) and particularly the high priority given to the wild pollinator conservation (IPBES 2016).

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10. Supplementary Information

Table S1. Number of bumblebee specimens observed on the four thistle species concerned by the regulations. Q = queen; M = male; W = worker; U = unknown. *Bombus* sp. are *Bombus* unspecified specimens in the database.

Bombus species	*Carduus crispus*					*Cirsium arvense*					*Cirsium palustre*					*Cirsium vulgare*					Four species					Total number of specimens
	Q	M	W	U	Total	Q	M	W	U	Total	Q	M	W	U	Total	Q	M	W	U	Total	Q	M	W	U	Total	
All *Bombus* species	1	2	6	6	15	201	170	86	12	469	95	425	919	57	1496	280	324	126	306	1036	577	921	1137	381	3016	88975
Bombus sp.						1	1	35		37		4		2	6			1		1	1	5	36	2	44	428
Bombus barbutellus												3			3		2			2		5			5	146
Bombus bohemicus						2	11			13	3	96		2	101	9	28		5	42	14	135		7	156	1438
Bombus campestris							6			6		6			6	2	18			20	2	30			32	197
Bombus confusus													1		1								1		1	194
Bombus cryptarum							27	3		30	1	17			18						1	44	3		48	862
Bombus distinguendus							3		2	5	12		13	18	43	7	6	29	265	307	19	9	42	285	355	2132
Bombus flavidus												7			7							7			7	128
Bombus hortorum							2	1		3	5	3	11		19	12	24	33	12	81	17	29	45	12	103	4642
Bombus humilis												3	12		15		1			1		4	12		16	1755
Bombus hypnorum						1	4			5		3	5		8		2			2	1	9	5		15	552
Bombus jonellus						1	2	7		10	8	4	3	1	16	6		2		8	15	6	12	1	34	1523
Bombus lapidarius			2	1	3	18	13	4		35		4	95	4	103	25	59	16	4	104	43	76	117	9	245	5974
Bombus lucorum						3	13	15	3	34	4	35	33	3	75	10	7	9	1	27	17	55	57	7	136	7215
Bombus magnus									3	3	11		4	5	20	8		1	2	11	19		5	10	34	842
Bombus mesomelas								4		4		3	14		17		10			10		13	18		31	1317
Bombus monticola												24	17		41							24	17		41	3315
Bombus mucidus													6		6								6		6	264
Bombus muscorum								1	4	5	6		4	4	14	9		5	8	22	15		10	16	41	594
Bombus norvegicus												12			12		2			2		14			14	75
Bombus pascuorum		1	1	3	5	98	16	2		116	9	22	85	5	121	107	39	20	5	171	214	78	108	13	413	10633
Bombus pomorum													2		2								2		2	176
Bombus pratorum		1	2		3		3	6		9		36	41	10	87		4		1	5		44	49	11	104	3252
Bombus pyrenaeus												4	3		7		1			1		5	3		8	1040
Bombus quadricolor											3	13			16		2			2	3	15			18	122
Bombus ruderarius							1	2		3	1	39	36		76	2	11			13	3	51	38		92	2710
Bombus ruderatus												1			1	2				2	2	1			3	1805
Bombus rupestris											2	8			10	1	6			7	3	14			17	251
Bombus sichelii												1	24		25							1	24		25	1069
Bombus soroeensis	1				1	1	2	2		5	27	39	493	3	562	8	4			12	37	45	495	3	580	4910
Bombus subterraneus												1	2		3		6			6		7	2		9	635
Bombus sylvarum				1	1							1	2		3			1		1		1	3	1	5	2786
Bombus sylvestris											2	21			23	1	17		3	21	3	38		3	44	451
Bombus terrestris			1	1	2	76	66	4		146	1	10	9		20	71	73	8		152	148	149	22	1	320	21003
Bombus vestalis												4			4		1			1		5			5	174
Bombus veteranus													3		3		1	1		2		1	4		5	1622
Bombus wurflenii												1	1		2							1	1		2	1428

Chapitre 3

A one century monitoring of the bumblebee assemblages related to landscape in four localities of Belgium.

Chapter 3: "A one century monitoring of the bumblebee assemblages related to landscape in four localities of Belgium"

By Sarah Vray, Pierre Rasmont, Marc Dufrêne, Denis Michez, and Nicolas Dendoncker.

1. Background

In previous chapters, we showed the strong changes in the assemblages of bumblebees in Belgium and we highlighted the importance of floral resources availability for these essential pollinators. We now consider the importance of landscape changes on the changes observed on bumblebees. Indeed, we learned from Chapter 1 that the most declining species are living in open lands, whereas the least affected species prefer wooded habitats. Moreover, some species are more restrictive in their habitat preferences and are therefore more specialized in some particular habitat types. The study of the landscape composition and structure around the sampling sites could therefore provide new information on the relationship between bumblebees and landscapes. To do so, four localities well sampled by the Ball's collection of the last century were selected. In these localities, we carried out three years of sampling from 2013 to 2015, as well as a comprehensive and detailed analysis of the landscape composition and structure of the past and the current periods.

2. Summary

Bumblebees are declining in most parts of Western Europe. Many studies have highlighted the role of agricultural intensification and urbanisation in this decline, and some of them showed the influence of landscape composition and structure on bumblebees' populations. Yet very few studies explore what happened before these drivers of global land use change acted, and these are mostly based on spatial data of low resolution. Here, we perform a comparative approach based on detailed landscape composition and structure associated to bumblebees' records between the early twentieth century (1910-1930) and the recent period (2013-2015) in four localities representative of Belgium. We show that bumblebee assemblages drastically changed and that decline in richness was stronger in the localities where urbanisation and agricultural intensification were the most intense. The locality still presenting a high proportion of grassland, orchards and woodland and where landscape composition changed the least is still hosting a rich bumblebee fauna, even more diverse than in the past. We provide recommendations for land use management based on these findings. We also warn about the importance of other factors such as land use intensity, climatic conditions and altitude, which should be included in any study addressing land use changes.

3. Introduction

Numerous bumblebee (*Bombus*) species are undergoing a strong population decline in Western Europe and North America (Rasmont *et al.* 2005; Biesmeijer *et al.* 2006; Goulson *et al.* 2008; Williams & Osborne 2009; Cameron *et al.* 2011; Nieto *et al.* 2014). Among the 68 bumblebee species in Europe, 31 are decreasing, 20 are stable, and only 9 present positive population trends and expansion of their distribution (Nieto *et al.* 2014). This decline seems higher in the agricultural plains of Western-Europe than in the Mediterranean and mountainous regions where agriculture remains relatively extensive (Rasmont *et al.* 2005; Iserbyt *et al.* 2008) or even in decline (Iserbyt *et al.* 2015). The alarming regression of bumblebees can be explained by several factors including shortage in floral resources, habitat loss and fragmentation, and intensive use of agrochemicals and pesticides, mostly resulting from agricultural intensification since the 1950s (Rasmont & Mersch 1988; Kosior *et al.* 2007; Goulson *et al.* 2008). Agricultural landscapes, composed of small polycultures and grasslands rich in flowers separated by hedges and trees before the 1950s, gradually turned into homogeneous landscapes dominated by large monocultures, intensive pastures and early mowing hayfields (Christians 1998; Robinson & Sutherland 2002; Mazoyer & Roudart 2006). These changes reduced nesting sites availability for bumblebees, which nest in tufts of grass on the ground surface or in abandoned rodents' burrows (Alford 1975; Kells & Goulson 2003; Goulson *et al.* 2010). Moreover, the increasing use of nitrogenous chemical fertilisers led to the replacement of leguminous (Fabaceae) crops, known to be important for

bumblebees' diet, with other crops (*e.g*. sugar beet and corn) that do not constitute a food resource for these species (Rasmont & Mersch 1988; Rasmont 2007). Moreover, changes in landscape structure may also have great effects on bumblebees' assemblages. Among bees, bumblebees are the most susceptible to the deleterious effects of habitat fragmentation (Aguirre-Gutiérrez *et al.* 2015). For a bumblebee colony to survive, it must have access to floral resources within its foraging range, which is for several species inferior to 1 km and decreases as the proportion of patches rich in floral resources increases (Carvell *et al.* 2012). Green lanes and hedgerow networks are not only important for nest sites and floral resources (Croxton *et al.* 2002; Sepp *et al.* 2004; Osborne *et al.* 2008b; Morandin & Kremen 2013), but also for bumblebees' movements and orientation (Cranmer *et al.* 2012). In addition to the restriction of bumblebees foraging movements, habitat fragmentation could also reduce the ability of bumblebees to disperse between populations, leading to loss of genetic diversity and therefore to a higher vulnerability to local extinctions due to environmental and demographic stochasticity (Osborne *et al.* 1991; Brook *et al.* 2002; Fahrig 2003; Dauber *et al.* 2003; Steffan-Dewenter *et al.* 2006; Le Féon *et al.* 2010; Jha & Kremen 2013).

Most studies assessing the influence of landscape composition and structure on bumblebees' populations are based on contemporary data and very few go back before the onset of agricultural intensification, *i.e.* before 1950 (Senapathi *et al.* 2015; Aguirre-Gutiérrez *et al.* 2017; Mihoub *et al*. 2017). The rare studies doing so do not include high-resolution data of land cover. One exception is a recent

study in England (Senapathi *et al.* 2015), but its island fauna shows a lower diversity and different population trends than the European continent.

The aim of this study is therefore to qualify and quantify the relationship between landscape changes and bumblebees' populations before and after the beginning of large-scale urbanisation and agricultural intensification in Belgium. This country is a particularly suitable case for this kind of study. It presents a typical example of agricultural intensification in Western Europe (Christians 1998), and its bumblebee fauna has been studied since the early 19th century (Meunier 1888; Ball 1914, 1920, Rasmont 1988; Rasmont *et al.* 1993, 2005). Here, we perform correlative and comparative approaches based on landscape composition and structure, and bumblebees data recorded in early century (1910-1930) and recent period (2013-2015) in four localities representative of Belgium. These four localities were well sampled during the last century by F.J. Ball (Ball 1914, 1920) and were carefully resampled during 2013-2015. Landscape composition and structure were analysed at a high-resolution in each period and each locality. We predict that the strongest shifts in bumblebee' assemblages during the last century occured in landscapes where land cover changed the most at a spatial scale relevant for bumblebees. Furthermore, we expect that specific land cover types (*e.g.* heathland, grassland, woodland, orchards) are related to higher species richness than other types such as cropland and settlement.

4. Material and methods

4.1. Sampling sites and bumblebees dataset

Species richness generally increases with the number of samples and sample size (*i.e*. the number of recorded specimens per sample) until reaching a plateau corresponding to the maximum number of species that can be found in the sampled site (Gotelli & Colwell 2001). If the sample size is small, sampling may have missed the rarer species of the assemblage (Gotelli & Colwell 2001). Therefore, there is a trade-off between sampling a large number of localities with a small sampling effort and sampling a smaller number of localities with higher sampling effort to capture as many species as possible. The first option allows sampling a wider range of environments that differ in their landscape composition and history as well as their climate, but often records a smaller number of species focused on the most common ones. The second option is restricted to a smaller number of different environments but allows collecting a higher number of species with higher chances to record rare species. Here, we opted for the second option for two reasons: (1) our goal was to assess the changes in the bumblebee assemblages, and therefore to consider rare species and not just common ones, and (2) this option was already followed in the past samplings, where only few localities were intensively sampled (Ball 1914, 1920). We targeted localities representing as much as possible the land cover diversity of Belgian biogeographical regions and for which we had the highest number of bumblebee records for the period 1910-1930 (P1). We obtained these historic

data by digitizing the F.J. Ball's collection (Ball 1914, 1920) stored at the Royal Belgian Institute of Natural Sciences (RBINS) in Brussels. The four localities (Francorchamps, Moorsel, Trivières, and Torgny) are well distributed throughout the country and represent the two main biogeographical regions of Belgium, with different altitudes (Fig. 1). Their current climatic conditions show some differences, with Francorchamps and Torgny having lower temperatures and more precipitations, and Moorsel and Trivières having higher temperatures and fewer precipitations (Table S1). As climate data were unfortunately not available in 1910-1930 (P1) in these localities, we could not consider climate variables in addition to landscape variables in our analyses.

Figure 1. Localization of the four sampling localities consisting in former municipalities and their 3 km buffer, represented with the elevational gradient and European biogeographical regions. Trivières and Saint-Vaast are merged in "Trivières", and Torgny and Lamorteau are merged in "Torgny" in analyses. Altitude is around 30 m for Moorsel, 70 m for Trivières, 240 m for Torgny, and 495 m for Francorchamps.

As the information about the localisation of old records was limited to the locality mentioned on the label pinned under the specimen, we assigned them arbitrary geographical coordinates corresponding to the center of the locality (*i.e.* the church tower of the village). For recent sampling and further analyses, we considered the most precise spatial scale possible: the former municipality including the locality (*i.e.* the municipality before the merger of Belgian municipalities between 1975 and 1983). Two neighbouring former municipalities were merged together for Trivières (Trivières and Saint-Vaast) and for Torgny (Torgny and Lamorteau) in order to have a comparable area than the other two former municipalities (Fig. 1). In these four localities, we collected bumblebees from 2013 to 2015 (P2) at a rate of three days per locality in 2013 and in 2014, and four in 2015. Sampling days were spread between March and September in order to record both early-emergence and late-emergence species, and the dates were chosen to be as close as possible to the dates sampled a century ago. We recorded bumblebees in as many habitat types as possible inside the former municipalities, consisting in 11 sites in Moorsel, 10 in Trivières, 19 in Francorchamps and 13 in Torgny (Fig. S1). The field methods used historically by the collectors who contributed to the F.J. Ball's collection are unknown but probably consisted mainly in unlimited collections with nets (Ball 1914, 1920, Rasmont pers. comm.). For recent samplings, we applied to all our sampling sites one of the most effective and widely used sampling protocol (Westphal *et al*. 2008). These samplings consisted in transect (straight-ahead) walks where all bumblebees encountered were captured with a net during suitable weather

conditions for pollinators (minimum of 15°C, no rain, dry vegetation, and a maximum of 6 on the Beaufort wind force scale). Each specimen was then identified under binocular following Rasmont & Terzo (2010). For each record, we collected the information about: (1) the bumblebee species; (2) the recorder; (3) the date, (4) the caste (queen, male, or worker) and (5) the plant species on which the specimen was observed. The list of plant species visited by bumblebees in each locality is provided in Supplementary Material (Table S2). For each sampling session, we noted the list of flowering plants and their proportion in the sampling site, as well as several attributes that could be of use to interpret the results (*e.g.* type of crop, grazing intensity, time of mowing). In total, the final dataset consisted in 16 296 specimens in 1910-1930 (P1) and 3949 in 2013-2015 (P2).

4.2. Land cover analyses

As the effects of the landscape context on bumblebees are different according to the spatial scale (Steffan-Dewenter *et al.* 2002; Westphal *et al.* 2006), we considered two buffers around the former municipality border for land cover analyses based on the foraging range of bumblebees according to several studies (Walther-Hellwig & Frankl 2000; Kreyer *et al.* 2004; Knight *et al.* 2005; Greenleaf *et al.* 2007; Osborne *et al.* 2008a; Wolf & Moritz 2008; Hagen *et al.* 2011; Carvell *et al.* 2012): a buffer of 1 km, representing the maximum foraging range size of small species (*e.g. Thoracobombus* species), and a buffer of 3 km, consisting in the maximum foraging range size of bigger species (*e.g. B. terrestris* and *B. lapidarius*).

For the 1910-1930 period (P1), we georeferenced and vectorised topographic maps at the scale of 1:20.000 from the "Institut Cartographique Militaire" (Fig. S2a, c), obtained from the actual "Institut Géographique National" (IGN) of Belgium. As Torgny is at the border of France (Fig. 1), we georeferenced and vectorised aerial photographs from the year 1930 from the French IGN in order to obtain land cover information for the buffers of 1 and 3 km (Fig. S2c). For ancient topographic maps and aerial photographs, we manually classified land cover into eight types, based on photo-interpretation (Fig. S2b and d): cropland (including plants nursery and small market gardener), grassland (including hayfields), heathland (including moorland and peatland), gardens (including parks and lawns), orchards, settlement areas (including buildings, roads, and any bare surface), woodland (including brushwood), and others (water, marsh and swamp, rock, quarry, and sand).

For the recent period (P2), we used the "Top10Vector" from the IGN, which is a vector dataset containing the most geometrically accurate topographic data from Belgium for the year 2010 and consisting in 37 land cover types. We reclassified these land cover types into the same eight types than the earlier period. For the French part of Torgny, we used orthophotos from the French IGN for the year of 2012 that we manually vectorised and classified into the eight land cover types based on photo-interpretation.

For each locality, we calculated one landscape composition metric, the relative area of each land cover type (*i.e.* the area of each land use type divided by the total area), as well as three structure metrics, *i.e.*: mean patch area, number of patches,

and edge-length (*i.e.* the perimeter of woodland). As vector maps of the recent time period are much more detailed than the historic topographic maps, they are divided in a higher number of patches. Therefore, comparison of structure metrics can be carried out between localities but not between time periods. Moreover, the size of roads and urban areas are overestimated in ancient topographic maps, leading to an overestimation of settlement area in P1 and thus a probable underestimation of urbanisation between P1 and P2. All geographic analyses were performed using ArcGIS 10 software (ESRI 2011).

In order to have a proxy of land use intensity in each locality and each time period, we collected several additional data (Ministère de l'Agriculture et des Travaux Publics 1913; Ministère de L'Intérieur 1920; Belgian Federal Government 2017): population density (*i.e.* the number of inhabitants per hectare), proportional area of fallows, proportional area of some crops (cereals, sugar beet, potatoes, and leguminous), yield of some crops (barley, spelt, sugar beet, and potatoes), and livestock number and density (number of bovines, horses, sheep, and goats, as well as number of bovines per hectare of grassland, see Table 2).

4.3. Bumblebee assemblages analyses

In order to assess the sampling completeness of each locality in each time period, we performed individual-based rarefaction curves (Gotelli & Colwell 2001). We computed the expected (*i.e.* rarefied) species richness in random subsamples of 100 specimens using the Hurlbert's index formula (Hurlbert 1971) and its standard error based on Heck *et al.* (1975), as well as the slope of the rarefaction

curves for this sample size (*i.e.* the derivative of the rarefied species richness). We also calculated several indicators of species richness and diversity in each time period, *i.e.*: species richness, Shannon's diversity index (*i.e.* the uncertainty in identifying the species of an individual taken at random from the dataset, which reflects the richness and the evenness of the assemblage), 1-Simpson index (*i.e.* the probability that two individuals drawn at random are from different species), and Berger-Parker's index (*i.e.* proportional abundance of the most dominant species; Berger & Parker, 1970). In order to assess the relative dominance of each species in each locality and for each time period and limit the influence of sampling bias related to different sampling sizes, we estimated their rank-abundance based on their relative abundance (*i.e.* the ratio between the number of specimens of the species and the number of specimens of all *Bombus* species in the same locality and time period). We plotted rank-abundance curves (RAC) scaled on the log of species abundance (Whittaker 1965). However, some bias could still remain in some species group for which the workers are often very difficult to determine, leading to frequent mistakes. This is especially the case for species belonging to the *Bombus* subgenus (*i.e*. *B. cryptarum*, *B. lucorum*, *B. magnus*, and *B. terrestris*), for which many workers were not determined in P1.

In order to quantify the compositional dissimilarity of bumblebee assemblages between localities and periods, we calculated the Bray–Curtis dissimilarity coefficient (Bray & Curtis 1957) based on the logarithm of species abundances (*i.e*. number of specimens). We then represented the resulting dissimilarity matrix using a Principal Coordinates Analysis (PCoA, also known as

Multidimensional Scaling). This distance-based statistical method allows choosing more adapted distance measurement for quantitative species data than the Euclidian distance in Principal Components Analysis or the χ^2 distance in Correspondence Analysis (Legendre & Legendre 2012). In contrast to these two distances, the Bray-Curtis dissimilarity coefficient does not take into account double-zeros and gives the same contribution to differences in abundant species than in rare species (Legendre & Legendre 2012). All analyses were performed in R with packages "vegan" (Oksanen *et al.* 2017) and "BiodiversityR" (Kindt & Coe 2005).

4.4. Relationship between landscape and bumblebee assemblages

4.4.1. Past and present landscape composition

In order to measure and describe the relationship between land cover and species' assemblages in the four localities at the two time periods, we used a technique combining multiple regression with a PCoA: the Constrained Analysis of Principal Coordinates (CAP), also called Canonical Ordination of Principal Coordinates. This analysis is a Redundancy Analysis based on the results of a PCoA (Legendre & Andersson 1999; Anderson & Willis 2003). Using the Bray-Curtis distance, this analysis has several advantages for this type of study: it assumes non-linear relationships, it does not take into account double-absences, and it gives the same contribution to differences in abundant species than in rare species (Legendre & Legendre 2012). CAP allows exploring the relationship between a response matrix (here the logarithm of bumblebee abundances matrix)

and an explanatory matrix (here the land cover matrix), where the explanatory matrix constrains the ordination axes. The relationship between these two matrices can be represented by projecting species and land cover factors onto the canonical axes, which enables visualising interactions between the species distributions and the land cover types that characterise the sites. To test the significance of this relationship, we performed permutation test for CAP under reduced model (with 1000 permutations) for the overall results and for each axis (Legendre *et al.* 2011; Legendre & Legendre 2012). As our dataset contained eight lines (4 sites x 2 periods), we could only perform analyses with one or two land cover variables per CAP. As the landscape structure metrics greatly differ between the ancient and the recent maps, we only performed analyses on the landscape composition variables (*i.e.* the total area of each land cover type except the "other" land cover type). We also performed one CAP per agricultural statistic describing the intensity of land use. As each locality is situated in a different ecological region, we quantified the explained variability of the bumblebee dataset by altitude. Altitude is highly correlated to the continuous ecological gradient found in Belgium and explains almost all the geographic structures of several ecological variables given their spatial autocorrelation (Dufrene & Legendre 1991). We partially controlled the potential effect of the region and therefore of spatial autocorrelation by using the altitude as a covariate in a partial CAP. Partial CAP (pCAP) is an extension of CAP wherein the influence of one or more variables (*i.e.* covariates) can be controlled for.

4.4.2. Present landscape composition and structure

In order to assess the relationship between bumblebees and landscape with a greater precision than in P1 and to be able to include landscape structure metrics, we performed the same analyses (CAP) on the 41 sampling sites of the recent period for which we collected at least 30 specimens (Fig. S1). We calculated the same landscape metrics than at the former municipality scale but this time in a buffer of 500 m (representing the direct nearby landscape and the mean foraging range size of most species; Osborne *et al.* 1999; Wolf & Moritz 2008; Carvell *et al.* 2012) around each sampling site. A buffer with a larger surface caused too large overlapping of the buffers around sampling sites. We performed several CAP: on the landscape composition variables (*i.e.* total area of each land cover type), on the landscape structure variables (*i.e.* mean patch area, number of patches, and edge-length of woodland), as well as on mean patch area and on number of patches. We then performed pCAP partially controlled for the effect of spatial autocorrelation by using the altitude as a covariate in the models. For additional information, we calculated expected species richness in random subsamples of 30 specimens using the Hurlbert's index formula (Hurlbert 1971) for each sampling site. We then computed Spearman rank-correlation coefficients between the expected species richness and the landscape metrics. All analyses were performed in R with packages "vegan" (Oksanen *et al.* 2017) and "BiodiversityR" (Kindt & Coe 2005).

5. Results

5.1. Changes in landscape composition

The four localities follow contrasting land cover dynamics (Fig. 2 and 3), which are slightly different between the 1 and the 3 km buffer scales (Fig. 2). Francorchamps is dominated by woodland and is the only locality showing a relatively high proportion of heathland, which however decreased by half between P1 and P2 (Table 1). Torgny is dominated by grassland, cropland and woodland. Moorsel and Trivières are dominated by cropland, gardens, grassland, and settlement areas. Cropland areas sharply decreased in all localities, with a reduction in the relative area of most crop types (Table 2), and were mainly replaced with gardens, grassland, settlement and woodland. Yield per hectare of all crop types greatly increased (Table 2), representing a strong intensification in the use of the few remaining crops. Similarly, number of cattle and livestock density increased with the area of grassland (Table 2). Population density, settlement areas and gardens increased overall, with the western localities of the country (*i.e.* Moorsel and Trivières) showing higher expansion (Fig. 2 and Table 2). Torgny presents a decline in settlement areas (Table 1), which most likely results from the overestimation of this land cover type in P1 and should be interpreted more as a stability than a decline, as reflected by the relatively stable population density (Table 2).

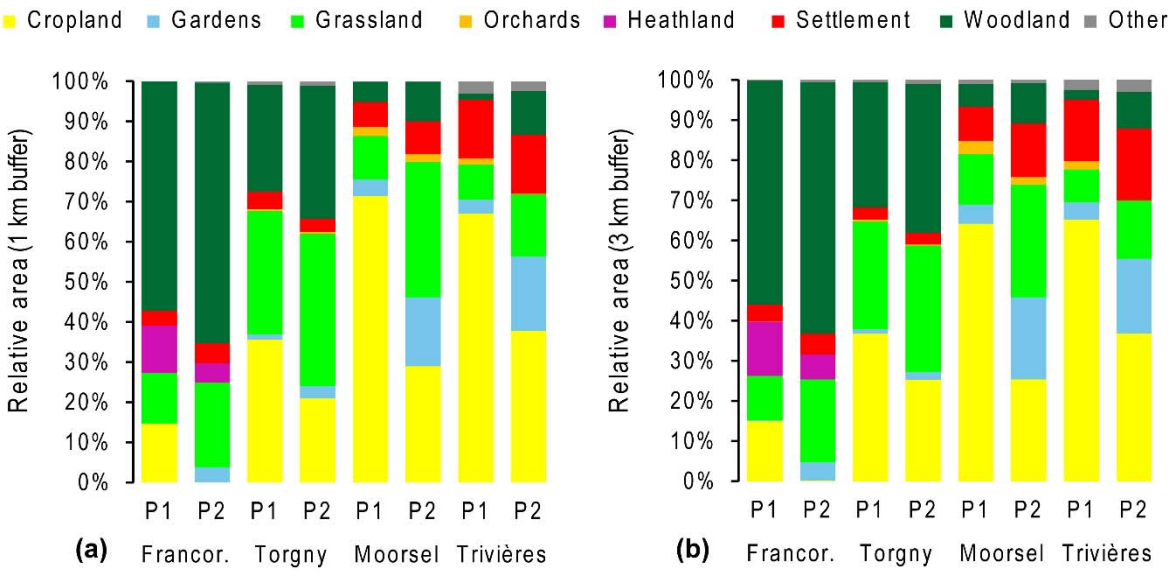

Figure 2. Relative area in a buffer of (a) 1 km and (b) 3 km around each former municipality, in P1 (1910-1930) and P2 (2013-2015). "Francor." is Francorchamps. P1 = 1910-1930, P2 = 2013-2015.

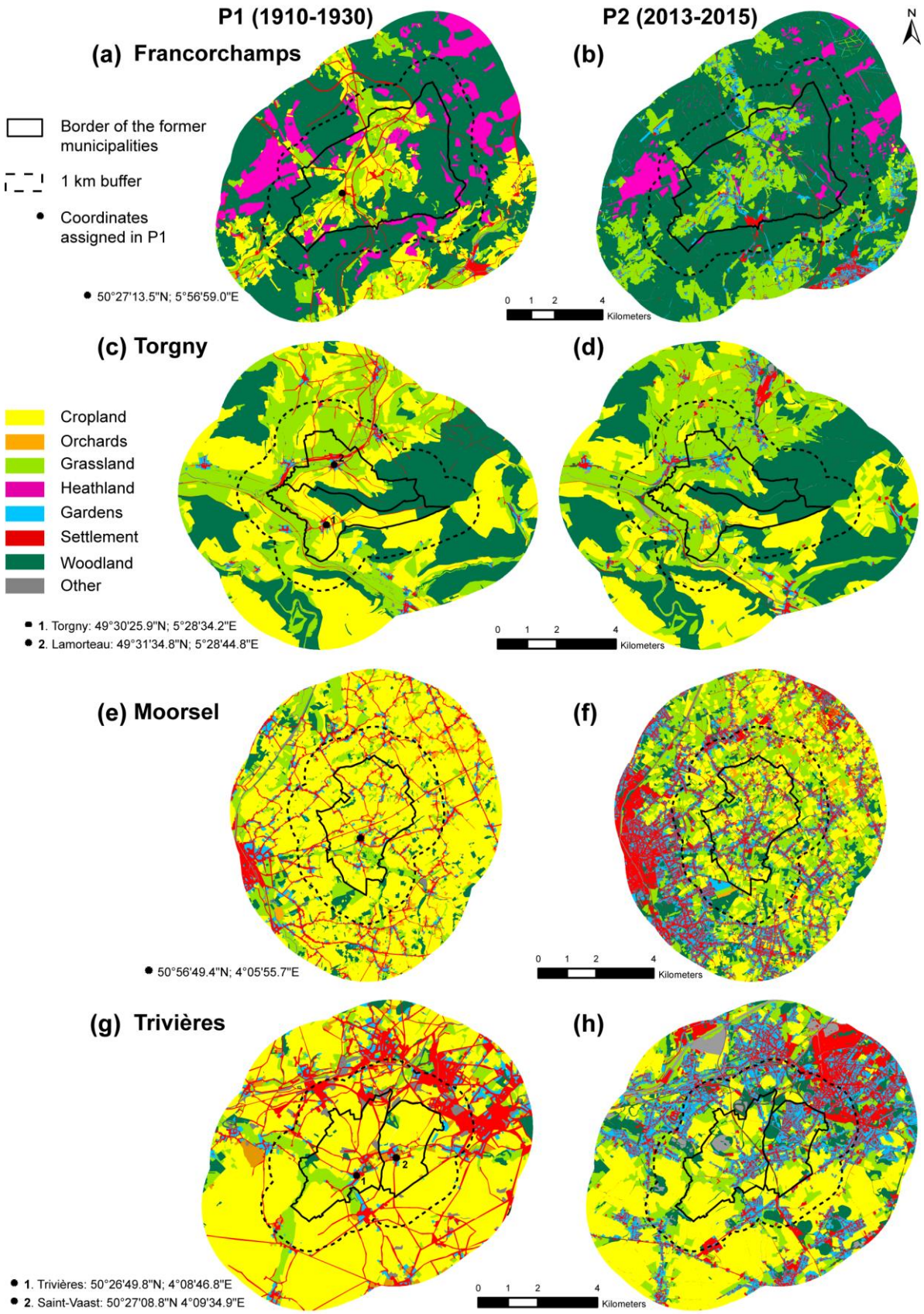

Figure 3. Land cover types in a 3 km buffer around the former municipalities of (a) Francorchamps in P1 and (b) in P2, (c) Torgny (and Lamorteau) in P1 and (d) in P2, (e) Moorsel in P1 and (f) in P2, and (g) Trivières (and Saint-Vaast) in P1 and (h) in P2.

Table 1. Area (in hectares) of each land cover type in the 1 and 3 km buffers around each former municipality, in P1 (1910-1930) and P2 (2013-2015).

	Francorchamps			Torgny			Moorsel			Trivières		
Area (ha)	**P1**	**P2**	**P2 - P1**	**P1**	**P2**	**P2 - P1**	**P1**	**P2**	**P2 - P1**	**P1**	**P2**	**P2 - P1**
1 km buffer												
Cropland	822.84	3.64	-819.21	1147.82	678.64	-469.18	1898.49	769.42	-1129.07	2119.56	1197.92	-921.64
Gardens	6.41	213.77	207.36	43.70	96.89	53.19	109.37	458.47	349.10	112.62	586.41	473.80
Grassland	712.26	1183.76	471.51	989.36	1225.05	235.69	288.41	895.30	606.90	277.43	489.22	211.79
Heathland	655.23	272.28	-382.95	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Orchards	4.84	0.00	-4.84	16.74	15.06	-1.67	61.71	51.15	-10.56	48.77	3.09	-45.68
Settlement	203.47	273.55	70.08	141.12	104.33	-36.79	164.70	219.26	54.56	459.55	466.34	6.79
Woodland	3221.40	3653.57	432.17	859.43	1068.83	209.40	133.27	262.53	129.26	50.05	347.55	297.50
Other	1.36	23.98	22.61	29.56	38.87	9.31	4.15	4.04	-0.11	97.67	75.44	-22.22
3 km buffer												
Cropland	1926.86	23.30	-1903.56	3312.50	2273.48	-1039.02	4965.30	1961.29	-3004.01	5685.50	3206.81	-2478.68
Gardens	31.00	584.02	553.02	101.80	175.03	73.24	372.30	1586.72	1214.42	375.78	1625.04	1249.26
Grassland	1420.80	2647.62	1226.83	2409.03	2836.25	427.22	969.66	2175.55	1205.88	710.68	1261.49	550.82
Heathland	1735.67	811.34	-924.33	0.00	0.01	0.01	0.00	0.07	0.07	0.00	0.00	0.00
Orchards	20.23	0.13	-20.10	38.21	22.07	-16.14	260.79	140.44	-120.35	188.19	9.72	-178.46
Settlement	519.92	651.05	131.13	277.53	263.61	-13.93	652.24	1041.21	388.97	1334.12	1562.31	228.19
Woodland	7178.66	8071.92	893.26	2803.68	3346.87	543.19	444.53	777.07	332.54	207.95	787.99	580.04
Other	27.10	67.57	40.48	59.17	84.41	25.24	77.55	60.51	-17.04	218.55	261.86	43.31

Table 2. Population density and agricultural statistics in each locality and time period.

	Francorchamps			Torgny			Moorsel			Trivières		
	P1	**P2**	**P2 - P1**	**P1**	**P2**	**P2 - P1**	**P1**	**P2**	**P2 - P1**	**P1**	**P2**	**P2 - P1**
Population density (inhabitant/ha)	0.65	0.77	0.12	0.62	0.67	0.05	7.92	10.82	2.90	10.80	11.98	1.18
Leguminous area (%)	0.52	0.00	-0.52	9.49	2.29	-7.20	7.96	0.00	-7.96	17.24	0.00	-17.24
Cereals area (%)	3.36	1.37	-1.98	27.26	0.07	-27.18	36.30	7.07	-29.23	35.37	11.81	-23.57
Potatoes area (%)	0.51	0.00	-0.51	6.07	0.03	-6.05	18.19	1.13	-17.06	2.63	2.74	0.11
Sugar beet area (%)	0.00	0.00	0.00	0.08	0.00	-0.08	0.02	0.05	0.03	9.69	2.33	-7.36
Fallows area (%)	0.00	0.00	0.00	2.60	0.13	-2.47	0.05	0.05	0.00	0.03	0.40	0.37
Yield of barley (100kg/ha)	31	95	64	21	75	54	25	98	73	31	96	65
Yield of potatoes (100kg/ha)	146	431	285	144	321	177	194	377	183	163	362	199
Yield of spelt (100kg/ha)	20	83	63	21	62	41	26	74	48	23	79	56
Yield of sugar beet (100kg/ha)	267	878	611	210	607	397	326	827	501	266	870	604
Bovines density per ha of grassland	3.06	3.22	0.16	2.13	3.50	1.37	7.42	6.49	-0.93	2.98	5.90	2.92
Number of bovines (thousands)	206.71	260.33	53.62	157.79	329.56	171.77	293.37	321.41	28.04	250.99	303.07	52.08
Number of goats (thousands)	5.58	1.54	-4.04	12.79	1.04	-11.75	53.53	8.16	-45.37	12.78	2.22	-10.56
Number of horses (thousands)	31.86	3.31	-28.55	23.03	2.40	-20.63	34.60	6.69	-27.91	57.57	3.58	-53.99
Number of sheep (thousands)	11.55	11.58	0.03	14.52	16.16	1.64	30.83	12.42	-18.41	47.13	8.09	-39.04

Concerning the landscape structure, the number of patches and mean patch area differ greatly between localities, with very similar trends between 1 and 3 km buffers (Fig. 4 and S3). In P1, Torgny and Trivières have fewer and larger patches than Francorchamps and especially Moorsel, indicating that their landscapes are less fragmented (Fig. 4a and c). This trend is conserved in P2 only for Torgny, which keeps large patches of grassland and cropland (Fig. 4b and d).

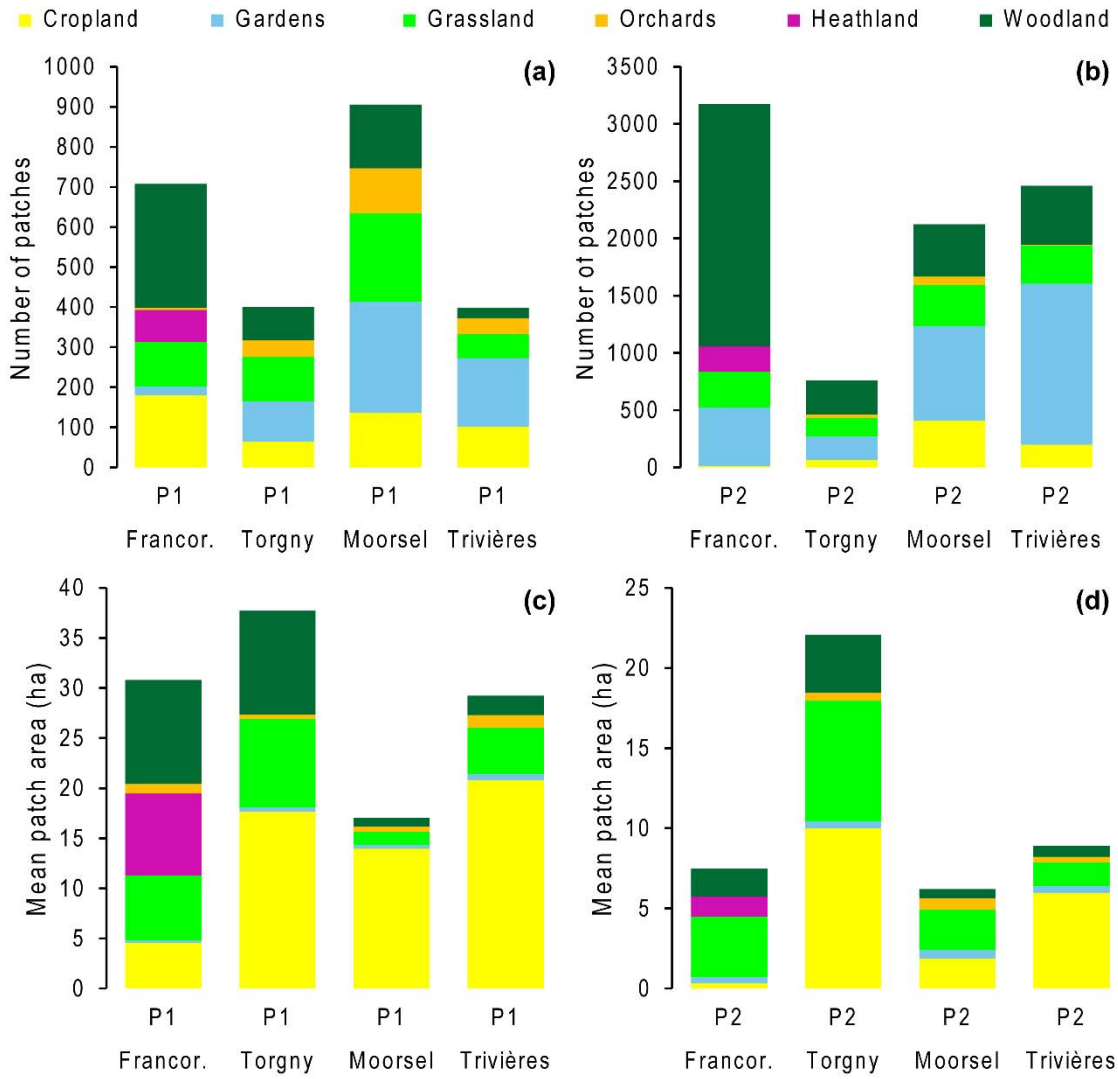

Figure 4. Number of patches in (a) P1 and (b) P2, and mean patch area in hectares in (c) P1 and (d) P2 in the 1 km buffer around each locality. "Francor." is Francorchamps. P1 = 1910-1930, P2 = 2013-2015.

5.2. Changes in bumblebee assemblages

A total of 28 species was recorded between 1910 and 1930, from 21 in Torgny to 26 in Francorchamps and Trivières (Table 3). This species richness fell to 19 species in 2013-2015, with large differences between the four localities: Moorsel and Trivières present the highest decline in species richness, whereas Francorchamps and Torgny seem to be less affected. Diversity indices show the same patterns between localities and time periods.

Table 3. Abundance, species richness, and diversity indicators in the four localities. Rarefied richness is the expected richness based on the Hurlbert's formula (Hurlbert 1971) with standard error (se) based on Heck *et al*. (1975), in a subsample of 100 specimens. Curve slope is the slope of the rarefaction curve for a sample size of 100 specimens (*i.e.* the derivative of rarefied species richness). P1 = 1910-1930; P2 = 2013-2015.

	Francorchamps		**Torgny**		**Moorsel**		**Trivières**		**Total**	
	P1	**P2**	**P1**	**P2**	**P1**	**P2**	**P1**	**P2**	**P1**	**P2**
Abundance	6627	1093	1090	1016	4524	796	4055	1044	16296	3949
Richness	26	15	21	18	25	8	26	11	28	19
Rarefied richness (± se)	10.7 (± 1.7)	10.1 (± 1.1)	11.6 (± 1.6)	15.4 (± 1.1)	17.2 (± 1.4)	6.6 (± 0.6)	14.1 (± 1.5)	7 (± 1)	18.5 (± 1.9)	12.8 (± 1.6)
Curve slope	0.044	0.019	0.040	0.029	0.037	0.006	0.036	0.015	/	/
Shannon	1.42	1.75	1.71	2.29	2.51	1.31	2.22	1.39	2.29	1.93
1-Simpson	0.58	0.74	0.71	0.85	0.89	0.60	0.85	0.68	0.81	0.77
Berger-Parker	0.63	0.44	0.48	0.27	0.24	0.61	0.22	0.47	0.38	0.42

Expected species richness in a subsample of 100 specimens decreased by almost 6 species, with a strong decline in Moorsel and Trivières, whereas it stayed stable in Francorchamps and increased in Torgny (Table 3). However, for this last locality, species rarefaction curves show an almost achieved plateau for P2 but not for P1 (Fig. 5), suggesting that sampling did probably not achieve the maximum species richness in P1. The same is observed for Francorchamps in P2, suggesting

that more species could have been found in this locality if more specimens had been sampled.

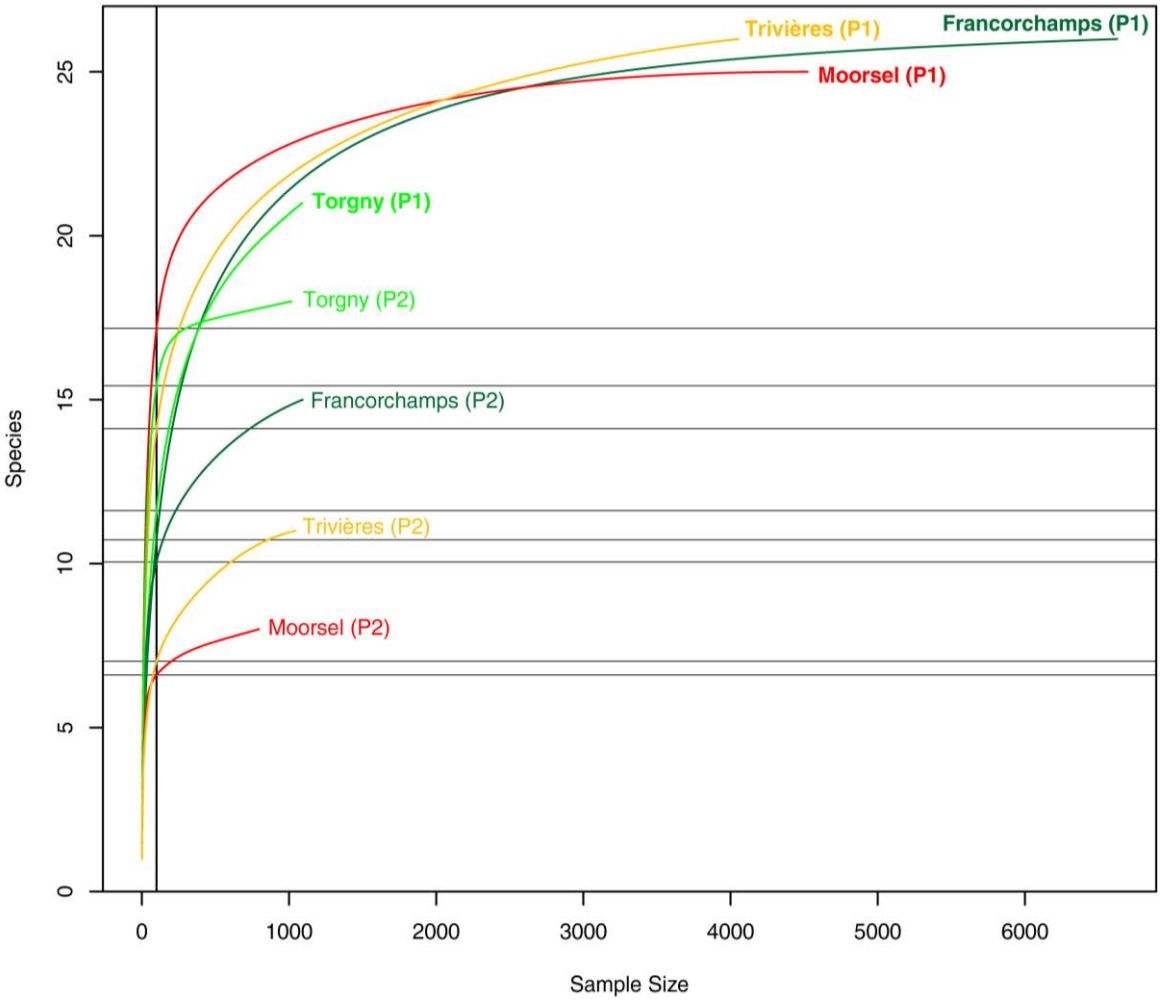

Figure 5. Individual-based species rarefaction curves for each locality in each time period, showing the relationship between the number of individuals and the number of species. The vertical line represents the expected species richness (*i.e.* Hurlbert's index) in a subsample of 100 specimens. P1 = 1910-1930; P2 = 2013-2015.

Table 4. Species abundance, proportional abundance, and rank-abundance in each locality and each time period (P1 = 1910-1930; P2 = 2013-2015). Results for species with [a] could be biased by the misidentification of workers.

	Francorchamps						**Torgny**						**Moorsel**						**Trivières**					
	Abundance		Proportion		Rank		Abundance		Proportion		Rank		Abundance		Proportion		Rank		Abundance		Proportion		Rank	
Species	**P1**	**P2**	**P1**	**P2**	**P1**	**P2**	**P1**	**P2**	**P1**	**P2**	**P1**	**P2**	**P1**	**P2**	**P1**	**P2**	**P1**	**P2**	**P1**	**P2**	**P1**	**P2**	**P1**	**P2**
B. barbutellus	17	0	0.26	0.00	15	/	4	7	0.37	0.69	13	17	108	0	2.39	0.00	12	/	11	1	0.27	0.10	18	11
B. bohemicus	47	24	0.71	2.20	10	8	11	16	1.01	1.57	9	14	2	0	0.04	0.00	24	/	0	0	0.00	0.00	/	/
B. campestris	5	4	0.08	0.37	21	11	11	11	1.01	1.08	10	16	162	1	3.58	0.13	9	8	13	2	0.32	0.19	16	8
B. confusus	5	0	0.08	0.00	22	/	1	0	0.09	0.00	18	/	9	0	0.20	0.00	21	/	2	0	0.05	0.00	22	/
B. cryptarum [a]	75	46	1.13	4.22	7	7	4	1	0.37	0.10	14	18	3	0	0.07	0.00	23	/	6	0	0.15	0.00	19	/
B. cullumanus	1	0	0.02	0.00	26	/	0	0	0.00	0.00	/	/	0	0	0.00	0.00	/	/	1	0	0.02	0.00	25	/
B. distinguendus	11	0	0.17	0.00	18	/	0	0	0.00	0.00	/	/	169	0	3.74	0.00	8	/	50	0	1.23	0.00	12	/
B. hortorum	268	47	4.04	4.31	4	6	21	38	1.93	3.74	7	6	344	33	7.60	4.15	5	6	113	24	2.79	2.30	10	6
B. humilis	2	0	0.03	0.00	25	/	8	17	0.73	1.67	11	13	4	0	0.09	0.00	22	/	433	0	10.68	0.00	4	/
B. hypnorum	190	16	2.87	1.47	5	9	1	22	0.09	2.17	19	12	373	43	8.24	5.40	4	5	2	26	0.05	2.49	23	5
B. jonellus	19	0	0.29	0.00	14	/	0	0	0.00	0.00	/	/	42	0	0.93	0.00	18	/	1	0	0.02	0.00	26	/
B. lapidarius	706	76	10.65	6.97	3	4	528	269	48.44	26.48	1	1	709	82	15.67	10.30	2	2	803	186	19.80	17.82	3	3
B. lucorum [a]	59	226	0.89	20.71	8	2	2	31	0.18	3.05	17	8	12	5	0.27	0.63	20	7	4	8	0.10	0.77	20	7
B. magnus [a]	85	0	1.28	0.00	6	/	0	0	0.00	0.00	/	/	2	0	0.04	0.00	25	/	4	0	0.10	0.00	21	/
B. muscorum	4	0	0.06	0.00	23	/	1	0	0.09	0.00	20	/	138	0	3.05	0.00	10	/	125	0	3.08	0.00	8	/
B. pascuorum	4176	484	63.01	44.36	1	1	91	195	8.35	19.19	4	2	1082	483	23.92	60.68	1	1	871	486	21.48	46.55	1	1
B. norvegicus	0	2	0	0.18	/	12	0	0	0	0	/	/	0	0	0	0	/	/	0	0	0	0	/	/
B. pomorum	0	0	0.00	0.00	/	/	5	0	0.46	0.00	12	/	0	0	0.00	0.00	/	/	137	0	3.38	0.00	6	/
B. pratorum	757	100	11.42	9.17	2	3	17	30	1.56	2.95	8	10	268	70	5.92	8.79	6	4	14	39	0.35	3.74	15	4
B. ruderarius	37	0	0.56	0.00	11	/	35	29	3.21	2.85	5	11	71	0	1.57	0.00	14	/	87	0	2.15	0.00	11	/
B. ruderatus	13	0	0.20	0.00	16	/	0	0	0.00	0.00	/	/	194	0	4.29	0.00	7	/	859	0	21.18	0.00	2	/
B. rupestris	4	1	0.06	0.09	24	14	95	47	8.72	4.63	3	5	20	0	0.44	0.00	19	/	12	0	0.30	0.00	17	/
B. soroeensis	33	1	0.50	0.09	12	15	220	31	20.18	3.05	2	9	0	0	0.00	0.00	/	/	0	0	0.00	0.00	/	/
B. subterraneus	7	0	0.11	0.00	20	/	0	0	0.00	0.00	/	/	63	0	1.39	0.00	15	/	48	0	1.18	0.00	13	/
B. sylvarum	31	0	0.47	0.00	13	/	26	49	2.39	4.82	6	4	49	0	1.08	0.00	17	/	127	0	3.13	0.00	7	/
B. sylvestris	54	8	0.81	0.73	9	10	1	16	0.09	1.57	21	15	58	0	1.28	0.00	16	/	2	2	0.05	0.19	24	9
B. terrestris [a]	13	56	0.20	5.13	17	5	4	173	0.37	17.03	15	3	113	79	2.50	9.92	11	3	123	268	3.03	25.67	9	2
B. vestalis	0	2	0.00	0.18	/	13	4	34	0.37	3.35	16	7	90	0	1.99	0.00	13	/	23	2	0.57	0.19	14	10
B. veteranus	8	0	0.12	0.00	19	/	0	0	0.00	0.00	/	/	439	0	9.70	0.00	3	/	184	0	4.54	0.00	5	/

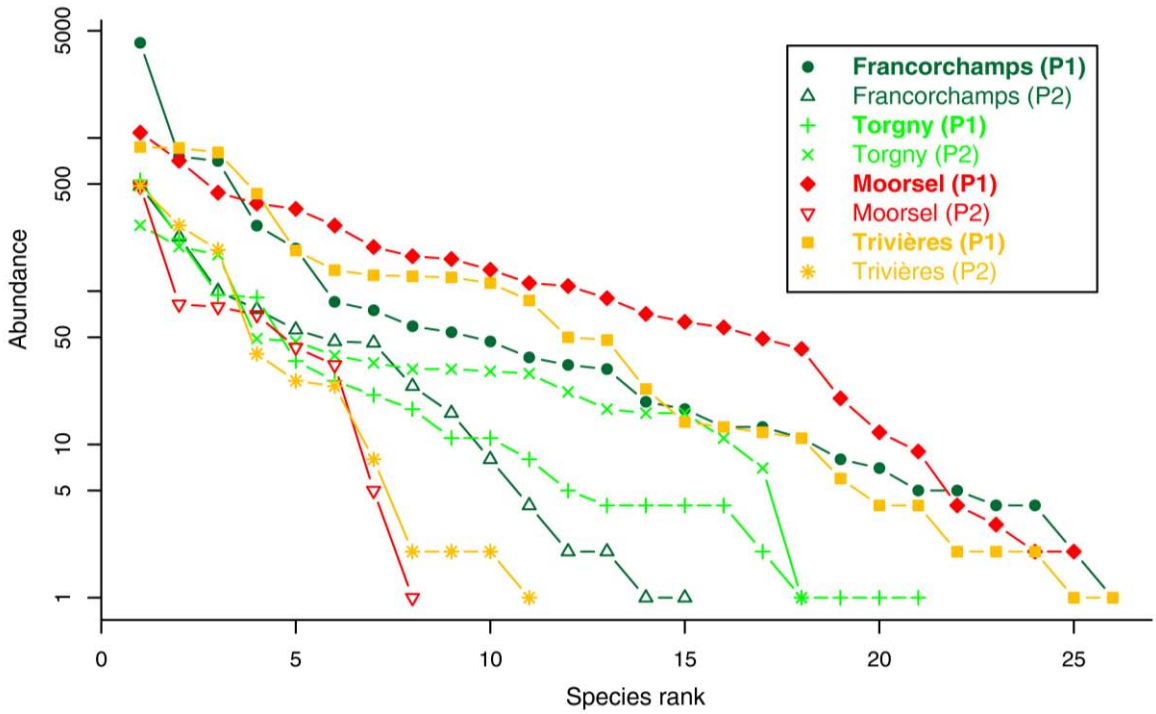

Figure 6. Rank-abundance curves for each locality in both time periods, scaled on the logarithm of the abundance. P1 = 1910-1930; P2 = 2013-2015.

Community composition and species dominance differ between localities and time periods (Table 4). In both time periods, the dominant species was *B. lapidarius* in Torgny and *B. pascuorum* in the three other localities. However, even if the dominant species remained the same in both time periods, its proportion changed in different ways according to the locality (see the Berger-Parker's index in Table 3). In Moorsel and Trivières, the proportional abundance of the dominant species increased between P1 and P2, whereas it decreased in Francorchamps and Torgny. More generally, rank-abundance curves show a steeper decline in P2 than in P1 in all localities (Fig. 6), indicating lower species evenness in the recent time period, with the exception of Torgny.

Table 5. Bray-Curtis dissimilarity coefficient calculated between sites and time periods. A value of 0 indicates a perfect similarity between assemblages, and a value of 1 indicates sites with no common species. "Francor." = Francorchamps. P1 = 1910-1930; P2 = 2013-2015. Numbers in bold are < 0.5.

		P1				**P2**		
		Francor.	Torgny	Moorsel	Trivières	Francor.	Torgny	Moorsel
P1	Torgny	0.633						
	Moorsel	0.500	0.683					
	Trivières	0.598	0.638	**0.459**				
P2	Francor.	0.632	0.671	0.717	0.747			
	Torgny	0.580	**0.458**	0.601	0.621	0.560		
	Moorsel	0.737	0.732	0.752	0.769	0.510	0.642	
	Trivières	0.725	0.688	0.727	0.733	**0.497**	0.579	**0.337**

Bray-Curtis dissimilarity coefficients are higher between the two time periods than between the localities in the same time period, except for Torgny (Table 5). This indicates that species composition differs more between time periods than between sites for Francorchamps, Moorsel and Trivières. In both P1 and P2, Moorsel and Trivières' bumblebee assemblages are the most similar. On the contrary, Torgny has a higher similarity between P1 and P2 than with other sites in the same time period. These dissimilarities are easily visible on the PCoA graph (Fig. 7), which shows that only Torgny kept a similar bumblebee assemblage than the ones found in the four localities a century ago.

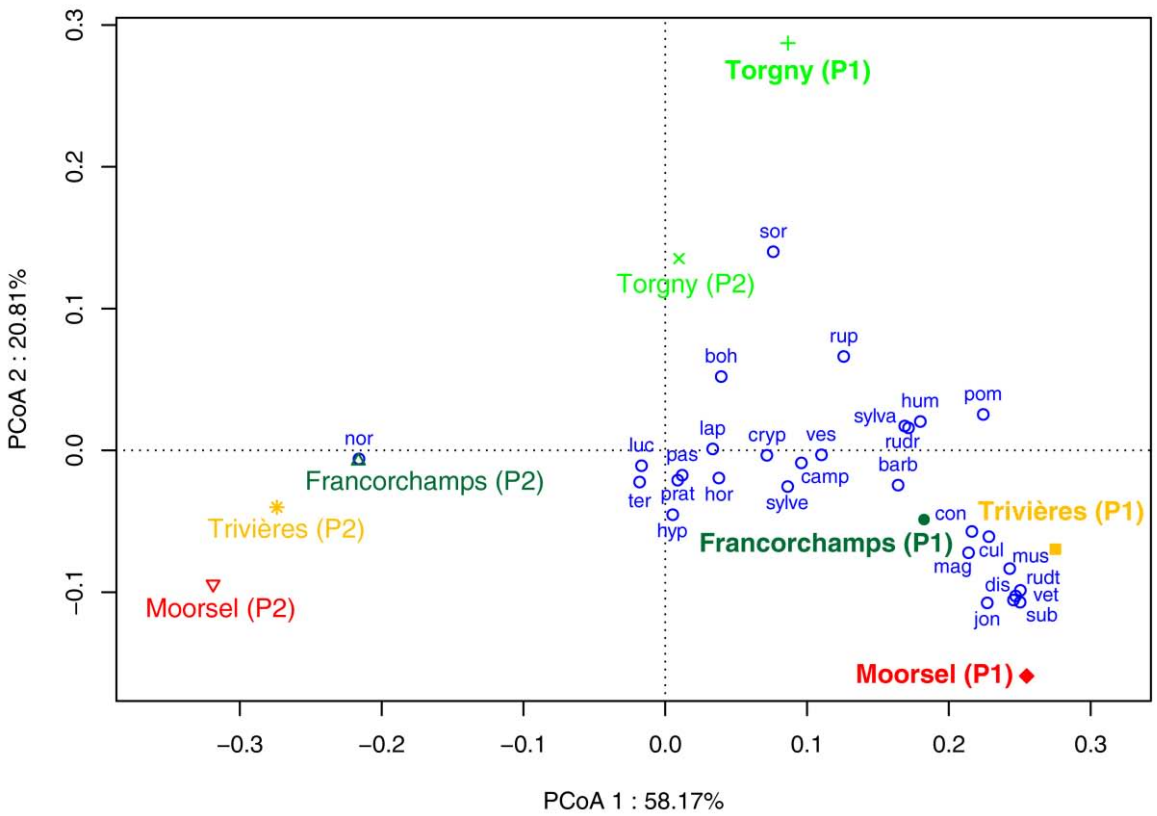

Figure 7. Principal Coordinate Analysis graph of the Bray–Curtis dissimilarity matrix based on the logarithm of species abundances, with *a posteriori* projection of species (in blue) as weighted averages of their contributions to the sites. The two first components account for 78.98% of the total variance. barb = *B. barbutellus*, boh = *B. bohemicus*, camp = *B. campestris*, con = *B. confusus*, cryp = *B. cryptarum*, dis = *B. distinguendus*, hor = *B. hortorum*, hum = *B. humilis*, hyp = *B. hypnorum*, jon = *B. jonellus*, lap = *B. lapidarius*, luc = *B. lucorum*, mag = *B. magnus*, mus = *B. muscorum*, nor = *B. norvegicus*, pas = *B. pascuorum*, pom = *B. pomorum*, prat = *B. pratorum*, rudr = *B. ruderarius*, rudt = *B. ruderatus*, rup = *B. rupestris*, sor = *B. soroeensis*, sub = *B. subterraneus*, sylva = *B. sylvarum*, sylve = *B. sylvestris*, ter = *B. terrestris*, ves = *B. vestalis*, vet = *B. veteranus*.

5.3. Relationship between landscape and bumblebee assemblages

5.3.1. Past and present landscape composition

We estimated the common variance between species and land cover, which represents here the variation in bumblebee assemblages' composition determined by the proportional area a land cover type alone or in association with the proportional area of a second land cover type, by using Constrained Analysis of

Principal Coordinates (Table 6). In the 1 km buffer, settlement and cropland explain significantly the bumblebee assemblages at a p-value of 0.05. In order to separate their relative effects, we performed partial CAP. The pCAP with cropland as explaining variable and settlement as covariate does not explain significantly the bumblebees' assemblages (F = 1.57, p-value = 0.22). The complementary pCAP shows that the variability explained by settlement drops from 45.9% to 23.6% when we remove the effect of cropland and that this relationship is no more significant at the p-value of 0.05 (F = 2.87, p-value < 0.1). Grassland and woodland assessed separately do not explain significantly bumblebees' assemblages at a p-value of 0.05 (but at a p-value of 0.1). But, put together, they explain significantly almost 59% of the bumblebee assemblages' variability. Results are less marked at the 3 km buffer scale (Table 6).

Table 6. Variability of the bumblebee assemblages explained by each land cover type, alone or in association with a second land cover type, in the 1 and 3 km buffers. "N.S." = non-significant at a p-value of 0.1. P-values "." < 0.1, **"*" < 0.05**, **"**" < 0.01**.

1 km buffer	Cropland	Settlement	Gardens	Orchards	Grassland	Heathland	Woodland
Alone	**35.2% ***	**45.9% ****	N.S.	N.S.	33.1% .	N.S.	31.8% .
+ Cropland	-	**58.8% ****	50.0% .	N.S.	50.7% .	46.1% .	N.S.
+ Settlement	**58.8% ****	-	**52.2% ***	**53.6% ***	**61.3% ***	51.6% *	52.5% .
+ Gardens	50.0% .	**52.2% ***	-	N.S.	**54.0% ***	N.S.	N.S.
+ Orchards	N.S.	**53.6% ***	N.S.	-	N.S.	N.S.	N.S.
+ Grassland	50.7% .	**61.3% ***	**54.0% ***	N.S.	-	**59.5% ***	**58.8% ***
+ Heathland	46.1% .	**51.6% ***	N.S.	N.S.	**59.5% ***	-	45.0% .
+ Woodland	N.S.	52.5% .	N.S.	N.S.	**58.8% ***	45.0% .	-
3 km buffer	Cropland	Settlement	Gardens	Orchards	Grassland	Heathland	Woodland
Alone	33.4% .	**44.7% ***	N.S.	N.S.	32.7% .	N.S.	35.7% .
+ Cropland	-	**60.2% ****	50.1% .	N.S.	50.1% .	N.S.	N.S.
+ Settlement	**60.2% ****	-	**58.0% ****	**54.1% ***	**60.4% ***	**51.7% ***	**53.3% ***
+ Gardens	50.1% .	**58.0% ****	-	N.S.	**53.3% ***	N.S.	N.S.
+ Orchards	N.S.	**54.1% ***	N.S.	-	N.S.	N.S.	N.S.
+ Grassland	50.1% .	**60.4% ***	**53.3% ***	N.S.	-	**60.5% ***	**58.3% ***
+ Heathland	N.S.	**51.7% ***	N.S.	N.S.	**60.5% ***	-	49.5% .
+ Woodland	N.S.	**53.3% ***	N.S.	N.S.	**58.3% ***	49.5% .	-

Concerning the statistics describing land use intensity (Table 2), two variables explain significantly the variability observed in bumblebees: population density, which explains 45% ($F = 4.96$, p-value < 0.05), and proportional area of sugar beet which explains 28% ($F = 2.35$, p-value < 0.05). Altitude does not explain significantly the composition of bumblebee assemblages between the four localities in the two time periods.

5.3.2. Present landscape composition and structure

In a 500 m buffer around the recent sampling sites, expected species richness in a subsample of 30 specimens is positively correlated to total area and mean patch area of grassland and woodland, and negatively correlated to total area and number of patches of cropland, gardens and settlement, as well as to mean patch area of cropland (Table 7). However, mean patch area of settlement is positively correlated to expected species richness. In other words, landscapes with fewer and larger patches of settlement but representing a small proportion (*i.e.* total area) of the landscape are correlated to a higher species richness than landscapes with many patches of settlement covering a wider area.

Table 7. Spearman rank-correlation coefficients between rarefied species richness (*i.e.* expected number of species in a subsample of 30 specimens) and landscape metrics. "N.S." = non-significant correlation (p-value > 0.05); p-values: **"*" < 0.05**, **"**" < 0.01**, **"***" < 0.001**.

Landscape metrics	Land cover types	Correlation with rarefied species richness	
Total area	Cropland	**-0.46**	**
	Grassland	**0.33**	*
	Heathland	0.19	N.S.
	Gardens	**-0.37**	*
	Orchards	-0.05	N.S.
	Settlement	**-0.45**	**
	Woodland	**0.36**	*
Edge-length	Edge of woodland	0.14	N.S.
Mean patch area	Cropland	**-0.45**	**
	Grassland	**0.38**	*
	Heathland	0.21	N.S.
	Gardens	-0.05	N.S.
	Orchards	-0.02	N.S.
	Settlement	**0.34**	*
	Woodland	**0.45**	**
Number of patches	Cropland	**-0.62**	***
	Grassland	-0.17	N.S.
	Heathland	0.19	N.S.
	Gardens	**-0.42**	**
	Orchards	-0.08	N.S.
	Settlement	**-0.44**	**
	Woodland	0.13	N.S.

The results of Constrained Analyses of Principal Coordinates, which estimate the common variance between species and landscape matrices, are given in table 8.

Table 8. Variability of the bumblebee assemblage composition explained by landscape metrics as well as altitude. Each line is a different CAP. "N.S." = non-significant at a p-value of 0.1. P-values: "." < 0.1, **"*" < 0.05**, **"**" < 0.01**, **"***" < 0.001**.

	Variability explained				With altitude as covariate			
	%	F	p-value		%	F	p-value	
All landscape metrics	**63.42**	1.34	0.001	***	54.12	1.17	0.065	.
Landscape composition (total area)	**23.31**	1.39	0.005	**	17.85	1.13	0.162	N.S.
Landscape structure	**44.27**	1.27	0.007	**	35.79	1.05	0.277	N.S.
Edge-length	**5.80**	2.34	0.002	**	2.92	1.27	0.168	N.S.
Mean patch area	**24.25**	1.46	0.004	**	17.29	1.09	0.245	N.S.
Number of patches	**22.52**	1.33	0.009	**	16.63	1.03	0.381	N.S.
Altitude	**12.15**	5.26	<0.001	***	-	-	-	-

The CAP based on all landscape metrics shows that landscape composition and structure in a buffer of 500m around the sites explain significantly 63% of the variability in species distribution between the sites (Table 8). The two first canonical axes are significant (CAP1: F = 6.61, p-val = 0.001, CAP2: F = 4.60, p-val = 0.001) and represent together 24% of the variance explained (Fig. 8). The first axis mainly represents a gradient from wooded landscapes mixed with heathland and grassland to landscapes dominated by cropland and settlement with gardens. The second axis mainly represents a gradient from landscapes constituted of a high number of small grassland patches and wooded patches with a high density of edge to landscapes dominated by orchards and large patches of grassland. Projecting the species in the space defined by the two first canonical axes shows some species preferences in terms of landscape composition and structure (Fig. 8). *B. terrestris* and *B. lapidarius* tend to be the most observed in landscapes dominated by cropland and settlement with gardens, whereas *B. lucorum*, *B. cryptarum*, and *B. bohemicus* are mainly observed in woody landscapes with heathland found in the Francorchamps' sampling sites. *B. rupestris*, *B. sylvarum*, and *B. soroeensis* tend to be related to sampling sites rich in orchards and grassland found in Torgny.

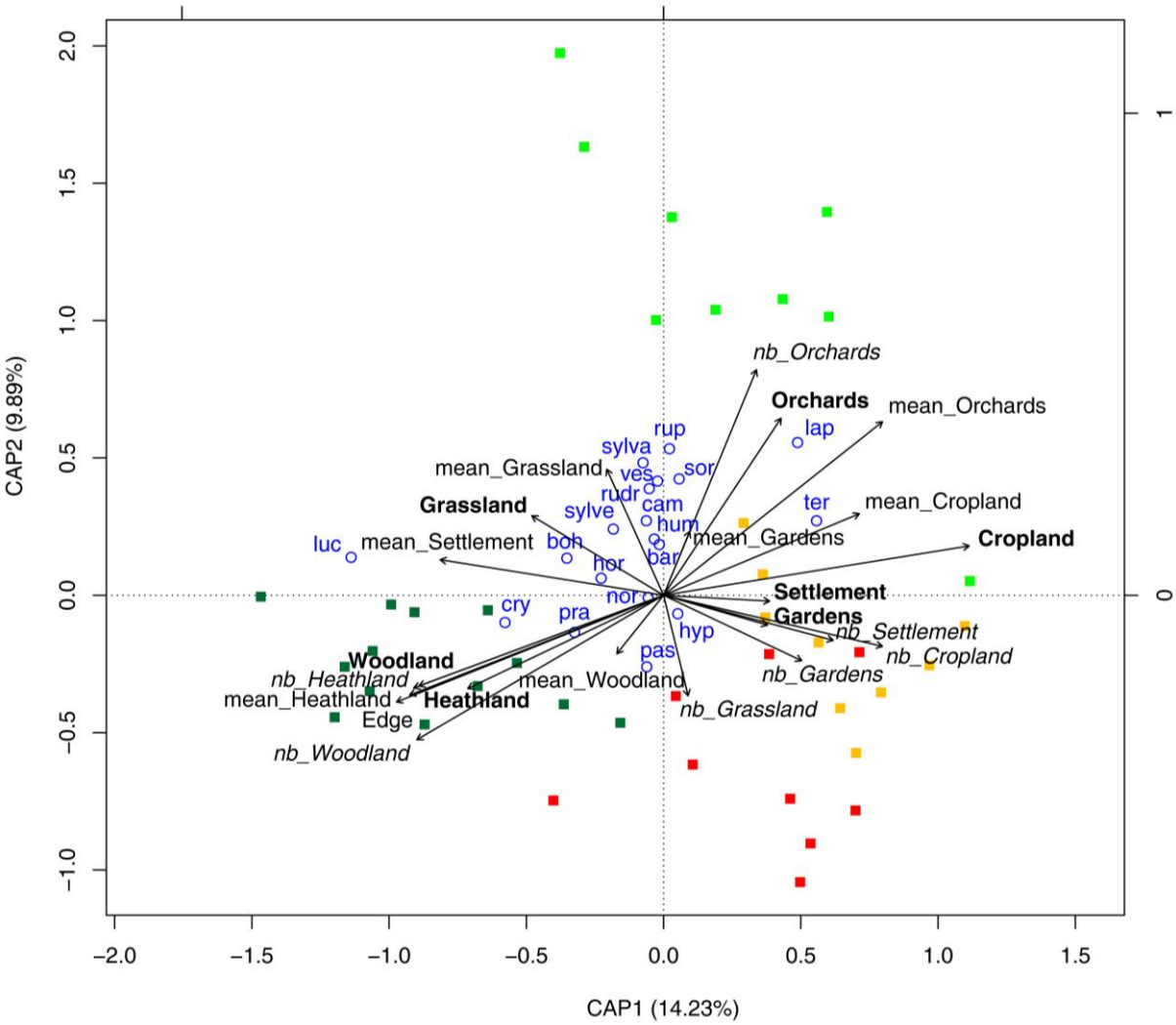

Figure 8. CAP projection of the species abundances (in blue) constrained by the landscape metrics in a buffer of 500 m (in black), as well as the sampling sites of the recent time period, on the two first axes. The bottom and left-hand scales are for the sampling sites and the response variables (*i.e.* species), the top and right-hand scales are for the explanatory variables (*i.e.* landscape metrics). Francorchamps' sampling sites are in dark green, Torgny's in light green, Moorsel's in red, and Trivières' in orange. Landscape metrics in bold are total area, landscape metrics with "mean_..." are mean patch area, and landscape metrics in italic with "nb_..." are number of patches. bar = *B. barbutellus*, boh = *B. bohemicus*, cam = *B. campestris*, cry = *B. cryptarum*, hor = *B. hortorum*, hum = *B. humilis*, hyp = *B. hypnorum*, lap = *B. lapidarius*, luc = *B. lucorum*, nor = *B. norvegicus*, pas = *B. pascuorum*, pra = *B. pratorum*, rudr = *B. ruderarius*, rup = *B. rupestris*, sor = *B. soroeensis*, sylva = *B. sylvarum*, sylve = *B. sylvestris*, ter = *B. terrestris*, ves = *B. vestalis*.

Altitude also explains significantly the bumblebee distribution across sampling sites (Table 8). When we remove the effect of altitude in a pCAP, the bumblebee variability explained by landscape metrics is no longer significant at the p-value of 0.5 (Table 8).

6. Discussion

6.1. Changes in bumblebees' assemblages and landscape

Our results show that bumblebee species richness severely decreased in our four studied localities, from 28 to 19 species between a century ago and today. These results follow the global trends observed in the whole country and in Europe (Rasmont *et al.* 1993; Kosior *et al.* 2007; Carvalheiro *et al.* 2013). A decrease of species richness and shifts in pollinator communities were also observed in 79% of the studied localities in England between 1930 and 2007 (Senapathi *et al.* 2015). In our results, the most severe decline is observed in the two western localities (Moorsel and Trivières), whereas the two eastern localities (Torgny and Francorchamps) tend to have a stable or even a higher diversity than in the past. The dominant species (*i.e. Bombus lapidarius* in Torgny and *B. pascuorum* in others) remains the same in each locality between the two time periods but its dominance (*i.e.* relative abundance) increases in the western and decreases in the eastern localities. Except for Torgny, dissimilarities between past and present bumblebee assemblages in the same locality are higher than dissimilarities between localities in the same time period, indicating a strong change in bumblebees' assemblages composition since a century ago.

Like bumblebee assemblages, land cover and land use drastically changed between the recent period and a century ago in most localities, with a similar global pattern than between 1930 and 2007 in England (Senapathi *et al.* 2015). Landscapes of a hundred years ago were dominated by low productive crops,

grasslands with low livestock density, orchards, and woodland sometimes associated to heathland. Settlement and gardens were covering a relatively lower surface. During the last century, cropland areas sharply declined but the use of remaining crops intensified with a threefold increase of yield per hectare (Table 2). Woodland and grassland areas increased but cattle density per grassland area increased as well, reflecting a greater risk of overgrazing. As in England (Senapathi *et al.* 2015), heathland areas in Francorchamps decreased by half. Settlement areas and gardens expanded coupled with an increase in population density. These changes differed markedly between localities, with the western localities being the most urbanised. On the other hand, the locality of Torgny is the less affected by land cover and land use changes and its human population density barely increased. In our analyses, two land cover types explain significantly the variability observed in bumblebee assemblages between localities and time periods: cropland and settlement areas (which explain respectively 35% and 46% as well as 59% together in the 1 km buffer). These two land cover types show the most important changes in the localities where bumblebee assemblages changed the most. These results confirm the importance of urbanisation and changes in agriculture in explaining the changes in bumblebee assemblages as it is the case in England (Senapathi *et al.* 2015) or in other studies with a more limited time scale (Mänd *et al.* 2001; Carvell 2002; Goulson *et al.* 2006; Xie *et al.* 2008; Ahrné *et al.* 2009; Le Féon *et al.* 2010; Potts *et al.* 2010; Ollerton *et al.* 2014).

In the recent time period, our results show that landscape composition and structure around the sampling sites explain more than 63% of the variability in

bumblebee assemblages recorded in the sites. As several studies (Pywell *et al.* 2006; Öckinger & Smith 2006; Hatfield & LeBuhn 2007; Ahrné *et al.* 2009; Le Féon *et al.* 2010; Diaz-Forero *et al.* 2013; Deguines *et al.* 2016), we show that bumblebee species richness is higher in landscapes dominated by large patches of grassland than in landscapes dominated by settlement, gardens, and a high number of large patches of cropland. Moreover, species richness is positively correlated to the total area as well as the mean patch area of woodland. Francorchamps and Torgny are the two richest localities in grassland and woodland and harbour the highest species richness today. However, in regions highly dominated by woodland, the proportion of woodland around grasslands tend to be negatively correlated to bumblebee abundance and species richness, probably because woods do not present high amount of floral resources for bumblebees and their high proportion could therefore restrain their foraging area (Winfree *et al.* 2007; Diaz-Forero *et al.* 2013). The highly wooded locality of Francorchamps harbours indeed less species than Torgny and hosts the most generalist species (e.g. *B. pascuorum*, *B. terrestris*, *B. lapidarius*), few species preferring wooded habitats (*e.g. B. lucorum*), and few rarer species present thanks to its remaining heathland (*i.e. B. cryptarum*, *B. soroeensis*). Landscapes too closed by woodland do not seem very favourable to accommodate a great diversity of bumblebee species, but a small proportion of woodland around open habitats (*i.e.* grassland and heathland) is still beneficial for many species because they (and their edges) are good nesting and overwintering sites (Diaz-Forero *et al.* 2011, 2013). Like some other studies (Ahrné *et al.* 2009; Deguines *et al.* 2016), our

results show a negative correlation between species richness and the total area and number of patches of settlement and gardens. However, mean patch area of settlement is positively correlated to species richness. This could be explained by the fact that the two richest localities (Torgny and Francorchamps) present larger (but still very few) patches of settlement, whereas the two others present smaller patches of settlement but in higher numbers. Large patches of settlement do not seem to threaten bumblebees in regions with low level of urbanization, especially in the most wooded ones such as Francorchamps, as found by several other studies (Winfree *et al.* 2007; Goulson *et al.* 2010; Diaz-Forero *et al.* 2013). As suggested by Winfree *et al.* (2007), the deleterious effects of settlements may arise only when the proportion of natural habitats is very low and when the total area of settlements increases too much to the detriment of these natural habitats, as in Moorsel and Trivières.

6.2. Relationship between bumblebee species and landscape

Bumblebee species tend to have different preferences in terms of habitat and therefore in terms of landscape composition and structure (Rasmont & Mersch 1988; Svensson *et al.* 2000; Westphal *et al.* 2006; Goulson *et al.* 2006), and their sensitivity to land use change tend to be influenced by their ecological traits (Williams *et al.* 2010; De Palma *et al.* 2015). In our analyses based on the recent period, we show that some species tend to be more related to particular land cover types than others. Species known to be specialized on heathland (*i.e. B. cryptarum*, *B. jonellus*, and *B. magnus*; Goulson *et al.* 2006; Kleijn & Raemakers

2008; Moquet *et al.* 2017b) were more abundant in the only locality presenting heathland (*i.e*. Francorchamps) than in the others 100 years ago. Today, only *B. cryptarum* is still found (and even abundant) in this locality, and (almost) disappeared from the others. *B. jonellus*, which is also drastically decreasing at the Belgian scale (Rasmont *et al.* 1993), is more specialized on Ericaceae found in heathland than other species (Kleijn & Raemakers 2008; Moquet *et al.* 2017b). The few remaining heathlands in Francorchamps could no longer have been sufficient for the maintenance of this species. Heathlands, more precisely Ericaceae, are known to be important for several bumblebee species, especially in early spring and in late summer when other flower resources are scarce (Moquet *et al.* 2017a). However, like in Francorchamps, they drastically decreased in Europe following changes in agricultural practices, from extensive grazing mainly by sheep during the 18th and 19th centuries to soil dewatering and enriching with fertilizers for agricultural use or spruce plantations during the 20th century (Aerts & Heil 1993; Webb 1998). Species preferring wooded habitats benefit from landscapes presenting a high proportion of woodland (Diaz-Forero *et al.* 2011), such as *B. norvegicus*, *B. lucorum* and *B. bohemicus* in Francorchamps. The high increase of *B. lucorum* in this locality could be explained by its clear preference for wooded habitats and forest edges (Svensson *et al.* 2000; Rasmont *et al.* 2015). Probably as a result, its parasite, *B. bohemicus*, is also mostly found in Francorchamps, where it increased as well. However, wooded landscapes may be detrimental for species preferring open lands, such as *B. humilis*, *B. ruderarius*, *B. rupestris*, *B. soroeensis*, and *B. sylvarum* associated to grasslands, as well as *B.*

veteranus in other studies (Rasmont & Mersch 1988; Diaz-Forero *et al.* 2011). Furthermore, species with large foraging range tend to be negatively affected by wooded landscapes (Diaz-Forero *et al.* 2011), such as *B. terrestris* and *B. lapidarius*, maybe because a high proportion of woodland could represent potential barriers for these species with larger foraging distances (Walther-Hellwig & Frankl 2000; Kreyer *et al.* 2004; Goulson *et al.* 2010). Several studies (*e.g*. De Palma *et al.* 2015; Deguines *et al.* 2016) showed that generalist species, such as *B. hypnorum*, *B. lapidarius*, *B. pascuorum* and *B. terrestris*, tend to be less sensitive to agriculture intensification and to urbanisation and can still be found in high numbers in urbanised and intensive cropland landscapes. These species were indeed among the only ones still observed in Moorsel and Trivières in the recent samplings. In a study on European agricultural landscapes, Carré *et al.* (2009) showed that landscape context in a 3 km buffer around crop fields explained bumblebee diversity and abundance found in the fields differently between subgenus. They showed that the *Megabombus* subgenus (*e.g. B. hortorum*) tended to be positively impacted by semi-natural habitats (*i.e.* broad-leaved forest and shrubs patches) and the *Bombus* subgenus (*e.g. B. terrestris*) by urban areas, but that the *Thoracobombus* subgenus (*e.g. B. pascuorum*) was not affected by the landscape context (Carré *et al.* 2009). In our results, species preferences cannot be generalized to subgenus because of the high differences between species within subgenus. For example, within the *Bombus* subgenus, *B. terrestris* seems indeed to be related to urban and cropland areas in our results, but *B. lucorum* and *B. cryptarum* are more related to woody landscapes with

heathland. This is the same for the *Thoracobombus* subgenus, with *B. pascuorum* being present everywhere and the others (*e.g. B. humilis, B. sylvarum, B. ruderarius*) being rarer and more related to grasslands. These differences in the sensitivity of species to landscape composition could imply that urbanisation and agricultural intensification change bumblebee assemblage composition leading to a domination by common species, less sensitive to land use changes or favoured by urban and crop habitats (Carré *et al.* 2009; Deguines *et al.* 2016).

6.3. Conservation recommendations

Like many studies (Mänd *et al.* 2001; Carvell 2002; Goulson *et al.* 2006; Xie *et al.* 2008; Ahrné *et al.* 2009; Le Féon *et al.* 2010; Potts *et al.* 2010; Ollerton *et al.* 2014; Senapathi *et al.* 2015), our results suggest that urbanisation and intensification of agriculture could threaten bumblebees. In the especially urbanised and intensive agricultural landscapes like Moorsel and Trivières, it is important to conserve elements that supply floral resources and nest sites for all bumblebee species in order to avoid the homogenization of bumblebee assemblages as we observe today. In these two localities, our field observations revealed that the few places where floral resources and bumblebees can still be found are in some well-flowered gardens, some vacant lots with ruderal vegetation, some small vegetable crops, some orchards, and in some field margins and path edges, even if these latter are regularly cut, covered often with nitrophilous plants (*e.g*. *Urtica dioica*) and sometimes with invasive plants (*i.e. Reynoutria japonica* in Moorsel). In Trivières, the sampling sites with the highest abundance and diversity of

bumblebees are around a heap under early spontaneous succession (sites TRI01 and TRI02, Fig. S1d). Heaps are known to be rich in bee species and to host many rare species, thanks to their high flowering plants diversity (Rasmont & Barbier 1990, 2000; Tropek *et al.* 2013; Hendrychová & Bogusch 2016). Heaps under spontaneous succession tend to host a higher diversity and more rare species than heaps reclaimed, by *e.g.* planting trees (Hendrychová & Bogusch 2016). We found indeed that this type of heap shows a high abundance in floral resources throughout the flight season of bumblebees (*e.g. Ballota nigra*, *Carduus* spp., *Cirsium* spp., *Dipsacus fullonum*, *Echium vulgare*, *Epilobium* spp., *Lathyrus* spp., *Lotus corniculatus*, *Medicago* spp., *Prunus* spp., *Rubus* spp., *Salix* spp., *Trifolium* spp., *Vicia* spp.), contrary to another heap entirely wooded also present in the locality. It is clear that this heap with natural succession acts as a refuge for native flora and bumblebees in Trivières, confirming that spontaneously developing ecosystems in general have major importance in nature conservation (Prach *et al.* 2011; Tropek *et al.* 2013; Hendrychová & Bogusch 2016), especially in the very anthropic regions like Trivières and its surroundings.

Even if grassland increased between a century ago and today, cattle density also increased. The present pastures and meadows in Moorsel and Trivières are mainly temporary and intensive, and they did not contain a lot of floral resources for bumblebees during our sampling sessions. Nitrogen fertilizers and herbicides used in crops and grassland indirectly affect bumblebees by reducing the diversity of plants (Kleijn *et al.* 2009), and therefore the diversity and availability of foraging resources (Roulston & Goodell 2011), such as thistles which are very

important for male bumblebees' diet (Vray *et al.* 2017). Maintaining a high abundance and species richness of bumblebees requires a large proportion of grassland containing a high quantity and diversity of flowers thorough the entire flight season of bumblebees (Bäckman & Tiainen 2002; Pywell *et al.* 2006; Hatfield & LeBuhn 2007; Rundlöf *et al.* 2008; Ahrné *et al.* 2009; Diaz-Forero *et al.* 2013). With the intensification of agriculture, the remaining grassland are most of the time turned into intensive pastures or meadows mown early in the season for ensilage, which annihilates the role of grasslands for bumblebees and other pollinators by removing floral resources (Rasmont 1988; Carvell 2002). Similarly, cropland drastically decreased but the types of crops and the intensity of their use changed. In our results, sugar beet area explains 28% of the differences in assemblage composition between localities and periods. This could represent the modification of the three-field rotations in European farmland following the mainstreaming of nitrogenous chemical fertilizers, with the replacement of leguminous crops enriching the soil in nitrogen by industrial crops such as sugar beet. In Trivières and Moorsel, cropland were dominated by low yielding cereal crops, leguminous plants (mainly *Trifolium incarnatum*), and sugar beet in 1910 (Ministère de l'Agriculture et des Travaux Publics 1913). They are now dominated by cereals, corn, potatoes, and sugar beet with high productivity, with the total abandon of leguminous crops (Belgian Federal Government 2017). Reinstatement of leguminous crops could provide a very valuable resource for bumblebees, many of which depending greatly on these plants (Rasmont & Mersch 1988; Goulson & Darvill 2004; Rasmont *et al.* 2005; Carvell *et al.* 2006). Furthermore, a study

showed that inclusion of organic crops with broadleaved weeds like those found in large numbers a century ago within 500 meters of nesting sites strongly enhances bumblebee species richness (Holzschuh *et al.* 2008). Torgny, which is today the richest locality in bumblebee diversity among the four sampled localities, is the only one still presenting leguminous crops (mainly *Trifolium pratense* and *Medicago sativa*) and a greater abundance and diversity of wild flowering plants in the fields. It is also the locality where high-stem orchards are still well represented. Orchards could also represent good supplies of floral resources for bumblebee queens in the early season (Goulson 2010). In Trivières, orchards covered the same proportion than woodland a century ago but have almost disappeared today, probably resulting from the Mansholt Plan in the 1970s, which strongly encouraged the felling of most orchards (Christians 1998).

In farmland areas, landscapes with undisturbed elements (*e.g.* hedges, slopes and field margins in late mowing) may harbour more permanently and in greater number bumblebee colonies than homogenous landscapes (Banaszak 1992; Mänd *et al.* 2001; Croxton *et al.* 2002; Osborne *et al.* 2008b). Withered grass and tussocks found in hedges, wood edges and field boundaries in late mowing represent favourable nest sites and are very appreciated by nest-seeking queens (Svensson *et al.* 2000). Contrary to Moorsel and Trivières, these landscape elements remain abundant in Francorchamps and Torgny. The bocage between pastures and hayfields is still well developed and many roadsides are under late mowing regime and present high flower diversity, with many plant species appreciated by bumblebees (*e.g. Trifolium pratense*, *Origanum vulgare*, *Knautia*

spp., *Onobrychis viciifolia* in Torgny). Sowing bee-friendly plants on arable field margins tend to be favourable for bumblebees (Kells *et al.* 2001; Croxton *et al.* 2002; Carvell *et al.* 2004; Pywell *et al.* 2006; Terzo & Rasmont 2007) and could improve floral resources availability in the localities under intensive farming like Moorsel and Trivières.

Furthermore, urbanisation, accompanied by an increase in population density, was the most intense in Moorsel and Trivières and could explain a great part of the bumblebees' decline. However, in highly urbanized areas, gardens and urban parks could be favourable for bumblebees and pollinators in general if they offer sufficient flower resources and nesting sites (Tommasi *et al.* 2004; McFrederick & LeBuhn 2006; Osborne *et al.* 2008b; Garbuzov & Ratnieks 2014; Normandin *et al.* 2017), sometimes even more than in intensive farmland areas (Samnegård *et al.* 2011; Baldock *et al.* 2015). Making gardens and parks more "bee-friendly" by plant selection and small adjustments is not very expensive and could be greatly helpful for bumblebee populations and other pollinators (Garbuzov & Ratnieks 2014). As urban areas are continuously growing, it is important to improve their value for pollinators and to include them in conservation plans in addition to farmland areas.

6.4. Limitations of this study and recommendations for future research

The first limitation of this study is the restricted number of studied localities. It is hazardous to draw strong conclusions from statistical analyses on the relationship between the changes in bumblebee assemblages and the changes in land cover

based on four localities. Moreover, the four localities present a very different landscape composition and dynamics and some landscape variables are correlated between land cover types. The most wooded locality (Francorchamps) is the only one presenting heathland and has the lowest proportion of cropland. Similarly, this most wooded locality is, with Torgny, the least urbanised. Separating the effects of all the different land cover types is therefore quite difficult. Furthermore, as each locality is situated in a distinct biogeographical region, other parameters such as altitude and probably climate are also important for explaining the variability observed in bumblebee assemblages between the sites. In 100 years, climate change probably had an impact on bumblebee assemblages in addition to land cover changes, and its effects may have differed between the four localities. As shown in other studies, climate has a pronounced impact on bumblebee assemblages (Rasmont *et al.* 2015; Goulson *et al.* 2015; Aguirre-Gutiérrez *et al.* 2017) and may act in synergy with land cover and land use changes (Brook *et al.* 2008; Tylianakis *et al.* 2008; Schweiger *et al.* 2010; Potts *et al.* 2010; Oliver & Morecroft 2014; Marshall *et al.* 2017). Furthermore, we highlighted that studying land cover dynamics alone cannot take into account the whole phenomenon of intensification of agriculture. It is important to consider information about land use practices or pressures (*e.g.* the type of crop, the number of livestock per pasture, the month of meadows mowing). The amount of pesticides and fertilizers used in crops and grassland should also bring important information but the lack of data on chemical input greatly limits this perspective. Future studies should take into account as far as possible the full range of factors

such as land use, agricultural statistics, and climate in their analyses. For future research, it would be interesting to study a higher number of localities with better separated landscape dynamics across the country and to have replicates inside each biogeographical region with similar landscape trajectories, altitude and climatic conditions in order to clearly distinguish the effects of each variable on bumblebee assemblages.

7. Conclusion

Unlike other studies mostly based on contemporary data, our study explores the relationship between changing landscapes and bumblebee assemblages by relying on historical data of land cover and bumblebee records going back before the acceleration of urbanisation and agricultural intensification in the 1950s. Our results show a general decline and strong shifts in bumblebee assemblages, which greatly vary between regions with different landscape dynamics. Changes in bumblebee assemblages were far less intense in regions where landscapes changed the least during the last century and conserved a high density of natural habitats and a low density of settlement areas and intensive crops. In the highly urbanised and intensive agricultural landscapes, future land management could enhance bumblebee diversity by restoring landscape elements rich in floral and nesting resources, by *e.g.* reinstate leguminous crops, planting "bee-friendly" flowers and hedges, and by leaving areas in late mowing. Our study shows the importance but also the limitations of historical data and brings information

about the effects of landscape-level changes on bumblebees, which could be useful for land management and conservation biology.

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10. Supplementary material

Table S1. Climatic conditions in P2 (2013-2015) in the nearest meteorological stations of the four studied localities. Means are the average values of the three years, min. and max. are respectively the lowest and the highest values observed in the three years combined. Data are from the Belgian IRM (Institut Royal Météorologique).

Climatic conditions	Spa (near **Francorchamps**)	Meix-Devant-Virton (near **Torgny**)	Asse-ter-Heide (near **Moorsel**)	La Hestre (near **Trivières**)
Temperatures (°C)				
Annual (mean)	8.8	10.0	10.9	10.6
Coldest month (mean)	1.1	2.4	3.8	3.5
Coldest month (min)	-10.2	-12.2	-11.5	-10.2
Warmest month (mean)	17.8	19.5	19.2	19.2
Warmest month (max)	34.2	35.8	34.6	34.0
Precipitations (mm)				
Annual (mean)	1000	1150	865	899
Driest month (mean)	31	38	21	34
Driest month (min)	14	15	15	24
Wettest month (mean)	111	157	101	120
Wettest month (max)	183	210	131	187

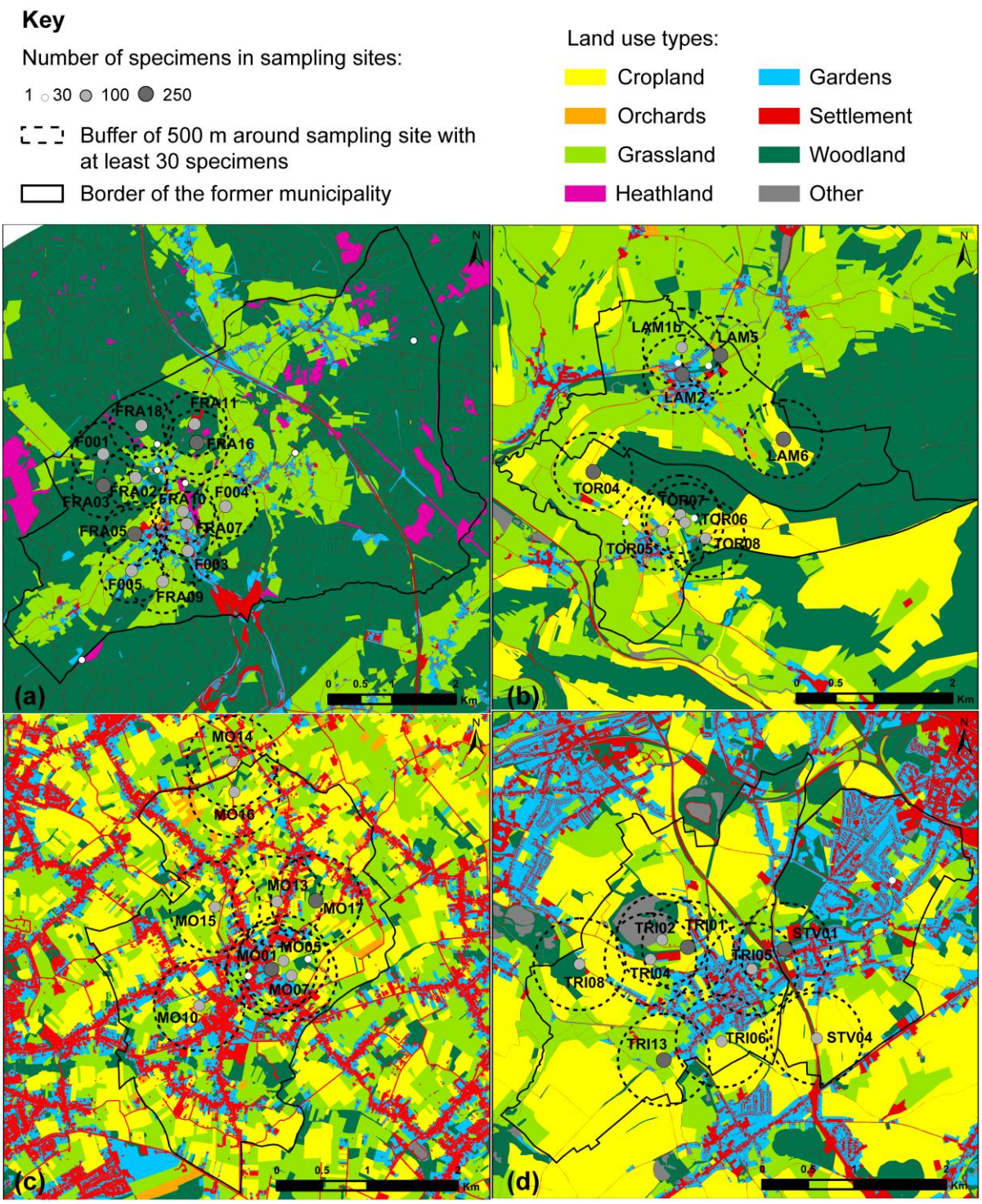

Figure S1. Land cover in P2 (2013-2015), sampling sites and the buffer of 500 m around sampling sites with at least 30 specimens in (a) Francorchamps, (b) Torgny, (c) Moorsel, and (d) Trivières.

Table S2. Absolute ("Abs.") and relative ("Rel. (%)") numbers of bumblebee observations on plant species in each locality in P2 (2013-2015).

Summary of the table: The flora visited by bumblebees varies between the localities. In Francorchamps, bumblebees were observed on a total of 41 plant genera, with three genera accounting for 57% of all the observations: *Trifolium* spp. (30%, with 16% *T. pratense* and 14% *T. repens*), *Epilobium* spp. (*E. angustifolium*, 14%), and *Cirsium* spp. (13%, with 5% *C. arvense* and 4% *C. palustre*). In Torgny, bumblebees were observed on 30 plant genera, with three genera accounting for 74% of all the observations: *Trifolium* spp. (46%, with 34% *T. pratense* and 12% *T. repens*), *Origanum* spp. (*O. vulgare*, 14%), and *Echium* spp. (*E. vulgare*, 13%). In Moorsel, bumblebees were observed on only 26 plant genera, with four genera accounting for 76% of all the observations: *Symphytum* spp. (*S. vulgare*, 22%), *Trifolium* spp. (22%, with 20% *T. repens*), *Lamium* spp. (*L. album*, 18%), and *Rubus* spp. (13%). In Trivières, bumblebees were observed on 44 plant genera, with the highest proportion on *Trifolium* spp. (33%, with 23% on *T. pratense* and 10% on *T. repens*), *Rubus* spp. (10%), and *Echium* spp. (*E. vulgare*, 8%).

	Francorchamps		**Torgny**		**Moorsel**		**Trivières**	
Plant species	Abs.	Rel. (%)	Abs.	Rel. (%)	Abs.	Rel. (%)	Abs.	Rel. (%)
Total	1072	100	918	100	745	100	988	100
Aesculus hippocastanum	1	0.09	0	0.00	0	0.00	0	0.00
Ajuga reptans	7	0.65	28	3.05	0	0.00	1	0.10
Alliaria petiolata	0	0.00	0	0.00	3	0.40	0	0.00
Anemone nemorosa	0	0.00	1	0.11	1	0.13	16	1.62
Aquilegia vulgaris	0	0.00	1	0.11	0	0.00	0	0.00
Arctium sp.	0	0.00	0	0.00	0	0.00	1	0.10
Arctium tomentosum	0	0.00	0	0.00	0	0.00	3	0.30
Ballota nigra	0	0.00	0	0.00	0	0.00	66	6.68
Borago officinalis	0	0.00	0	0.00	0	0.00	5	0.51
Buddleja davidii	0	0.00	1	0.11	0	0.00	52	5.26
Calluna vulgaris	12	1.12	0	0.00	0	0.00	2	0.20
Calystegia sepium	0	0.00	0	0.00	3	0.40	2	0.20
Campanula sp.	0	0.00	1	0.11	0	0.00	0	0.00
Cardamine pratensis	2	0.19	4	0.44	0	0.00	0	0.00
Carduus sp.	0	0.00	0	0.00	0	0.00	46	4.66
Centaurea jacea	13	1.21	0	0.00	0	0.00	0	0.00
Centaurea macrocephala	0	0.00	0	0.00	16	2.15	0	0.00
Centaurea nigra	1	0.09	0	0.00	0	0.00	3	0.30
Centaurea sp.	18	1.68	12	1.31	4	0.54	15	1.52
Cirsium arvense	57	5.32	0	0.00	4	0.54	7	0.71
Cirsium palustre	39	3.64	0	0.00	0	0.00	0	0.00
Cirsium sp.	34	3.17	0	0.00	0	0.00	21	2.13
Cirsium vulgare	12	1.12	0	0.00	1	0.13	8	0.81
Convolvulus arvensis	0	0.00	0	0.00	0	0.00	1	0.10
Crataegus monogyna	0	0.00	0	0.00	0	0.00	1	0.10
Crepis sp.	4	0.37	10	1.09	0	0.00	24	2.43
Cytisus scoparius	20	1.87	0	0.00	2	0.27	0	0.00

Dactylorhiza maculata	1	0.09	0	0.00	0	0.00	0	0.00
Digitalis purpurea	3	0.28	0	0.00	0	0.00	0	0.00
Dipsacus fullonum	0	0.00	1	0.11	0	0.00	5	0.51
Echium vulgare	0	0.00	122	13.29	0	0.00	75	7.59
Epilobium angustifolium	155	14.46	0	0.00	0	0.00	5	0.51
Epilobium hirsutum	0	0.00	0	0.00	1	0.13	7	0.71
Eupatorium cannabinum	1	0.09	0	0.00	0	0.00	0	0.00
Foeniculum vulgare	1	0.09	0	0.00	0	0.00	0	0.00
Genista sp.	2	0.19	0	0.00	0	0.00	0	0.00
Geranium robertianum	4	0.37	0	0.00	0	0.00	0	0.00
Geranium sanguineum	0	0.00	0	0.00	0	0.00	1	0.10
Geranium sp.	1	0.09	3	0.33	0	0.00	0	0.00
Glechoma hederacea	2	0.19	13	1.42	7	0.94	18	1.82
Helianthus annuus	0	0.00	0	0.00	2	0.27	0	0.00
Hieracium aurantiacum	7	0.65	0	0.00	0	0.00	0	0.00
Hyacinthoides non-scripta	0	0.00	0	0.00	0	0.00	9	0.91
Hypericum perforatum	0	0.00	3	0.33	1	0.13	2	0.20
Hypericum sp.	0	0.00	0	0.00	0	0.00	3	0.30
Impatiens glandulifera	0	0.00	0	0.00	14	1.88	0	0.00
Knautia arvensis	0	0.00	41	4.47	0	0.00	0	0.00
Lamiastrum galeobdolon	1	0.09	0	0.00	1	0.13	0	0.00
Lamium album	20	1.87	0	0.00	134	17.99	8	0.81
Lamium maculatum	0	0.00	4	0.44	0	0.00	0	0.00
Lamium purpureum	27	2.52	3	0.33	0	0.00	1	0.10
Lathyrus latifolius	0	0.00	0	0.00	0	0.00	21	2.13
Lathyrus odoratus	0	0.00	0	0.00	0	0.00	1	0.10
Lathyrus tuberosus	0	0.00	0	0.00	0	0.00	3	0.30
Leucanthemum vulgare	0	0.00	0	0.00	0	0.00	1	0.10
Linaria sp.	0	0.00	0	0.00	5	0.67	1	0.10
Linaria vulgaris	0	0.00	13	1.42	0	0.00	0	0.00
Lotus corniculatus	15	1.40	21	2.29	2	0.27	19	1.92
Lotus pedunculatus	4	0.37	0	0.00	0	0.00	0	0.00
Lotus sp.	1	0.09	0	0.00	0	0.00	0	0.00
Lupinus polyphyllus	1	0.09	0	0.00	0	0.00	0	0.00
Lychnis flos-cuculi	2	0.19	0	0.00	0	0.00	0	0.00
Mahonia aquifolium	0	0.00	0	0.00	1	0.13	0	0.00
Malus sp.	1	0.09	0	0.00	0	0.00	0	0.00
Malva moschata	0	0.00	0	0.00	0	0.00	1	0.10
Malva sp.	0	0.00	0	0.00	0	0.00	8	0.81
Medicago lupulina	0	0.00	0	0.00	0	0.00	3	0.30
Medicago sativa	0	0.00	0	0.00	0	0.00	16	1.62
Melampyrum arvense	0	0.00	4	0.44	0	0.00	0	0.00
Melilotus alba	0	0.00	0	0.00	0	0.00	3	0.30
Onobrychis viciifolia	0	0.00	13	1.42	0	0.00	0	0.00
Ononis repens	0	0.00	2	0.22	0	0.00	0	0.00
Origanum sp.	0	0.00	0	0.00	0	0.00	1	0.10
Origanum vulgare	2	0.19	130	14.16	0	0.00	0	0.00
Papaver rhoeas	0	0.00	0	0.00	0	0.00	1	0.10
Pentaglottis sempervirens	0	0.00	0	0.00	67	8.99	0	0.00
Plantago lanceolata	1	0.09	0	0.00	0	0.00	0	0.00
Polygonum bistorta	1	0.09	0	0.00	0	0.00	0	0.00

Primula veris	0	0.00	2	0.22	0	0.00	0	0.00
Primula vulgaris	0	0.00	0	0.00	2	0.27	0	0.00
Prunus avium	0	0.00	0	0.00	0	0.00	2	0.20
Prunus sp.	0	0.00	0	0.00	0	0.00	3	0.30
Ranunculus sp.	2	0.19	1	0.11	5	0.67	0	0.00
Reseda lutea	0	0.00	0	0.00	0	0.00	3	0.30
Reseda luteola	0	0.00	0	0.00	0	0.00	3	0.30
Rhinanthus angustifolius	0	0.00	0	0.00	0	0.00	1	0.10
Rhinanthus sp.	24	2.24	1	0.11	0	0.00	0	0.00
Rosa canina	0	0.00	0	0.00	0	0.00	3	0.30
Rubus sp.	55	5.13	11	1.20	97	13.02	94	9.51
Salix caprea	0	0.00	0	0.00	0	0.00	8	0.81
Salix sp.	6	0.56	0	0.00	0	0.00	10	1.01
Silene dioica	0	0.00	0	0.00	0	0.00	1	0.10
Solidago virgaurea	1	0.09	0	0.00	0	0.00	0	0.00
Spiraea sp.	1	0.09	0	0.00	0	0.00	0	0.00
Stachys palustris	25	2.33	0	0.00	0	0.00	0	0.00
Stachys sp.	7	0.65	1	0.11	0	0.00	0	0.00
Stachys sylvatica	22	2.05	0	0.00	0	0.00	0	0.00
Symphytum officinale	0	0.00	10	1.09	165	22.15	0	0.00
Tagetes patula	4	0.37	0	0.00	0	0.00	0	0.00
Taraxacum officinale	15	1.40	3	0.33	0	0.00	1	0.10
Taraxacum sp.	39	3.64	34	3.70	0	0.00	6	0.61
Teucrium scorodonia	30	2.80	0	0.00	0	0.00	0	0.00
Trifolium campestre	0	0.00	3	0.33	0	0.00	1	0.10
Trifolium pratense	170	15.86	314	34.20	16	2.15	232	23.48
Trifolium repens	146	13.62	106	11.55	151	20.27	94	9.51
Trifolium sp.	3	0.28	0	0.00	0	0.00	0	0.00
Tussilago farfara	2	0.19	0	0.00	0	0.00	2	0.20
Urtica dioica	0	0.00	0	0.00	3	0.40	0	0.00
Vaccinium myrtillus	7	0.65	0	0.00	0	0.00	0	0.00
Vaccinium uliginosum	20	1.87	0	0.00	0	0.00	0	0.00
Valeriana officinalis	0	0.00	0	0.00	0	0.00	1	0.10
Verbena officinalis	0	0.00	0	0.00	0	0.00	4	0.40
Veronica sp.	5	0.47	0	0.00	0	0.00	0	0.00
Veronica spicata	0	0.00	0	0.00	18	2.42	0	0.00
Vicia cracca	5	0.47	0	0.00	5	0.67	0	0.00
Vicia sepium	3	0.28	0	0.00	0	0.00	0	0.00
Vicia sp.	7	0.65	1	0.11	12	1.61	32	3.24
Viola reichenbachiana	0	0.00	0	0.00	2	0.27	0	0.00

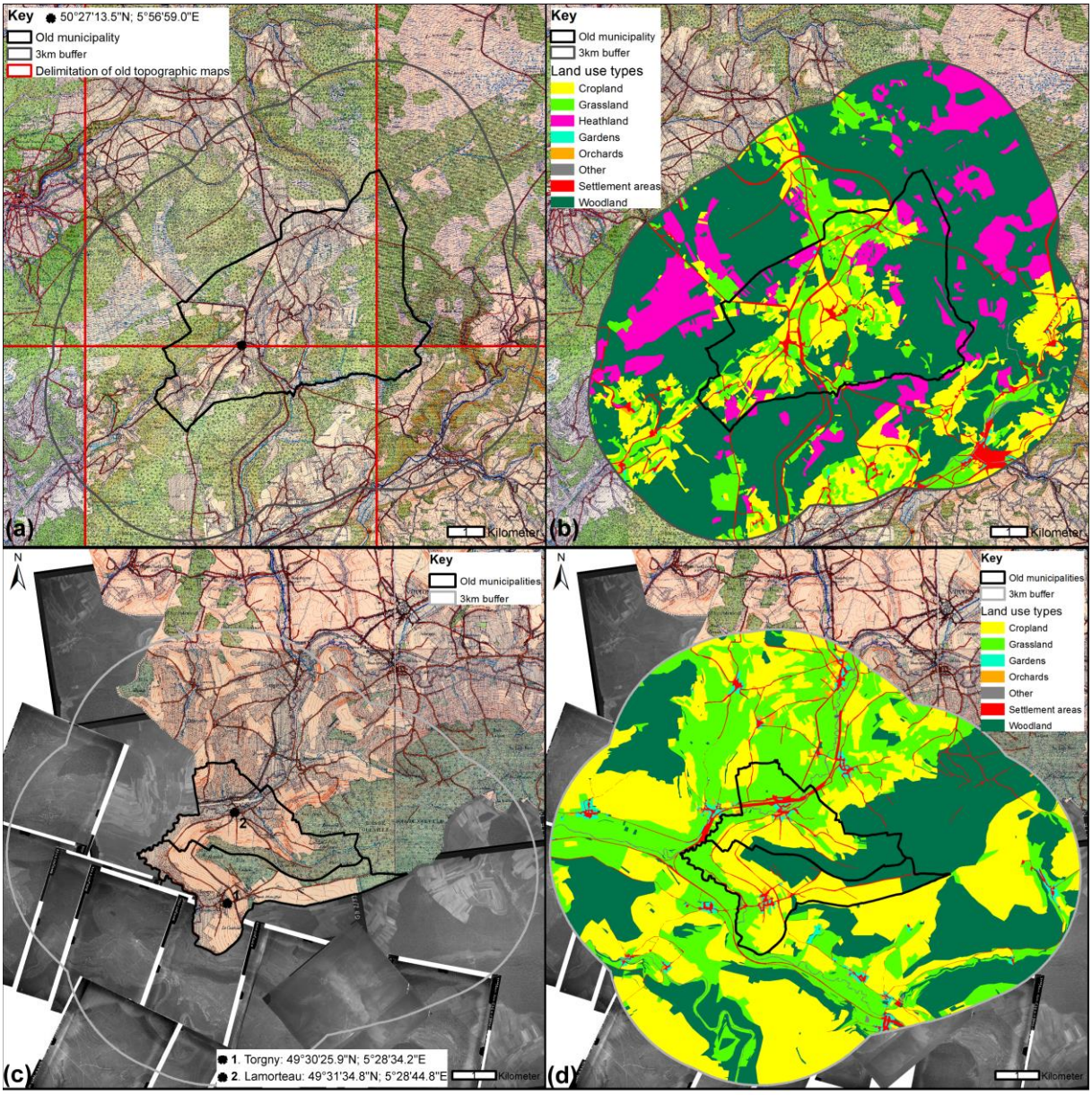

Figure S2. Classification process in the eight land cover types from topographic maps for Francorchamps (a and b) and from topographic maps (for the Belgian part) and aerial photographs (for the French part) for Torgny (c and d) for P1 (1910-1930).

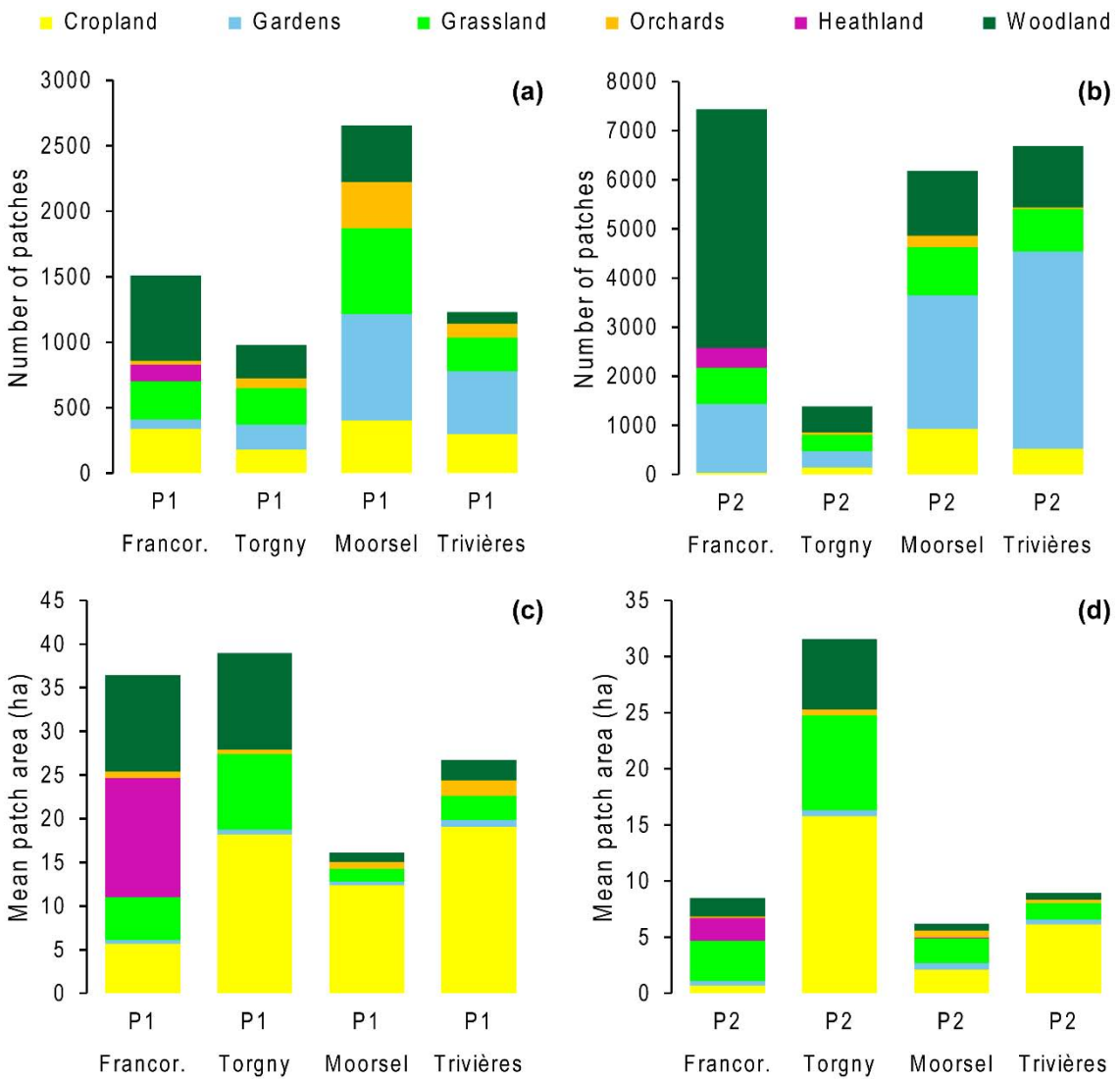

Figure S3. Number of patches in (a) P1 and (b) P2, and mean patch area in hectares in (c) P1 and (d) P2 in the 3 km buffer around each locality. P1 = 1910-1930, P2 = 2013-2015.

Chapitre 4

Bumblebee species distribution modelling based on land use and climate in Belgium along the last century.

Chapter 4: "Bumblebee species distribution modelling based on land cover and climate in Belgium along the last century"

By Sarah Vray, Leon Marshall, Denis Michez, Pierre Rasmont, and Nicolas Dendoncker.

1. Background

In the previous chapters, we showed the indisputable decline of many bumblebee species involving consequent changes in bumblebee assemblages in Belgium. We also observed that this decline and these changes in bumblebee assemblages were very different between localities with contrasting landscape dynamics. Not all species have the same habitat preferences and respond similarly to landscape changes. It hence seems important to account for (changes in) local land cover to explain the composition and the changes observed in bumblebee assemblages. Moreover, recent studies have shown that climate also plays a major role in these changes (Rasmont & Iserbyt 2012; Iserbyt & Rasmont 2012; Rasmont *et al.* 2015a; Kerr *et al.* 2015; Aguirre-Gutiérrez *et al.* 2017; Marshall *et al.* 2017). The aim of this study is to model the distribution of each bumblebee species at the Belgian scale according to climatic and land cover variables in the same three time periods as in chapter 1 (*i.e.* P1: 1910-1930; P2: 1970-1989; P3: 1990-2016), and to determine what are the key factors explaining the observed changes in species distribution. In addition, this may provide insight into what could be anticipated for the future of these species.

2. Summary

It is widely acknowledged that climate and land cover changes are currently the most pervasive drivers of ecosystem change worldwide and will also play an important role in the future. Therefore, a thorough understanding of the mechanisms involved in the responses of populations and communities to these environmental changes is a pre-requisite to project and mitigate the long-term effects of these changes on biodiversity. Here, we perform species distribution modelling of bumblebees in Belgium from 1910 to 2016 based on five land cover and five climate variables. We assess the relative influence of environmental variables on bumblebee distributions between time periods, types of variable, and species. Our results highlight the importance of precipitation variables reflecting droughts in defining bumblebee distributions. The also great influence of settlement and cropland areas, which vary between periods and species with different population trends, suggest that they had a great impact on species distribution changes during the last century. We also show that the influence of environmental variables can change between the periods investigated (*e.g.* decrease of the influence of land cover), and that these variations should be taken into account for future projections.

3. Introduction

There is no doubt that changes in climate, land cover and land use threaten biodiversity across ecosystems and regions (Walther *et al.* 2002; Parmesan & Yohe 2003; Thomas *et al.* 2004; Tscharntke *et al.* 2005; Tylianakis *et al.* 2008; Krauss *et al.* 2010). These global environmental changes can show synergistic or opposite effects (Travis 2003; Brook *et al.* 2008; Schweiger *et al.* 2010), and their impacts can vary in space, in time, and between taxa (Aguirre-Gutiérrez *et al.* 2017). The combination of climate and land cover changes has already caused the loss of local populations in a variety of taxa, which is the first step towards species extinction at the global scale (Ceballos & Ehrlich 2002; Sinervo *et al.* 2010; Collen *et al.* 2011). These two drivers of global biodiversity change therefore lead to drift in species spatial distribution, with population decline and extinction in the least favourable regions, and sometimes the dispersal in more favourable regions (Bowler & Benton 2005; Thomas *et al.* 2006; Huntley *et al.* 2010). These population changes result inevitably in modifications in the composition and functioning of local communities and ecological networks (Davis *et al.* 1998; Memmott *et al.* 2004; Brooker *et al.* 2007; Schleuning *et al.* 2016). Understanding how populations and communities respond to climate and land cover changes is a prerequisite to project their long-term effects on biodiversity and design efficient conservation strategies. As changes in climate and land cover are often spatially and temporally desynchronized, their combined effects on species distributions has to be analysed in a large temporal and geographical framework (Fox *et al.* 2014; Aguirre-Gutiérrez *et al.* 2017). Among the methods used to assess the

combined effects of climate and land cover conditions on species distribution, species distribution models (SDMs) are the most commonly used (Thuiller 2003, 2004; Guisan & Thuiller 2005; Graham & Hijmans 2006; Beaumont *et al.* 2007; Elith & Leathwick 2009; Morin & Thuiller 2009; Franklin 2010; Drew *et al.* 2011; Martin *et al.* 2013; Polce *et al.* 2013). Generally, these models are used to predict species distributions into environments where survey data are lacking by relating occurrence of species to environmental predictors (Elith *et al.* 2011). One of their applications is to project species distributions under climate and land cover changes scenarios based on present species distribution associated to present climate and land cover (Drew *et al.* 2011; Marshall *et al.* 2017). However, knowing the relation between environmental variables and population changes that occurred in the past could provide key elements in understanding the relative influence of drivers of species distribution across space and time, and therefore could help to better project future changes in species distribution (Randin *et al.* 2006; Kharouba *et al.* 2009; Austin & Van Niel 2011; Aguirre-Gutiérrez *et al.* 2017).

Bumblebees are among the most essential pollinators for their services to both natural ecosystems and agricultural production (Ollerton *et al.* 2011). Since the 1950s, they are undergoing a strong decline in Western Europe (Rasmont *et al.* 1993; Kosior *et al.* 2007; Nieto *et al.* 2014) fostered by, among others, habitat loss and fragmentation through urbanization and agricultural intensification (Goulson *et al.* 2008; Ahrné *et al.* 2009; Le Féon *et al.* 2010), as well as climate change (Rasmont *et al.* 2015a; Kerr *et al.* 2015). Unlike other pollinator insects for

which the decline slowed down after the 1990s, many bumblebee species were still declining during the recent decades (Rasmont *et al.* 1993, 2005; Carvalheiro *et al.* 2013). Among the 68 species recorded in Europe, 31 are decreasing, 20 are stable, and 9 present positive population trends and expansion of their distribution (Nieto *et al.* 2014). However, despite their high importance for pollination and their massive decline, few studies based on historical SDMs have been conducted on bumblebees in Europe. In a study conducted in the Netherlands, the most important variables explaining the distribution of bees (including bumblebees), butterflies and hoverflies were related to landscape composition and temperature, and their importance changed between 1951 and 2014 (*e.g.* increase of the importance of temperature in the recent decades; Aguirre-Gutiérrez *et al.* 2017). However, almost no information is available on the relationship between species assemblages, land cover and climate before the 'great acceleration' that started globally in the 1950s (Houghton 1994; Steffen *et al.* 2011), and hence considering the beginning of agricultural intensification before the 1950s. One exception is a study in the UK, where the impact of land cover on bee and wasp communities was assessed over the last 80 years, however no SDM was performed at the country scale and climate was not considered (Senapathi *et al.* 2015).

Given these gaps, the first aim of our study is to assess if land cover and climate variables can be used to model the historical distribution of bumblebee species (*Bombus* spp.) at the country scale (*i.e.* Belgium) along the last century. For this, we use a combined dataset of climate, land cover, and occurrence records

of 31 bumblebee species from 1910 to 2016 in Belgium. We then estimate which variables best define their projected distribution, in general and for each species separately. As each species has its own preferences in terms of habitat and climate (Reinig 1972, Rasmont 1988, Rasmont *et al.* 2015a), we expect that the influence of each environmental variable will change between species. Moreover, as the more drastic changes in land cover started earlier (*i.e.* in the 1950s; Fuchs *et al.* 2014; European Environment Agency 2017) than human-induced climate changes (*i.e.* in the 1990s; Klein Tank 2004; IPCC 2014), we also estimate if the influence of land cover and climate on bumblebee distributions changes over time. This study therefore attempts to provide elements of understanding on how species of these critical pollinators respond to environmental changes, which is essential to predict and mitigate their decline in the future and thereby to maintain a sustainable ecosystem service of pollination.

4. Material and methods

4.1. Study region

Belgium is a small country (30 528 km^2) located in Western Europe. It has a temperate oceanic climate, characterized by mild and rainy winters and cool and wet summers, with annual mean temperature of 10.8°C and annual precipitation of 850 mm between 1990 and 2014 in central Belgium (IRM 2017a). Belgium provides a typical example of urbanization and agricultural intensification in Western Europe (Christians 1998; Stoate *et al.* 2001; Robinson & Sutherland 2002; Antrop 2004; Mazoyer & Roudart 2006). Its population density increased

from 243 inhabitants per km^2 (7.4 million in total) in 1910 to 369 (11.3 million) in 2016 (Belgian Federal Government 2017). Belgian agriculture has gradually changed from a mixed set of crops and meadows before the 1950s to a specialization in animal husbandry, with a great proportion of crops transformed into corn forage crops or intensive pastures and hayfields (Christians 1998). The development of farm machinery and fertilizers in the 1950s, as well as the application of the so called Mansholt Plan during the 1970s, induced a drastic reduction of landscape diversity (*e.g.* suppression of crop rotation, removal of hedgerows, polycultures transformed into monocultures through land consolidation, felling of most orchards), which had dramatic consequences on biodiversity (Stoate *et al.* 2001; Robinson & Sutherland 2002). Starting in the 1990s and mainstreamed in the 2000s, agri-environmental schemes in Europe allowed the integration of environmental concerns into the Common Agricultural Policy (Kleijn & Sutherland 2003; Batáry *et al.* 2015). Several conservation measures were developed to counter the decline in pollinators, including for example the provision of floral resources (Pywell *et al.* 2006; Carvell *et al.* 2007; Terzo & Rasmont 2007; Batáry *et al.* 2010).

4.2. Bumblebee dataset and subdivision in time periods

We used a database consisting of 178 800 bumblebee records from compiled museum, survey records and opportunistic citizen data encompassing the period from 1810 to 2016. These data are recorded in the database *Banque de Données Fauniques de Gembloux et Mons* (BDFGM, Rasmont & Pauly 2010). Centenary

records come mostly from the Hymenoptera collection of F. J. Ball (Ball 1914, 1920) stored at the Royal Belgian Institute of Natural Sciences, and covering mainly the 1910-1930 period. The most recent records (*i.e.* from 2000s) are predominantly opportunistic citizen data from Belgian naturalist databases. As the spatial precision of old records is rather poor, we aggregated all species occurrences at a spatial resolution of 5 x 5 km grid cells (Fig. S1). Taking into account the temporal distribution of sampling effort between years and the main changes in environmental drivers, we subdivided the dataset into three time periods of similar length: 1910-1930 (P1), 1970-1989 (P2), and 1990-2016 (P3). The major changes between P1 and P2 were land cover and land use changes through agricultural intensification and urbanization (Christians 1998; Stoate *et al.* 2001; Robinson & Sutherland 2002; Antrop 2004; Mazoyer & Roudart 2006), whereas human-induced climate changes occurred mainly between P2 and P3 (Klein Tank 2004).

4.3. Land cover and climate data

We obtained land cover data from the "Historic Land Dynamics Assessment" (HILDA) version 2.0 (Fuchs *et al.* 2013, 2014). These land cover maps are available for every decade from 1900 to 2010 at a spatial resolution of 1 x 1 km. Land cover is classified in six types: settlement (including green urban areas), cropland (including orchards and agro-forestry), forest (including transitional shrub and woodland, tree nurseries, reforested areas for forestry purposes), grassland (including natural grassland, wetlands, and pastures), water, and other

land (including sparsely vegetated areas, beaches, bare soil). In order to obtain the same spatial resolution as the bumblebee distribution data, we aggregated land cover data at a resolution of 5x5km by calculating the proportion of each land cover type inside each 25km^2 grid cell (Fig. S2) using the Geospatial Modelling Environment software (Beyer 2012). In order to limit the predictability of the data (as the sum of the land cover variables in a grid cell is 100, the last variable embedded in the dataset is fully predictable), we removed the "water" land cover type as it is meaningless for bumblebees (Table 1).

We generated climate data with the ClimateEU v4.63 software, available at http://tinyurl.com/ClimateEU, from the project "ClimateEU: historical and projected climate data for Europe" (Wang *et al.* 2012; Hamann *et al.* 2013). These data consist in monthly minimum and maximum temperatures as well as monthly precipitation for each year. We extracted these data at the 5 x 5 km resolution and aggregated them as an average for each period. We then calculated the 19 bioclimatic variables widely used in ecology (Busby 1991; Hijmans *et al.* 2005). They represent trends in average and extreme climatic conditions that are closely associated with physiological constraints of plants and animals (Parmesan *et al.* 2000; Booth *et al.* 2014). In order to avoid collinearity in model projections, we only included variables presenting Pearson's rank-correlation coefficients inferior to the absolute value of 0.7 (Dormann *et al.* 2013). When two variables were correlated, we kept the one assumed to be the most relevant for bumblebees, especially the variables reflecting extreme high temperature and drought (Rasmont & Iserbyt 2012; Iserbyt & Rasmont 2012; Thomson 2016; Ogilvie *et al.*

2017). As seasonality (*i.e.* difference between maximum temperature of the warmest month and minimum temperature of the coldest month) was highly correlated to the other selected variables, we chose another variable related to continentality: the mean diurnal temperature range (*i.e.* difference between monthly maximum and monthly minimum temperatures). Continentality (*i.e.* difference between continental and marine climates) is partly characterized by the higher range of temperatures occurring over land than over water, caused by the lower heat capacities and evaporation rates of land surfaces than of water (Snow 2005). As seasonality, mean diurnal range generally increases with distance from the ocean (*e.g.* Mikolaskova 2009; Scheitlin 2013). Belgium presents indeed a gradient from low diurnal range and rainfall near the coast in the north-west to higher diurnal range and rainfall in the south-east (Fig. S3). The three variables of temperature and two variables of precipitation included in the models are presented in Table 1 and Figure S3.

Table 1. Land cover and bioclimatic variables selected for modelling, and their average value in Belgium in each period (P1: 1910-1930; P2: 1970-1989; P3: 1990-2016). Mean diurnal range is the mean of the monthly temperature ranges (monthly maximum minus monthly minimum). The driest quarter corresponds to the three consecutive months with the lowest cumulative precipitation. Similarly, the warmest quarter corresponds to the warmest three consecutive months of the year. Each variable is an average for each period.

Environmental variables (code)	**Units**	**Average value**		
		P1	**P2**	**P3**
Land cover				
Settlement area (Sett)	%	14.54	18.48	19.20
Cropland area (Crop)	%	39.70	25.89	27.70
Forest area (Wood)	%	18.65	21.83	23.16
Grassland area (Grass)	%	25.94	32.71	28.89
Other area (Other)	%	0.17	0.17	0.17
Water area: not included in the models	%	0.99	0.92	0.88
Climate				
Mean diurnal temperature range (ΔT)	°C	8.2	7.3	7.5
Maximum temperature of the warmest month (MTWM)	°C	21.8	21.5	22.5
Mean temperature of the driest quarter (TDQ)	°C	6.8	6.5	8.6
Precipitation of the driest quarter (PDQ)	mm	166.2	188.4	169.8
Precipitation of the warmest quarter (PWQ)	mm	232.6	215.8	237.9

4.4. Species distribution modelling

Out of the 31 *Bombus* species recorded in Belgium, we considered those present in at least 10 grid cells per time period for species distribution modelling in order to have at least the same number of occurrences as the number of variables. Three species were deleted from the dataset because represented by less than 10 records in total: *Bombus cullumanus* (Rasmont 1982), *B. quadricolor* (Peeters *et al.* 1999), and *B. wurflenii* (Debaille & Rasmont 1997). We modelled species distribution using Maxent version 3.4.0, which uses the machine-learning technique called maximum entropy modelling (Phillips *et al.* 2006; Phillips & Dudík 2008; Elith *et al.* 2011). This method is one of the most efficient for species distribution modelling based on presence-only data, including museum collection data (Hirzel *et al.* 2002; Elith *et al.* 2006, 2011; Pearce & Boyce 2006; Phillips *et al.* 2009; Elith & Leathwick 2009). In order to improve the models performance and limit model overfitting, we used the linear, quadratic, and product feature classes (*i.e.* types of transformation of the original covariates; Elith *et al.* 2011) to fit the models, as well as a background corresponding to the entire country. We used the area under the receiver operating characteristic curve (AUC) values to assess model performance (Hanley & McNeil 1982; Fielding & Bell 1997; Phillips *et al.* 2006; Elith *et al.* 2006). To account for variation within algorithm, we limited the total background selection to 1000 grid cells and produced five runs with different background selections. We then selected the best model (*i.e.* with the highest AUC) for each species in each period. As AUC value is a measure that depends on both sample size and species' prevalence in presence-only SDMs (*e.g.*

it increases when the number of sampling records decreases; Jiménez-Valverde 2012; van Proosdij *et al*. 2016), it can not be compared between models based on different datasets (*e.g.* between species and between time periods). In order to test whether the SDM differed significantly from a distribution expected by chance, we used a null-model methodology (Raes & ter Steege 2007; van Proosdij *et al*. 2016). This methodology allows using all records to develop and test one single SDM, contrary to other methods that randomly split the available species records into a training and a test samples and produce several models per species (Fielding & Bell 1997). We tested the AUC value of each best model against the expected AUC value of a null distribution (*i.e.* a null-model) based on random collection data (Gotelli & McGill 2006). As sampling sites in each period are not spread over the complete country (Fig. S1), they may not have captured the full range of values of each environmental variable occurring in the country. This potential environmental bias can have a negative effect on the predictive performance of the SDM (Kadmon *et al.* 2003). If the random null-model is based on the entire area but the sampling is environmentally biased, its AUC is more likely to differ significantly from the SDM's AUC (Kadmon *et al.* 2003; Raes & ter Steege 2007). We solved this problem by restricting the randomly drawn distribution of the null-model to all grid cells sampled in the time period and not to the entire country. As we were interested in whether the SDM performed significantly better than the one expected by chance, we used a one-sided 95% confidence interval (C.I.) of the AUC value. A significant model therefore indicated that the relations between species' presence grid cells and the values of

explanatory variables in these grid cells are stronger than the relations expected by chance (Raes & ter Steege 2007). This means that species with a significant model had specific niche requirements that were captured by the model in the grid cells where they were sampled.

For each species, we produced habitat suitability maps based on the modelled distribution obtained from the best model in each period. Habitat suitability maps represent a measure of the probability of species presence across sites. The maps were calculated based upon the ratio of the density of covariates at occurrence sites and the density of covariates across the entire study area, in order to provide a set of representative conditions suggesting areas of suitable habitat (Elith *et al.*, 2011).

4.5. Influence of environmental variables on bumblebee distributions

In order to assess the influence of each environmental variable on bumblebee distributions, we used their "permutation importance" value. This measure is determined by randomly permuting the values of the environmental variable among the training presence and background points and measuring the resulting decrease in AUC. A large decrease indicates that the model depends heavily on the variable. This measure is normalized to give percentages. Therefore, a null value assumes no influence of the variable on the model, and the highest the value, the more influence the variable has on the model. The more an environmental variable varies across the study region and achieves (un)favourable values for the studied species, the more it determines the species distribution across this region

(Pearson & Dawson 2003). As the "Other" land cover type represents less than 1% of the relative area of the study region and probably does not have any biological significance for bumblebees, its "permutation importance" values are extremely low compared to the other environmental variables (see Table 3 in the results). Therefore, we chose not to consider this variable in further analyses.

To test whether the influence of environmental variables differed significantly between types of variable and periods, we used linear mixed-effects models with a Gaussian structure fit by maximum likelihood (Zuur *et al.* 2009). We set the square root of variable influence (*i.e*. "permutation importance" value) as response variable and the variable type (land cover, precipitation, temperature), the period (P1, P2, P3), and their interaction as explanatory variables. As several measures were performed on each species, we added the species identity as a random factor in the models. We then performed post-hoc pairwise multiple comparison tests with Tukey contrasts (Hothorn *et al.* 2008) to test the significance of differences in influence of variable types in the model. For the interaction between variable types and periods, we computed the least-squares means (*i.e.* predicted marginal means) of all the factors' combinations and compared them using the Tukey method (Lenth 2016). We then assessed which particular environmental variables had the highest influence on bumblebee distribution in each period and if their influence changed between time periods. To do this, we did exactly the same analyses but this time not grouping the variables in three types but considering each environmental variable separately, *i.e*. by replacing the explicative term "variable type" (3 levels) with "variable" (9

levels) in linear mixed-effects models. All analyses were performed in R version 4.3.1 (R Core Team 2017). Modelling, Tukey's post-hoc tests, and least-squares means comparison were performed using the packages "nlme" version 3.1-131 (Pinheiro *et al.* 2017), "multcomp" version 1.4-6 (Hothorn *et al.* 2008), and "lsmeans" version 2.27-2 (Lenth 2016), respectively.

Additionally, we assessed the effect of each environmental variable on each SDM by analysing the response curves, which show how each environmental variable affects the SDM prediction. We classified each response curve into four categories: positive linear effect, negative linear effect, concave quadratic effect (curved inwards, *i.e*. the species prefers intermediate values of the variable), convex quadratic effect (curved outwards, *i.e*. the species prefers extreme values, which is in theory less probable biologically speaking), and no effect (*i.e*. flat curve). We then assessed the dominant effect of each variable in each period by counting the number of species in each effect category.

After these generalized analyses, we assessed which environmental variables had the highest influence on the distribution of each species separately. We present the results by ordering species according to their European population trend (*i.e.* increasing, stable, decreasing, Rasmont *et al.* 2015b), very similar to the Belgian trends (Rasmont *et al.* 1993).

5. Results

5.1. Changes in environmental conditions between time periods

Environmental conditions significantly change between time periods in the 1364 5x5km grid cells of Belgium (Fig. 1). Wilcoxon signed rank test performed between periods two by two return a p-value < 0.001 for all climate and land cover variables, except for the "Other" and the "Water" variables for which too many zeroes made it impossible to compute exact p-values. The most important changes between P1 and P2 are mainly land cover changes, whereas the most important changes between P2 and P3 are mainly temperature changes (Fig. 1). As predicted, the main land cover changes between P1 and P2 is a sharp decrease of cropland in favour of grassland, settlement and woodland (Table 1; Fig. 1a). Between P2 and P3, grassland areas decrease to the profit of cropland, woodland and settlement. Concerning climate changes, temperatures slightly decrease between P1 and P2 but increase between P2 and P3 (Fig. 1b), with average value of maximum temperature of the warmest month increasing by 1.07°C and mean temperature of the driest quarter by 2.11°C. Precipitation of the driest quarter increase between P1 and P2 but decrease between P2 and P3, whereas the opposite happens for precipitation of the warmest quarter (Fig. 1c).

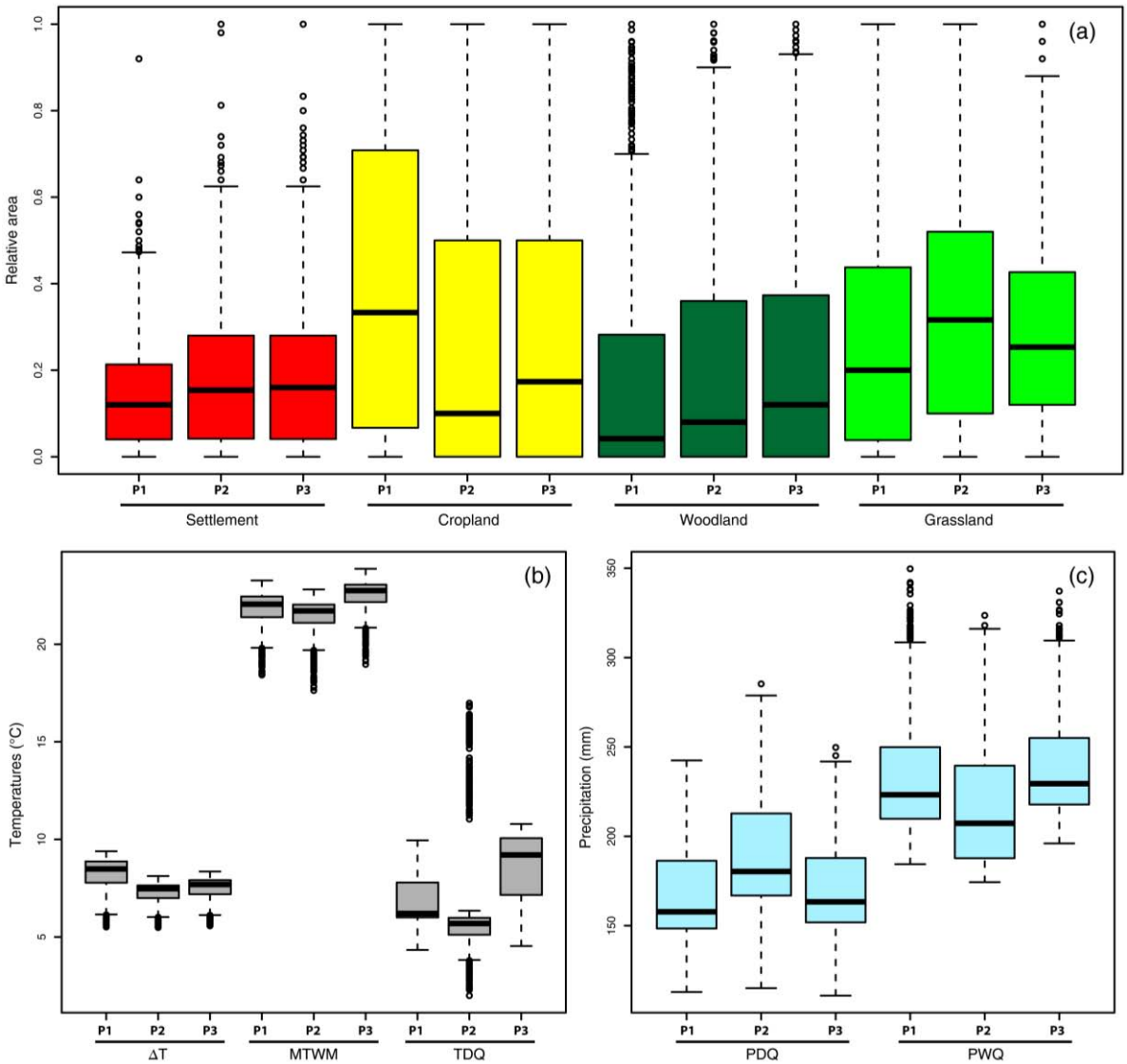

Figure 1. Boxplots showing the median and the quartiles of the environmental conditions in Belgium in the three periods (P1: 1910-1930; P2: 1970-1989; P3: 1990-2016), with (a) land cover, (b) temperature, and (c) precipitation variables. "ΔT": mean diurnal range; "MTWM": maximum temperature of the warmest month, "TDQ": mean temperature of the driest quarter, "PDQ": precipitation of the driest quarter; "PWQ": precipitation of the warmest quarter. "Water" and "Other" land cover types represent each less than 1% of the relative area and are therefore not shown.

5.2. Species distribution modelling

We obtain significant species distribution models for 19 species in P1, 18 in P2, and 16 in P3 (Table 2). All significant SDMs show a high performance (average AUC ± SD: 0.81 ± 0.06). This means that the chosen environmental variables well describe the distribution of these species. For eight species in P1, *B. veteranus* in P2, and three species in P3, the SDM's AUC is not significantly higher than the null-model's AUC (Table 2). This means that these SDMs do not perform significantly better than a randomly drawn model, and therefore that the relations between grid cells with species' presence and the environmental variable values in those grid cells are not stronger than can be expected by chance. Ten species show a significant SDM in each of the three periods (Table 2).

Table 2. Number of grid cells recorded, AUC of the null-model with a 95% confidence interval, and AUC of the SDM of each species in each period (P1: 1910-1930; P2: 1970-1989; P3: 1990-2016). Species are ordered by their European population trend according to the IUCN: increasing (green), stable (yellow), decreasing (red), and unknown trends (white). "/" are for species recorded in less than 10 grid cells, whereas AUC in brackets are when the SDM's AUC is not significantly higher than the null-model's AUC in a 95% confidence interval. AUC values are not comparable between species and between periods. "*" are the ten species for which a significant SDM is available for the three periods.

Species	Number of grid cells			AUC of the null-model in a 95% C.I.			AUC of the SDM		
	P1	P2	P3	P1	P2	P3	P1	P2	P3
B. hypnorum	44	263	573	0.787	0.663	0.614	(0.784)	0.714	0.660
B. lapidarius	104	297	824	0.764	0.659	0.617	0.820	0.747	(0.611)
B. pascuorum	138	573	965	0.745	0.647	0.617	0.752	0.669	(0.585)
B. pratorum	90	473	683	0.776	0.642	0.608	(0.768)	0.691	0.644
*B. terrestris**	70	323	392	0.758	0.657	0.626	0.768	0.726	0.709
*B. vestalis**	37	28	70	0.810	0.791	0.712	0.863	0.897	0.786
*B. bohemicus**	34	91	67	0.811	0.733	0.727	0.824	0.834	0.777
B. campestris	66	42	167	0.765	0.768	0.674	(0.754)	0.843	0.734
*B. hortorum**	106	316	438	0.772	0.655	0.628	0.782	0.738	0.652
B. jonellus	33	6	54	0.821	/	0.737	0.862	/	0.905
*B. lucorum**	58	238	118	0.769	0.663	0.712	0.814	0.732	0.772
B. norvegicus	5	19	18	/	0.856	0.848	/	0.929	(0.785)
*B. sylvestris**	46	149	115	0.782	0.694	0.702	0.867	0.796	0.773
B. barbutellus	60	11	4	0.760	0.768	/	0.772	0.953	/
B. confusus	20	0	0	0.864	/	/	0.905	/	/
B. distinguendus	73	2	0	0.747	/	/	0.844	/	/
B. humilis	38	9	7	0.803	/	/	(0.794)	/	/
B. muscorum	52	5	2	0.780	/	/	(0.754)	/	/
B. pomorum	14	0	0	0.757	/	/	0.981	/	/
*B. ruderarius**	70	113	54	0.756	0.716	0.741	0.790	0.794	0.779
B. ruderatus	63	3	6	0.772	/	/	0.809	/	/
*B. soroeensis**	24	26	41	0.834	0.825	0.769	0.871	0.888	0.874
B. subterraneus	37	8	0	0.819	/	/	(0.800)	/	/
*B. sylvarum**	48	18	13	0.794	0.842	0.781	0.820	0.931	0.982
B. veteranus	53	17	8	0.777	0.849	/	(0.773)	(0.833)	/
B. cryptarum	47	77	44	0.788	0.721	0.757	(0.779)	0.837	0.849
B. magnus	44	6	36	0.800	/	0.775	0.822	/	0.911
*B. rupestris**	57	12	67	0.781	0.771	0.728	0.830	0.838	0.808

In figure 2, we present the habitat suitability maps of three examples of species with a different European population trend according to the IUCN (Rasmont *et al.* 2015b): the increasing *B. terrestris,* the stable *B. bohemicus*, and the decreasing *B. sylvarum*. The habitat suitability maps of other species are provided in supplementary material (Fig. S4-10). The modelled distribution of *B. terrestris* shows a low performance, which means that its relatively widespread distribution (Fig. 2a) is poorly explained by the chosen environmental variables, although it performs significantly better than a random model. Although less widespread, its parasite (*i.e. B. vestalis*) has a very similar modelled distribution (Fig. S10c). The modelled distribution of the stable *B. bohemicus* tends to be restricted in the south of the country (Fig. 2b), and is quite different from its host species (*i.e. B. lucorum*, Fig. S6). The modelled distribution of the decreasing *B. sylvarum* shows a progressive restriction of habitat suitability to the south-east of Belgium (Fig. 2c).

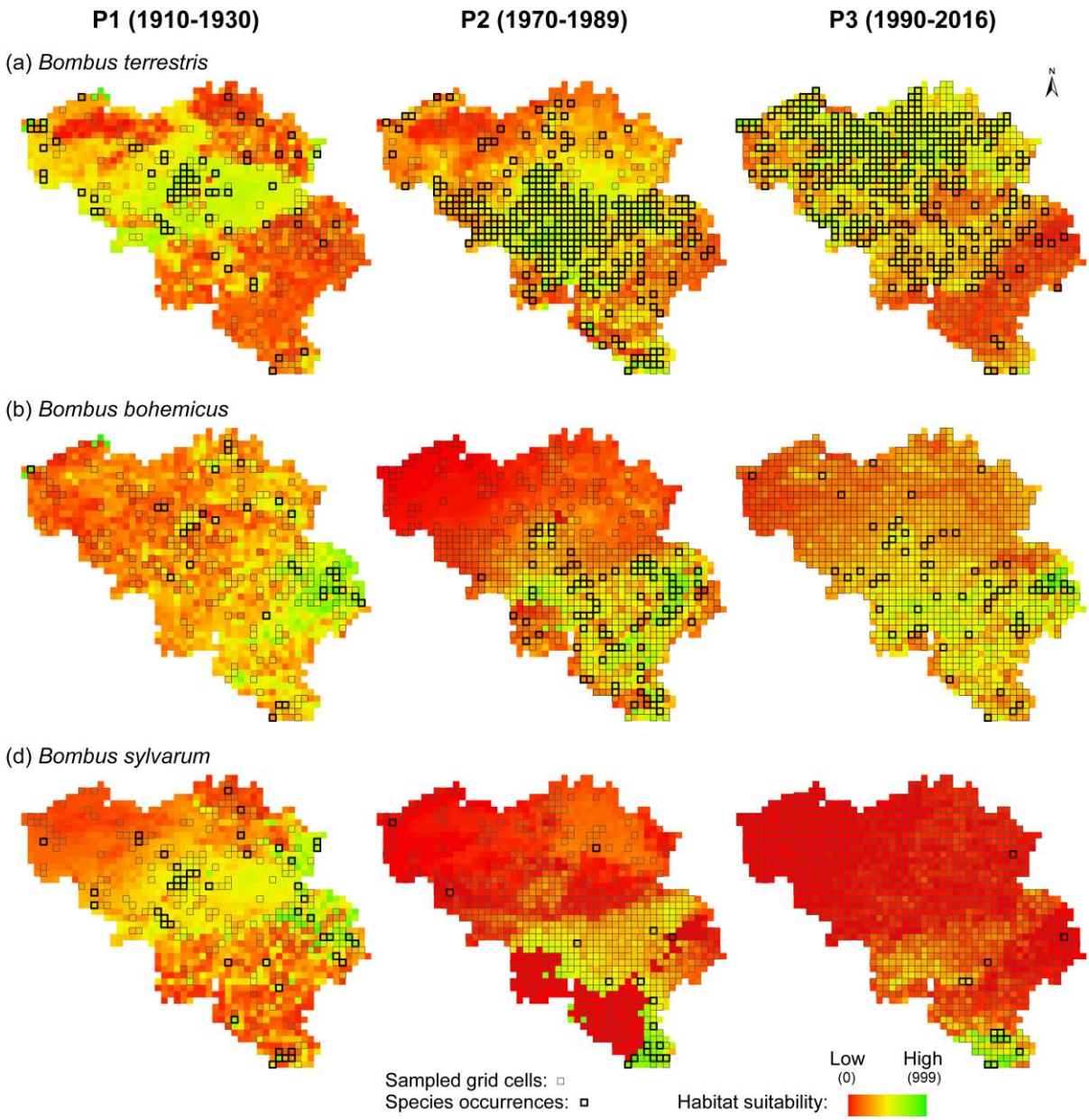

Figure 2. Examples of habitat suitability maps obtained from the species distribution models.

5.3. Influence of environmental variables on bumblebee distributions

Overall, the importance of environmental variables in defining bumblebee distributions ranges from 0 to 68%, and values vary between variable types (Table 3).

Table 3. Influence (*i.e.* "permutation importance" value, in percent) of environmental variables on bumblebee distributions, all periods combined. "SD": standard deviation. "Sett": settlement area; "Crop": cropland area; "Wood": woodland area; "Grass": grassland area; "PDQ": precipitation of the driest quarter; "PWQ": precipitation of the warmest quarter; "ΔT": mean diurnal range; "MTWM": maximum temperature of the warmest month, "TDQ": mean temperature of the driest quarter. The land cover "other" is not included in the category "land cover" and in further analyses.

	Mean	SD	Minimum	1st quartile	Median	3rd quartile	Maximum
All variables	**10.95**	**10.38**	**0.00**	**3.16**	**8.68**	**15.45**	**68.26**
Land cover	**12.06**	**10.06**	**0.00**	**4.98**	**10.51**	**16.34**	**60.20**
- Sett	11.03	8.79	0.00	4.53	10.52	15.51	44.98
- Crop	12.60	11.95	0.00	5.66	9.87	17.56	60.20
- Wood	15.28	11.11	0.22	8.65	13.70	20.69	51.58
- Grass	9.32	6.89	0.00	4.41	8.65	12.39	38.13
- (Other)	(1.47)	(2.18)	(0.00)	(0.00)	(0.06)	(2.84)	(7.04)
Precipitation	**12.96**	**13.15**	**0.00**	**2.51**	**9.34**	**18.53**	**68.26**
- PDQ	12.48	14.53	0.00	1.17	8.59	20.01	68.26
- PWQ	13.43	11.73	0.00	6.48	10.01	17.41	60.83
Temperature	**8.13**	**7.88**	**0.00**	**2.34**	**6.01**	**11.23**	**38.34**
- ΔT	8.47	8.19	0.00	2.88	6.64	13.38	37.55
- MTWM	8.52	7.54	0.00	2.60	7.64	11.60	36.15
- TDQ	7.40	7.99	0.00	2.30	4.53	9.26	38.34

Results of the linear mixed-effects model are presented in supplementary material (Table S1). Post-hoc Tukey's tests reveal that temperature variables have a significantly lower influence than precipitations (p-value < 0.01) and land cover variables (p-value < 0.001) on bumblebee distributions all time periods combined (Fig. 3a). When the comparisons are performed within a single time period, precipitation and land cover variables have a significantly higher influence than temperature respectively in P2 (p-value < 0.05) and P3 (p-value < 0.01; Fig. 3b; Table S2). The significant higher influence of precipitations in P2 comes from the very high influence of precipitation of the driest quarter during this period (PDQ, Fig. 4), which is significantly higher than all the other climate variables (p-values <0.05 for PWQ, ΔT, MTWM, and p-value < 0.01 for TDQ), and than grassland area

(p-value < 0.01; Table S3). Within land cover types, woodland and grassland are the most explicative variables in P1, but their influence significantly decreases between P1 and P2 (Fig. 4; Table S3). On the contrary, cropland area is among the lowest explicative variables in P1, but its influence significantly increases and it becomes the most important variable in P3 (Fig. 4; Table S3).

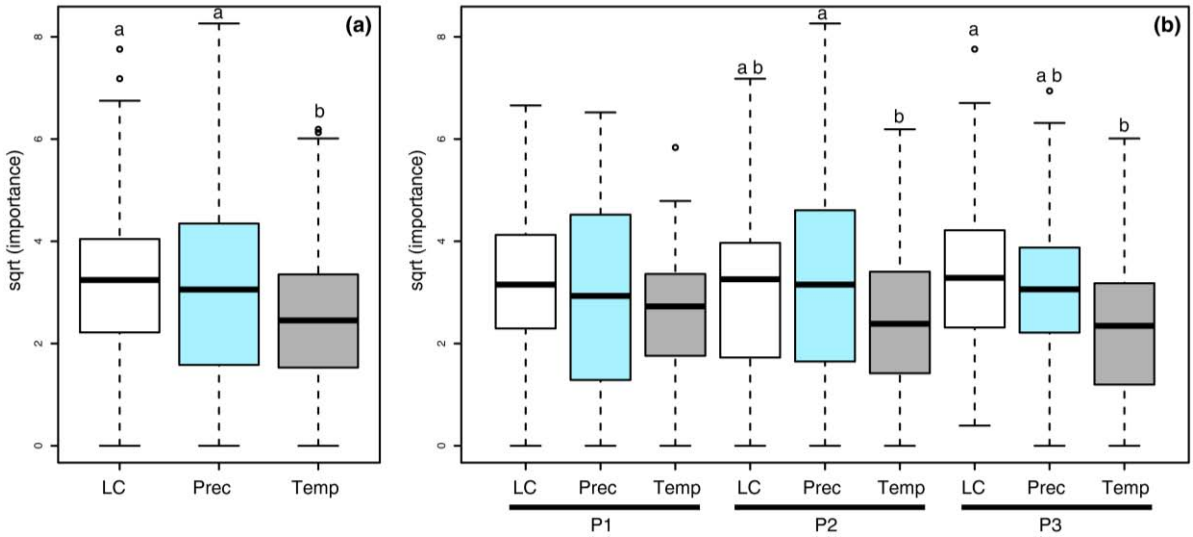

Figure 3. Boxplots showing the median and the quartiles of the influence (*i.e.* "permutation importance" value) of environmental variables on bumblebee species distribution models according to (a) their type and (b) the interaction between periods and variable types. P1: 1910-1930; P2: 1970-1989; P3: 1990-2016; "LC": land cover variables; "Prec": precipitation variables; "Temp": temperature variables. Lower case letters give the results of significant statistical tests between variable types within a time period. Letters for non-significant tests are not shown. Comparisons of variable types between time periods are not significant.

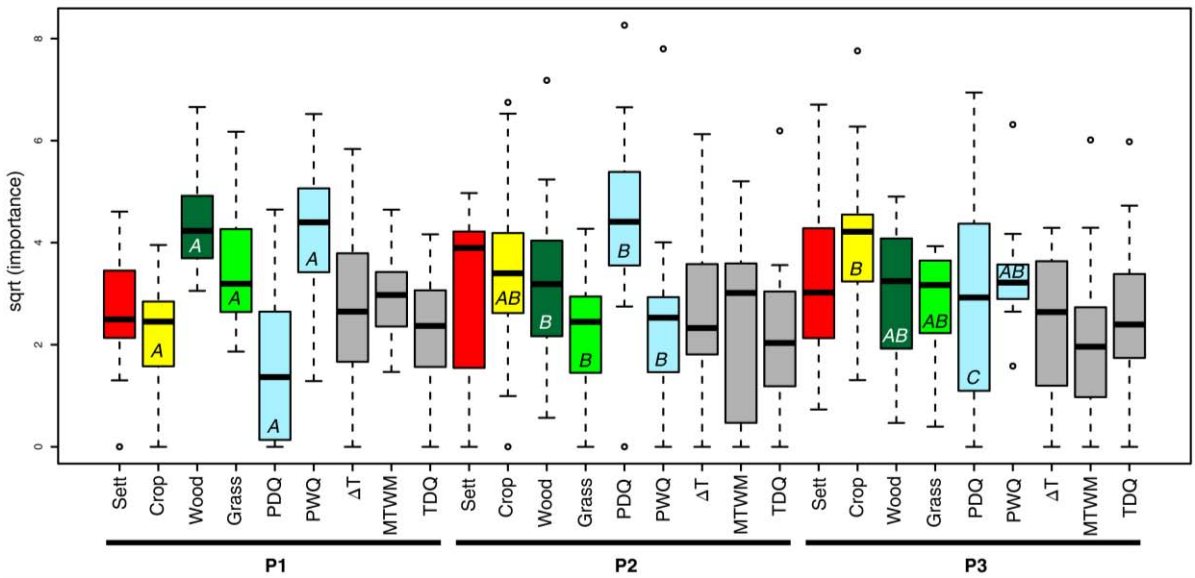

Figure 4. Boxplots showing the median and the quartiles of the influence (*i.e.* "permutation importance") of environmental variables on the bumblebee species distribution models in each period (P1: 1910-1930; P2: 1970-1989; P3: 1990-2016). "Sett": settlement area; "Crop": cropland area; "Wood": woodland area; "Grass": grassland area; "PDQ": precipitation of the driest quarter; "PWQ": precipitation of the warmest quarter; "ΔT": mean diurnal range; "MTWM": maximum temperature of the warmest month, "TDQ": mean temperature of the driest quarter. Upper case letters give the results of significant statistical tests of a single variable between time periods. Letters for non-significant tests are not shown. Results of significant statistical tests between variables within a time period shown in Table S3.

Concerning the effects of each environmental variable on species modelled distributions, they vary between variables and between time periods (Fig. 5). The most noteworthy changes concern the effects of settlement and especially cropland areas, which switch from a positive influence for most species in P1 to a negative influence in P2 and P3, whereas the opposite happens for grassland area.

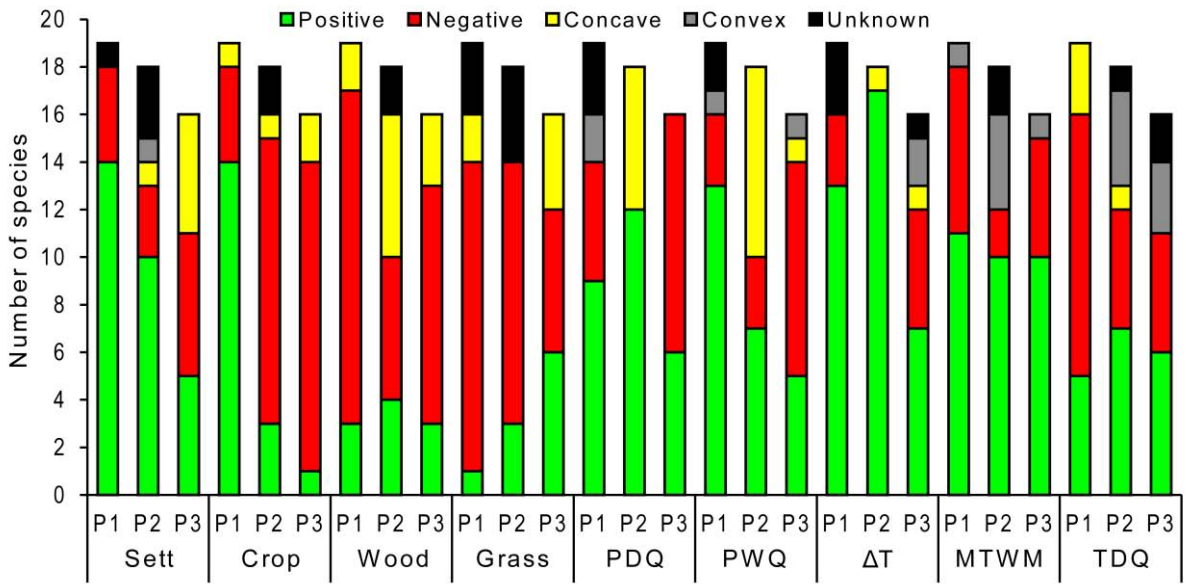

Figure 5. Number of species with a significant SDM per environmental variable and period, according to the effects of environmental variables. "Positive": positive linear effect; "Negative": negative linear effect; "Concave": concave quadratic effect (curved inwards, *i.e.* the species prefers intermediate values of the variable); "Convex": convex quadratic effect (curved outwards, *i.e.* the species prefers extreme values); "Unknown": unknown effect (*i.e.* flat curve). "P1": 1910-1930; "P2": 1970-1989; "P3": 1990-2016; "Sett": settlement area; "Crop": cropland area; "Wood": woodland area; "Grass": grassland area; "PDQ": precipitation of the driest quarter; "PWQ": precipitation of the warmest quarter; "ΔT": mean diurnal range; "MTWM": maximum temperature of the warmest month, "TDQ": mean temperature of the driest quarter.

Regarding specific variation, some species have their distribution influenced equally by the three types of variables (*e.g*. *B. hortorum*), several seem to be more influenced by land cover (*e.g. B. terrestris, B. norvegicus, B. ruderarius, B. ruderatus, B. pomorum*), and others by precipitation variables (*e.g. B. lapidarius, B. jonellus*; Fig. 6). The details per each environmental variable is provided in supplementary material (Fig. S11).

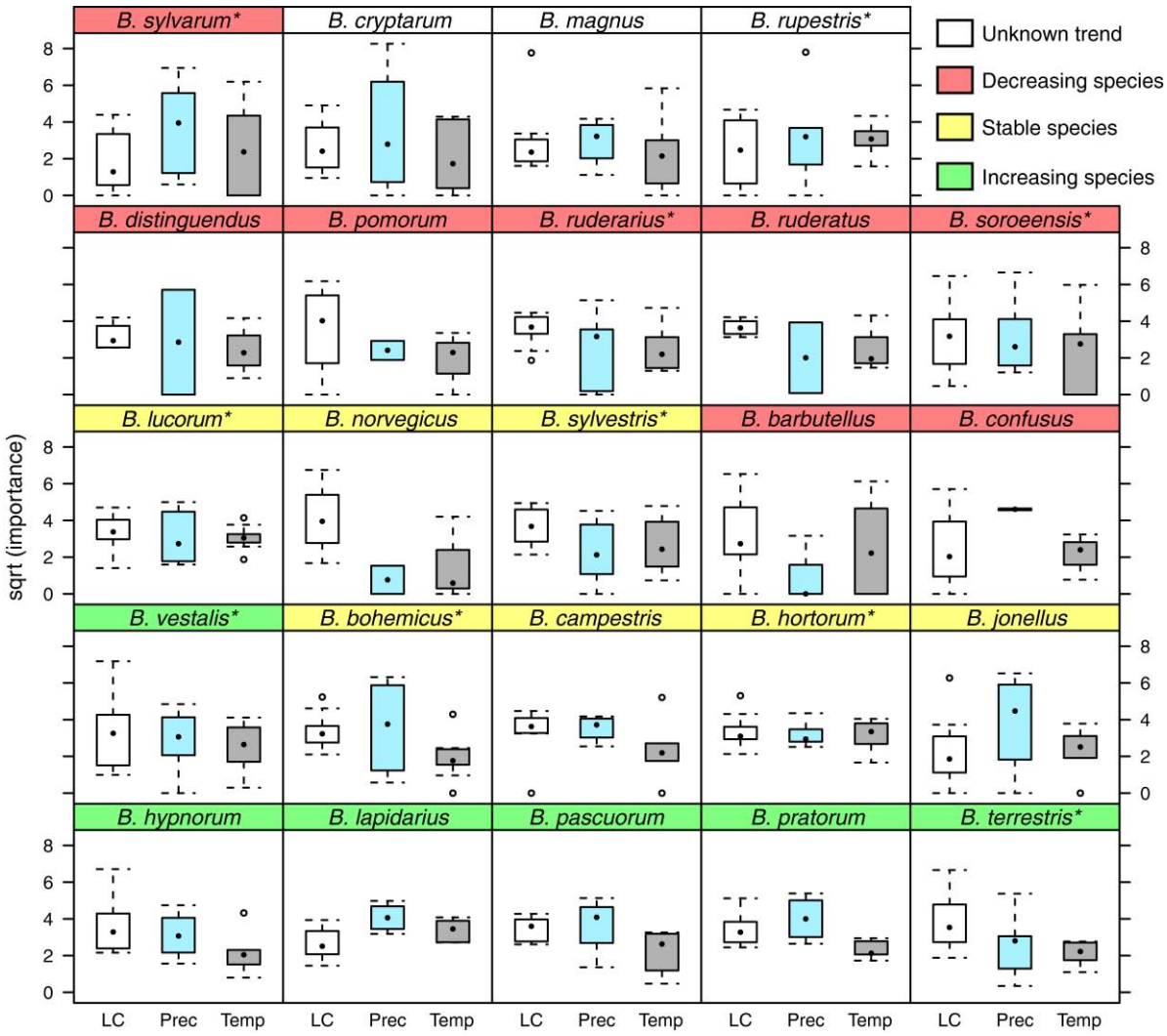

Figure 6. Boxplots showing the median and the quartiles of the influence (*i.e.* "permutation importance" value) of environmental variables types in defining each species distribution, sorted by their European population trend according to the IUCN. "LC": land cover variables; "Prec": precipitation variables; "Temp": temperature variables. "*" are the ten species for which a significant SDM is available for the three time periods.

6. Discussion

Using historical bumblebee records, climate and land cover data, we analysed species distribution in three time periods along the last century using species distribution modelling. Out of the 31 *Bombus* species recorded in Belgium, we obtain a significant modelled distribution for 19 species in P1 (1910-1930), 18 in P2 (1970-1989), and 16 in P3 (1990-2016). For most species, modelled

distributions show a high performance, meaning that environmental variables well define their distribution across Belgium at the 5x5km resolution. We show that the influence of climate and land cover in explaining the historical distribution of bumblebees in Belgium changes significantly across time and that bumblebee species show vastly different responses to these environmental variables.

6.1. Relative influence of environmental variables and their temporal variation

Contrary to a similar study in the Netherlands (Aguirre-Gutiérrez *et al.* 2017), our results show that variables related to precipitation (*i.e.* precipitation of the driest and the warmest quarters) and land cover influence more the bumblebee distributions than variables related to temperature, respectively in 1970-1989 and in 1990-2016. The fact that precipitation has a high influence in 1970-1989 could be partly explained by the severe local drought spreading from December 1975 to the end of summer 1976 in Belgium, which caused a high mortality in the herbaceous vegetation (Brochet 1977, Klein 2009, IRM 2017b). The severity of the drought greatly varied between regions of the country (Klein 2009). This high spatial variability of unfavourable values of precipitation could explain the high influence of these variables on bumblebee species distributions during this period (Pearson & Dawson 2003; Ogilvie *et al*. 2017). Droughts can have heavy impacts on bumblebee colonies by reducing the availability of floral resources (Memmott *et al.* 2007, Thomson 2016). With climate change, droughts are expected to be

more frequent, intense and associated with periods of heat waves (Meehl & Tebaldi 2004; IPCC 2014). Rainfall variables reflecting droughts should therefore be included in future projection models.

Even if temperature values increased between P2 and P3, their influence on bumblebee distributions remained among the less important variables in the three time periods. Yet, high temperatures during periods of heatwaves are known to be damaging for bumblebees (Rasmont & Iserbyt 2012; Martinet *et al.* 2015a), and Aguirre-Gutiérrez *et al.* (2017) observed an increase in importance of temperature in determining the distributions of pollinators in the recent decades. Our results suggest that while temperature increased, the variability of the temperature conditions occupied by bumblebees remained similar. The fact that the influence of temperature did not increase over time does not mean that the increase of temperature has not influenced the species range. Moreover, we cover a different region and a different set of climate variables (and hence processes) than Aguirre-Gutierrez *et al.* (2017), which may also explain the differences between the two studies.

Like in other countries of Western Europe (Houghton 1994; Fuchs *et al.* 2014; European Environment Agency, 2017), the major land cover changes occurred mainly between P1 and P2. Influence of each land cover types on bumblebee distribution also significantly changed between these two time periods. Whereas woodland and grassland areas increased, their influence significantly decreased. While settlement area increased, its influence on bumblebee distributions increased as well, and its effect became more and more negative over time. The

constant urbanisation of land is known to be a major threat for bumblebee populations and diversity in general (Ahrné *et al.* 2009; Jha & Kremen 2013; European Environment Agency, 2016). However, urban sprawl and the development of settlement areas did not occur at the same intensity in all regions of Belgium (Fig. S2). Some regions, especially in the south of the country, still have a small proportion of settlement areas and still accommodate a high diversity of bumblebees (Vray *et al.*, in prep.), including the most declining ones.

On the contrary, cropland did not seem to strongly restrict bumblebee distributions a century ago in Belgium, although it occupied a much larger area than today (Fig. S2). Most of the currently restricted species were relatively widespread across the country during 1910-1930, including regions with a high density (even higher than today) of cropland. The sharp decrease of cropland area occurring between P1 and P2 was coupled with a drastic increase of its influence on bumblebee populations between P1 and P3. Moreover, while the effect of this land cover type was positive for most species in P1, it became mostly negative in P2 and in P3. This means that, in addition to having increased its influence over time, cropland no longer has the same effect on the current distribution of bumblebees as it had a hundred years ago. This phenomenon can be explained by agricultural intensification. The fact that cropland has a high negative influence on the current distribution of the majority of bumblebee species is most likely coming from its management rather than from its extent. Indeed, agricultural intensification began in the 1950s in Western Europe, with the advent of agricultural moto-mechanization and chemical fertilizers occurring mainly from

1950 to 1970 (Christians 1998 ; Robinson & Sutherland 2002 ; Mazoyer & Roudart 2006), between our two first time periods. Agricultural practices further intensified with the Mansholt Plan during the 1970s, between our two last time periods (*e.g*. suppression of crop rotation, removal of hedgerows and homogenization of the agricultural landscape with large monocultures, and increased crop yields; Christians 1998 ; Robinson & Sutherland 2002 ; Mazoyer & Roudart 2006). In addition to confirming the negative effect of modern agriculture on bumblebees like in other studies (*e.g*. Kleijn *et al.* 2009; Le Féon *et al.* 2010; Senapathi *et al.* 2015), our results emphasize that the variable of cropland area of a century ago is not comparable to the current one. Moreover, with the growing development of agri-environmental measures since the 1990s and especially the 2000s (*e.g*. Kleijn & Sutherland 2003; Batáry *et al.* 2015), this variable is expected to continue to change in the future. This means that one must be extremely careful when analysing the influence of cropland area on species and biodiversity over time, and that cropland area is probably not an appropriate variable for (future) projections. This concern is even more important for future projections because the majority of future changes expected in the agricultural sector are changes in land use and not land cover (Laurance 2001).

6.2. Variations among species

Our results show that the influence of each climate and land cover variables varies between species. Some species have their distribution more influenced by land cover (*e.g. B. terrestris, B. norvegicus, B. ruderarius, B. ruderatus, B. pomorum*),

some by precipitation variables (*e.g. B. lapidarius, B. jonellus*), and some others seem to be equally influenced by the three types of variables (*e.g*. *B. hortorum*). These differences could be explained by species preferences and specialisation in terms of habitat and climatic conditions. Species are predicted to respond differently to environmental changes according to their specific ecological traits, in bumblebees (Goulson *et al.* 2005; Williams *et al.* 2007, 2010; Bommarco *et al.* 2010; De Palma *et al.* 2015, Aguirre-Gutiérrez *et al.* 2016) and in other organisms in general (*e.g*. Davies *et al*. 2004; Brook *et al*. 2008).

In parallel, we observe large differences in predicted geographical distribution between species. The generalist and increasing species at the European scale (*i.e. B. hypnorum*, *B. lapidarius*, *B. pascuorum*, *B. pratorum*, *B. terrestris*, and *B. vestalis*) present a widespread distribution across the country and are still well present in the north, where settlement and agriculture are the most developed and dominant today. They seem to be well adapted to urban environment and frequently occur in gardens and parks in urban and farmland areas where they generally dominate the assemblages (McFrederick & LeBuhn 2006; Ahrné *et al.* 2009). For many of these species, the distribution models show a low level of performance, sometimes even inferior to the null model and therefore not significant. This means that they are poorly influenced by the chosen land cover and climatic variables. As these widespread species occur in a wider geographic range including a higher number of environmental conditions, the eco-climatic envelope used by the models to produce their habitat suitability projections is wider and therefore less affected by changes in the environmental variables (Hernandez *et*

al. 2006; Mateo *et al*. 2010). Several studies showed that species with a wider geographic extent have generally a lower ecological specialisation, and are therefore less sensitive to global change and less subject to decline and extinction (Brook *et al.* 2008; Goulson *et al.* 2005; Bommarco *et al.* 2010; Williams *et al.* 2010; De Palma *et al.* 2015). This is for example the case for *B. terrestris* and *B. vestalis*, which are both widespread generalist species and increasing in Europe (Martinet *et al*. 2015b; Rasmont *et al.* 2015b).

On the contrary, the most currently restricted species (*e.g. B. barbutellus*, *B. soroeensis*, *B. sylvarum*), which are decreasing at the European scale, are mainly persisting only in the south-east of Belgium today. This region show higher rainfall, higher proportions of woodland, and lower proportions of cropland and settlement (Fig. S2). These decreasing species seem therefore to maintain their populations only in well-watered regions with lower levels of urbanisation and agriculture and higher levels of natural habitats reflected by the amount of woodland. These results tend to confirm the negative effects of settlement and modern agriculture especially on decreasing species as shown by other studies (Tscharntke *et al.* 2005; Ahrné *et al.* 2009; Kleijn *et al.* 2009; Jha & Kremen 2013; Le Féon *et al.* 2010; European Environment Agency 2016). Results very similar to ours were obtained in the UK (Senapathi *et al.* 2015), where the sites where urbanisation and cropland expansion were the most intense lost the highest number of species. On the contrary, the sites that lost the lowest number of species were those where woodland remained the most abundant or increased during the last 80 years.

However, two other geographically restricted species show a restriction of their range in the east and especially north-east of the country: *B. jonellus* and *B. magnus*. These two species are particularly specialized on heathland (Moquet *et al.* 2017), which is the most abundant where their habitat suitability projection is the highest. Although heathland is not included in our land cover variables, our environmental variables are able to clearly define the distribution of these species. This could be explained by the fact that heathland ecosystems require very specific climate (*e.g*. low temperatures and high levels of humidity; Peñuelas *et al.* 2009) that could have been partly captured by our bioclimatic variables.

6.3. Caveat and perspectives for future research

The very different locations of the sampled grid cells between the three time periods may have led to sampling bias. Indeed, sampling was mostly performed around cities in P1 (*e.g*. Brussels), in the south of the country in P2 and in the north in P3 (Fig. S1). This particularly affects the modelled distributions of widespread species (*e.g*. *B. terrestris*), which gives the impression of shifting northward between P2 and P3. The fact that the north seems particularly unsuitable for these species in P2 but becomes more suitable in P3 is most likely due to these spatial sampling biases. Moreover, as the north and the south of the country show different climate and land cover trends (Fig. S2-3), this may partly explain the differences in influence observed between periods. For example, the greatest influence of precipitation in P2 may be due to the fact that sampling was mostly performed in the south, where precipitation is higher.

As in Aguirre-Gutiérrez *et al.* (2017), our results show that the influence of climate and land cover on bumblebee species distributions does vary over time. The fact that the chosen environmental variables have a given influence on the distribution of a species during one period does not necessarily mean that these will have the same influence in another period. Neglecting these changes could misrepresent the future projection of species distributions based on present data. We particularly noted that cropland area is probably not an appropriate variable for projections over time. Considering variables reflecting the management of crops in addition to their areal extent could add valuable information for modelling bumblebee species, which greatly suffer from the intensification of agriculture (*e.g*. Kleijn *et al.* 2009; Le Féon *et al.* 2010). For future projections, including these concerns in land cover and land use changes scenarios should make the models more likely and reliable (Rounsevell *et al*. 2006; Martin *et al*. 2013).

Similarly, models could probably be improved by considering land cover data with better spatial and thematic resolutions. Our land cover data, which are currently the only available at the Belgian and the century scales, may not have fully captured the landscape information relevant for bumblebees because consisting in a one-kilometre resolution and only six types (four of which were useful for SDMs). The low spatial resolution did not allow the calculation of habitat fragmentation variables as Aguirre-Gutiérrez *et al.* (2017) did, given that the majority of movements of bumblebees are below one kilometre (Walther-Hellwig & Frankl 2000; Osborne *et al.* 2008; Wolf & Moritz 2008). Yet, landscape-

scale processes should be considered in projecting species distributions. For now, most of species distribution models does not take into account the fine processes occurring at the meta-population or the meta-community levels (Brooker *et al.* 2007; Franklin 2010).

Dispersal ability of a species greatly influences its responses to climate and land cover changes (*e.g*. Tscharntke *et al.* 2005; Bowler & Benton 2005; Bommarco *et al.* 2010; Darvill *et al.* 2010), and therefore the influence of these environmental factors on its distribution (Brooker *et al.* 2007; Ehrlén & Morris, 2015, Aguirre-Gutiérrez *et al.* 2016). A region considered suitable based on the climate and land cover variables chosen for the modelling will not necessarily be occupied by the species if this species is unable to reach it (Schurr *et al.* 2012). Dispersal abilities of bumblebee species are beginning to be studied but are still poorly known (*e.g*. Darvill *et al.* 2010; Lepais *et al.* 2010). Similarly, species interactions within assemblages and communities should also be considered (Araújo & Luoto 2007; Brooker *et al.* 2007; Giannini *et al.* 2013). For example, a parasite will not occupy a region where its host is not present. Although this seems logical, distribution modelling of parasite species never takes into account the distribution of their host species (but see Giannini *et al.* 2013 on bee parasites). This is often the case for bumblebees, for which the distribution of host species does not intervene in the distribution modelling of cuckoo (*i.e.* inquiline) species, simply because these interactions are highly variable and still poorly understood (Løken 1984; Williams 2008; Lhomme 2009). A cuckoo bumblebee can parasitize several host species, which may not necessarily be the same in all

regions (Løken 1984; Williams 2008; Lhomme 2009). Yet, these important interactions could greatly improve the models performance if they were more studied.

All these considerations about land management, landscape variables and species ecology would inevitably make the models much more complex, but they would allow them to attain a greater likelihood, more faithful to the real ecological processes at work, and also for future projections (Pacifici *et al.* 2015).

7. Conclusion

Our results show that the influence of land cover and climate variables on bumblebee distributions varies between periods and between species. We particularly emphasize the great influence of precipitation, especially precipitation during the driest and the warmest quarters of the year, as well as land cover. High density of settlement and cropland seem to be detrimental for declining species in the recent period, whereas increasing species do not seem to be negatively affected by these land cover types. Our results suggest that settlement and cropland areas had a great impact on species distribution changes during the last century. But we also show that the effect of cropland areas switched from a low positive effect a century ago to a very high negative effect today, most likely coming from agricultural intensification. In order to improve the models and for future projections, it would be interesting to consider variables reflecting the current and future managements of cropland, the

landscape structure, as well as the dispersal ability of the species and its interspecific interactions.

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10. Supplementary material

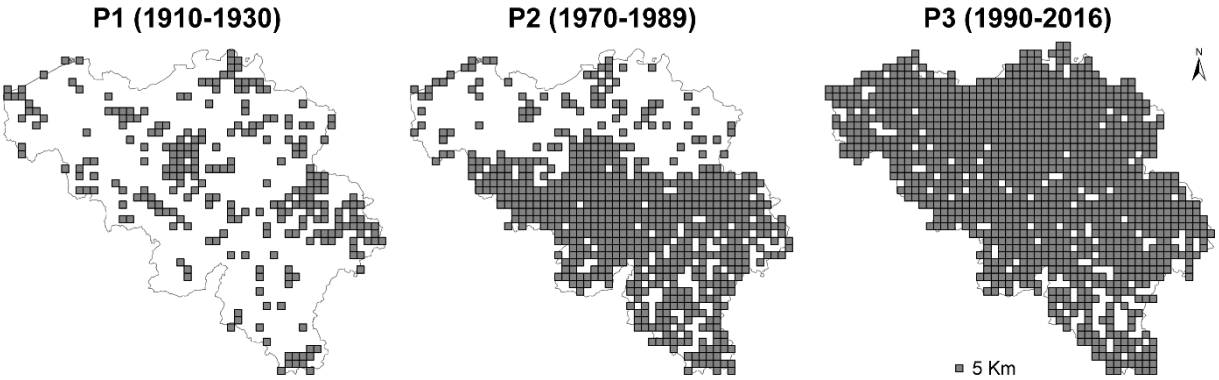

Figure S1. Maps of the sampled grid cells in each period in Belgium (spatial resolution: 5x5 km grid cells).

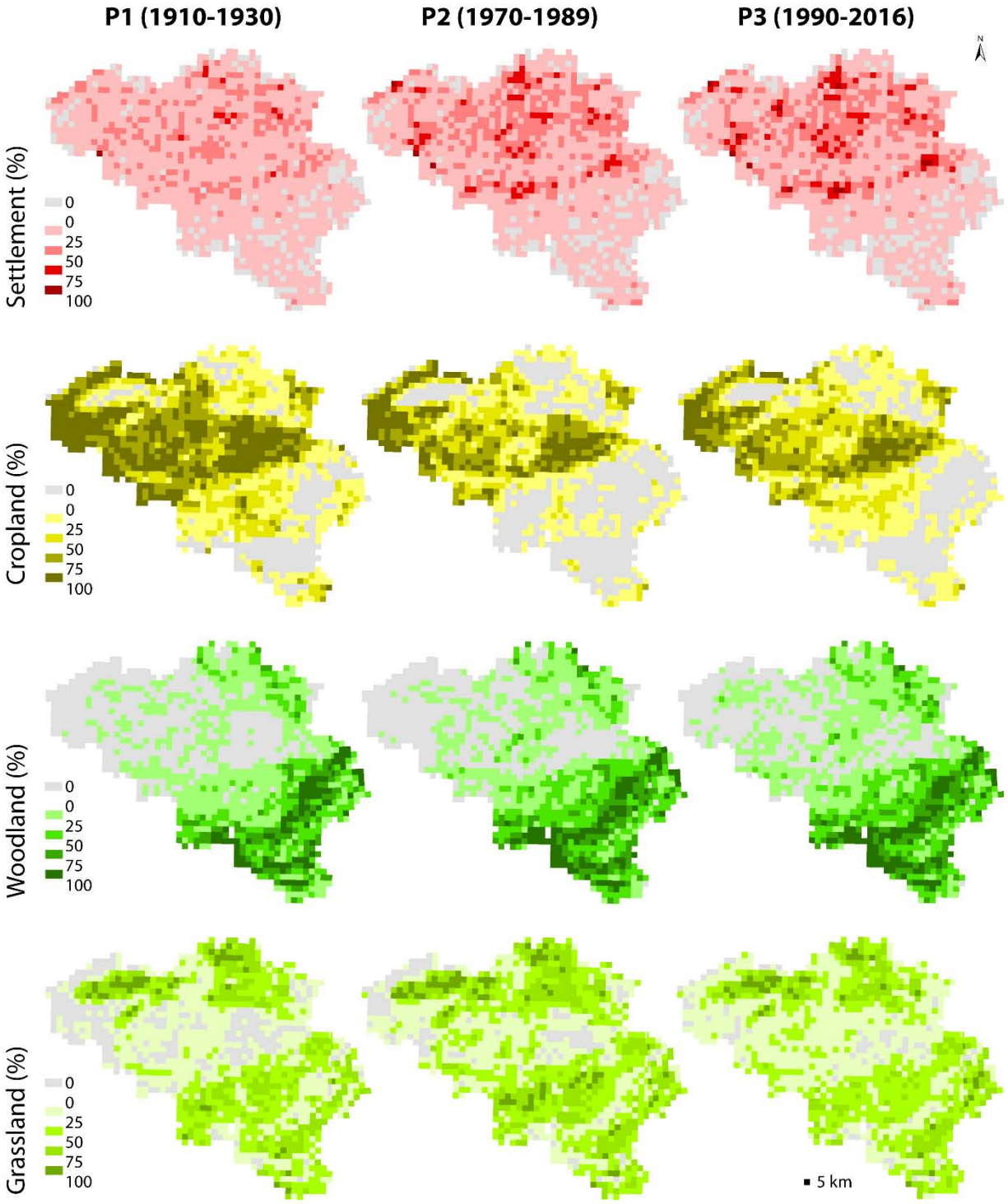

Figure S2. Proportional area of each land use type used for SDM in each period and each 5x5 km grid cell. The "other" land use type is not shown because representing very few grid cells in the north-west of the country (along the coast).

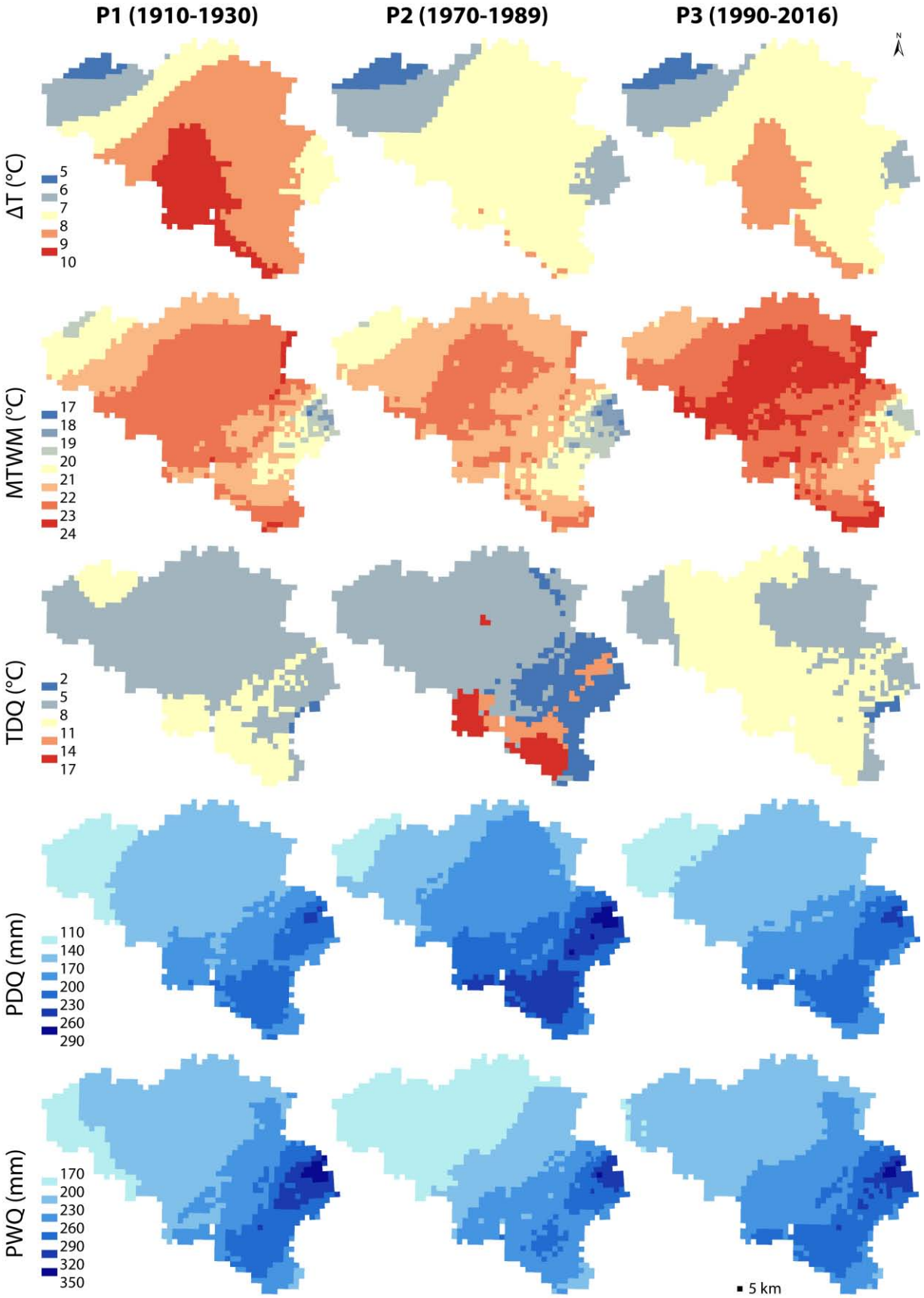

Figure S3. Climatic variables in each period and each 5x5 km grid cell. See the text for details.

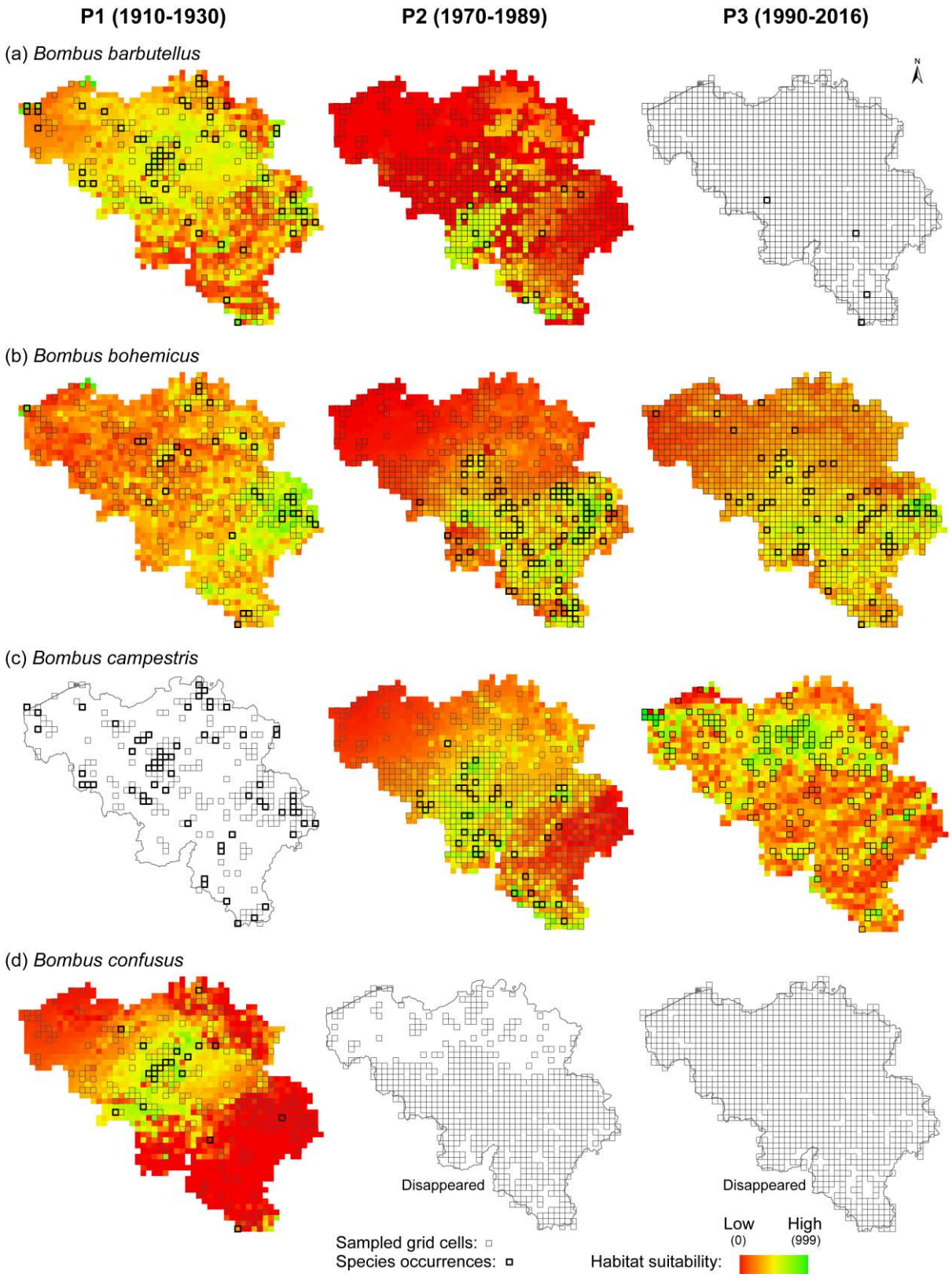

Figure S4. Species distribution maps. When the number of grid cells was inferior to 10 or when the SDM was not significant based on its AUC, only the map of the records is shown.

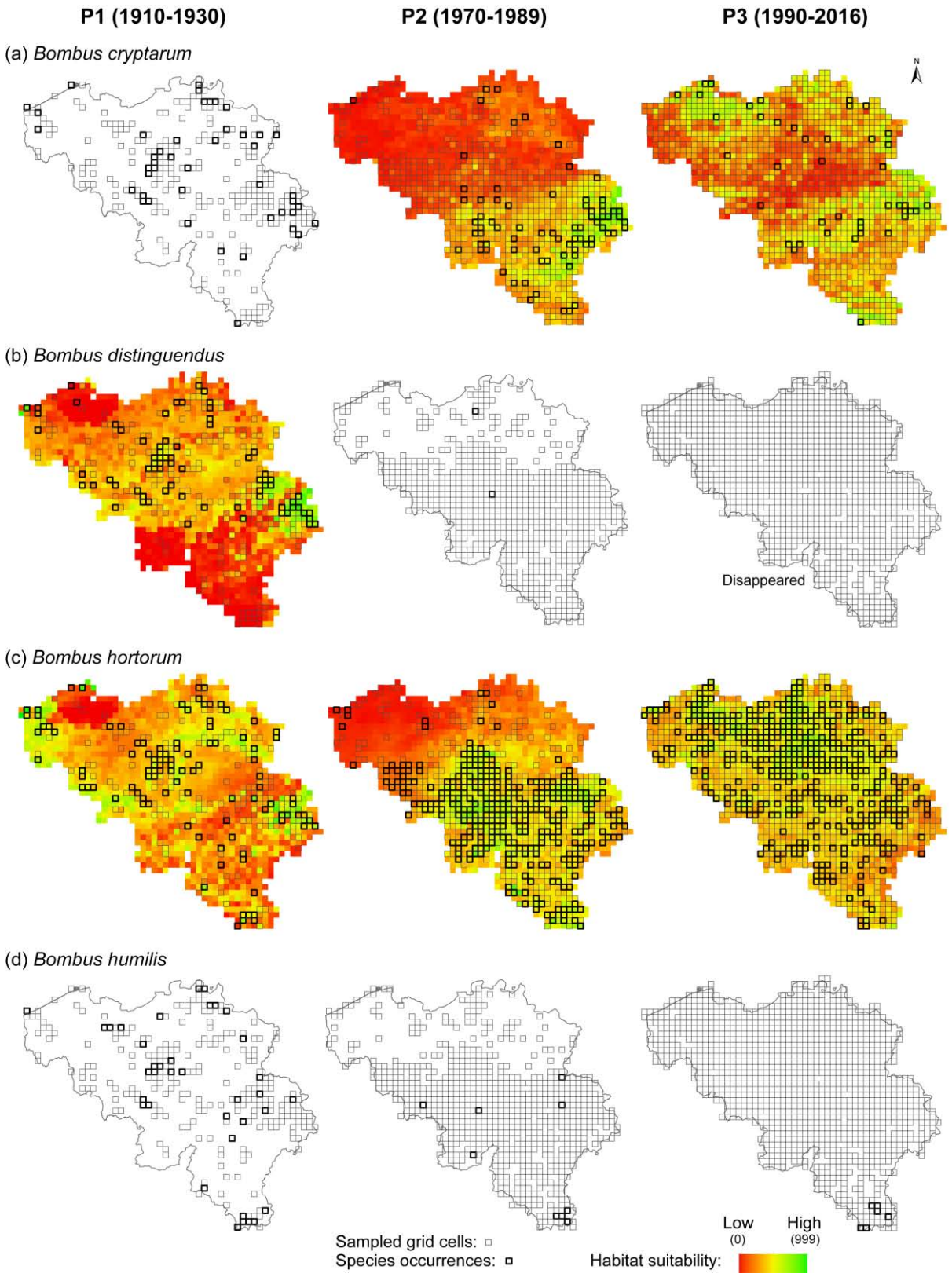

Figure S5. Species distribution maps. When the number of grid cells was inferior to 10 or when the SDM was not significant based on its AUC, only the map of the records is shown.

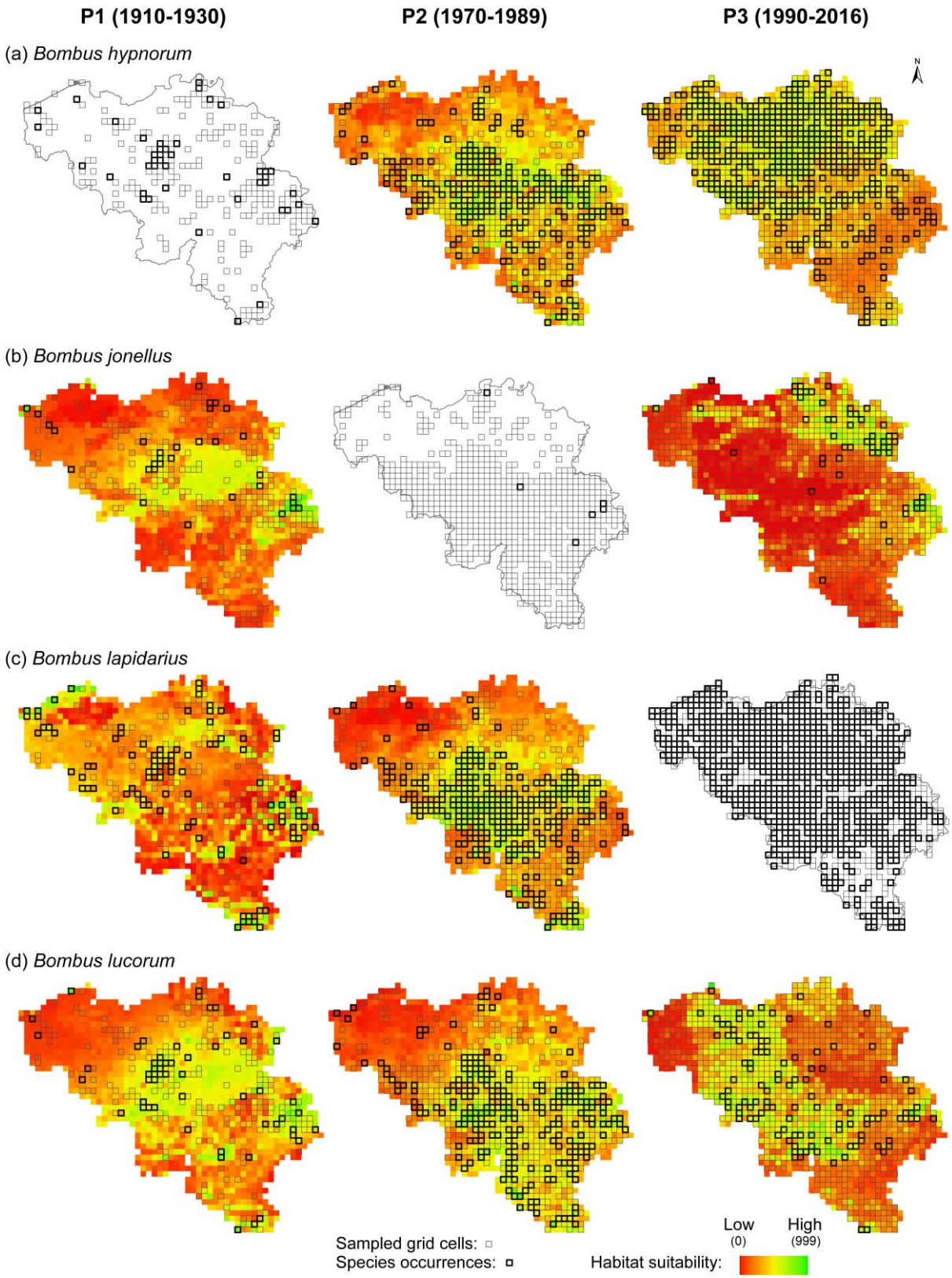

Figure S6. Species distribution maps. When the number of grid cells was inferior to 10 or when the SDM was not significant based on its AUC, only the map of the records is shown.

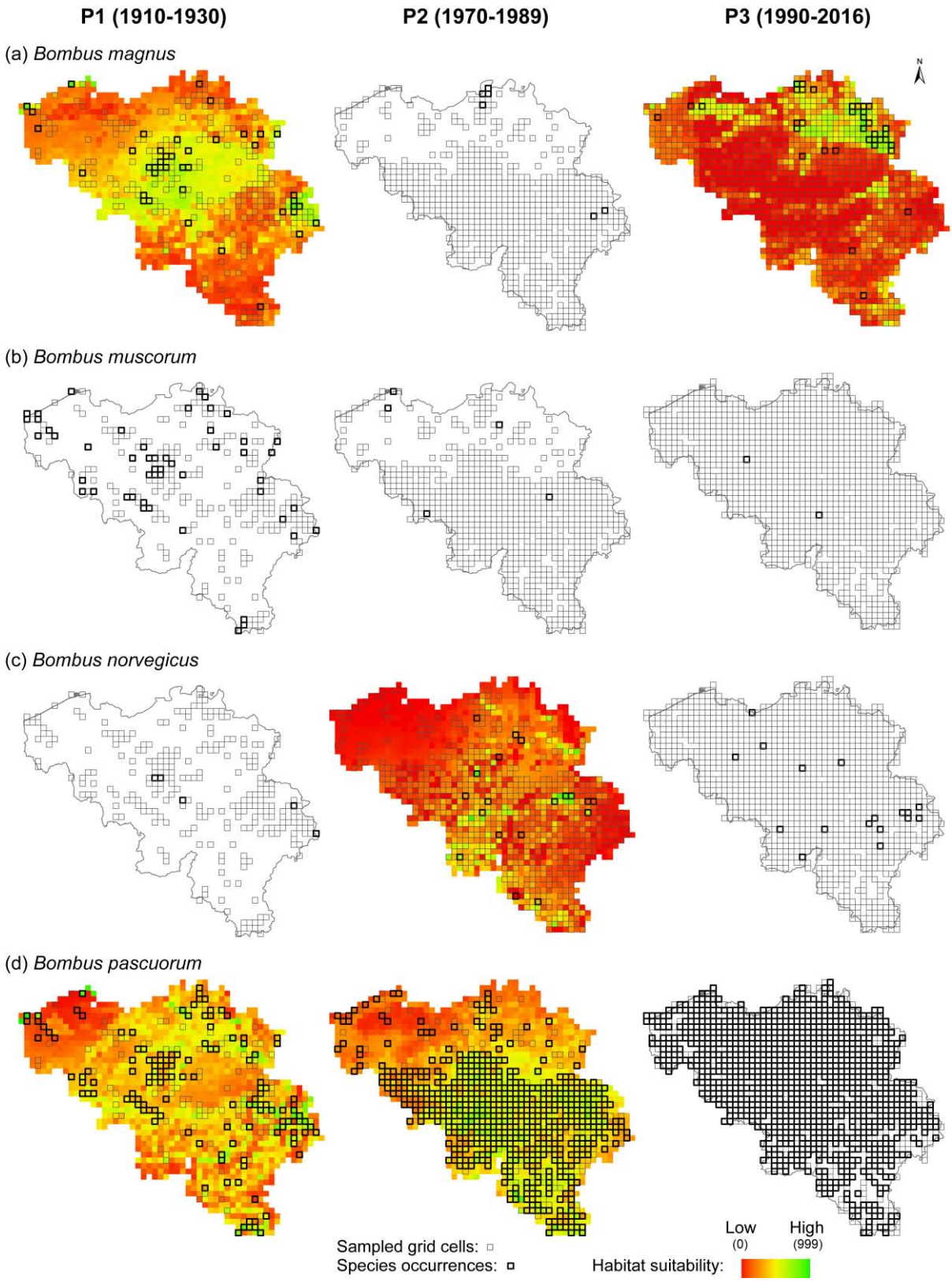

Figure S7. Species distribution maps. When the number of grid cells was inferior to 10 or when the SDM was not significant based on its AUC, only the map of the records is shown.

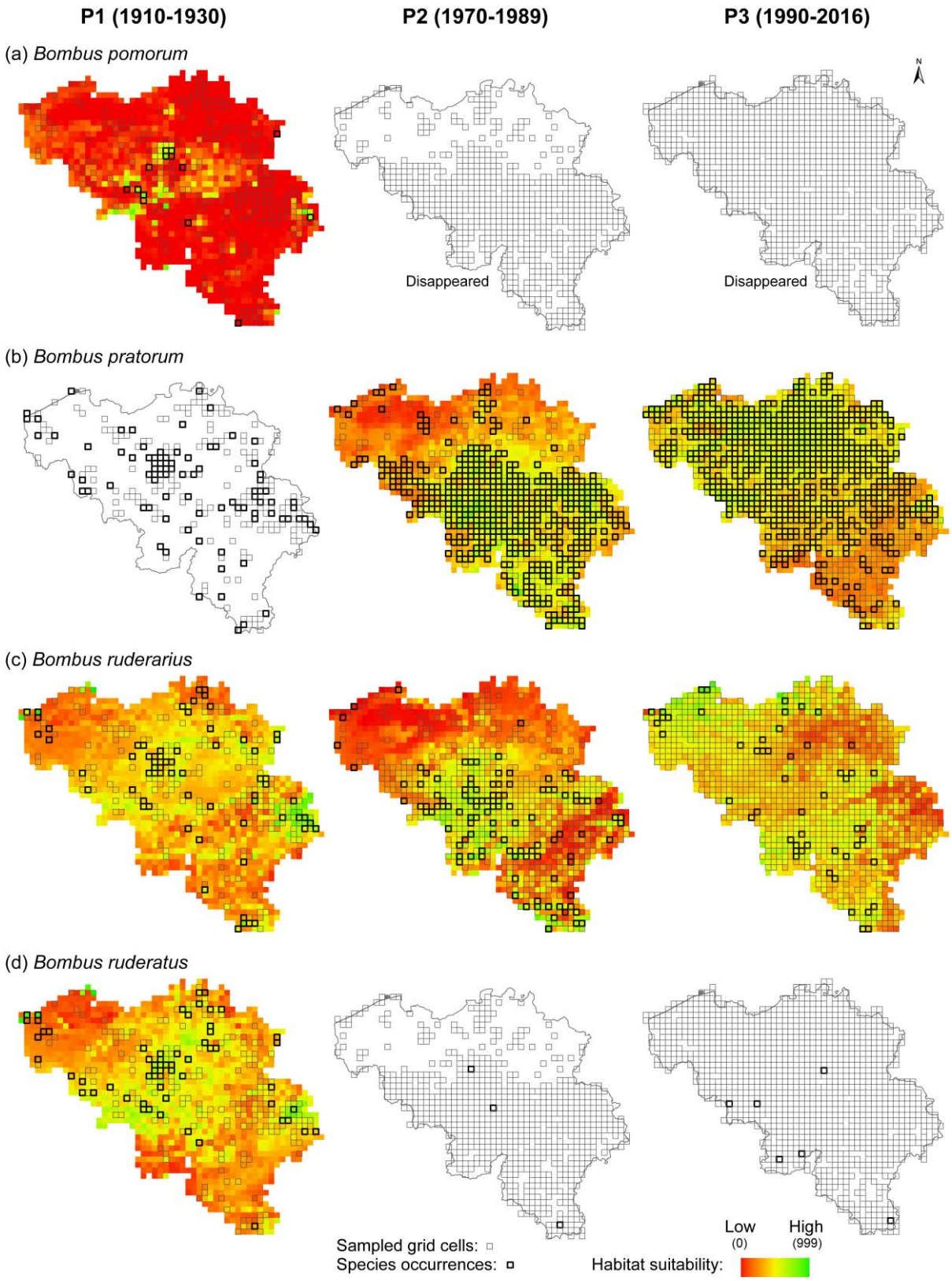

Figure S8. Species distribution maps. When the number of grid cells was inferior to 10 or when the SDM was not significant based on its AUC, only the map of the records is shown.

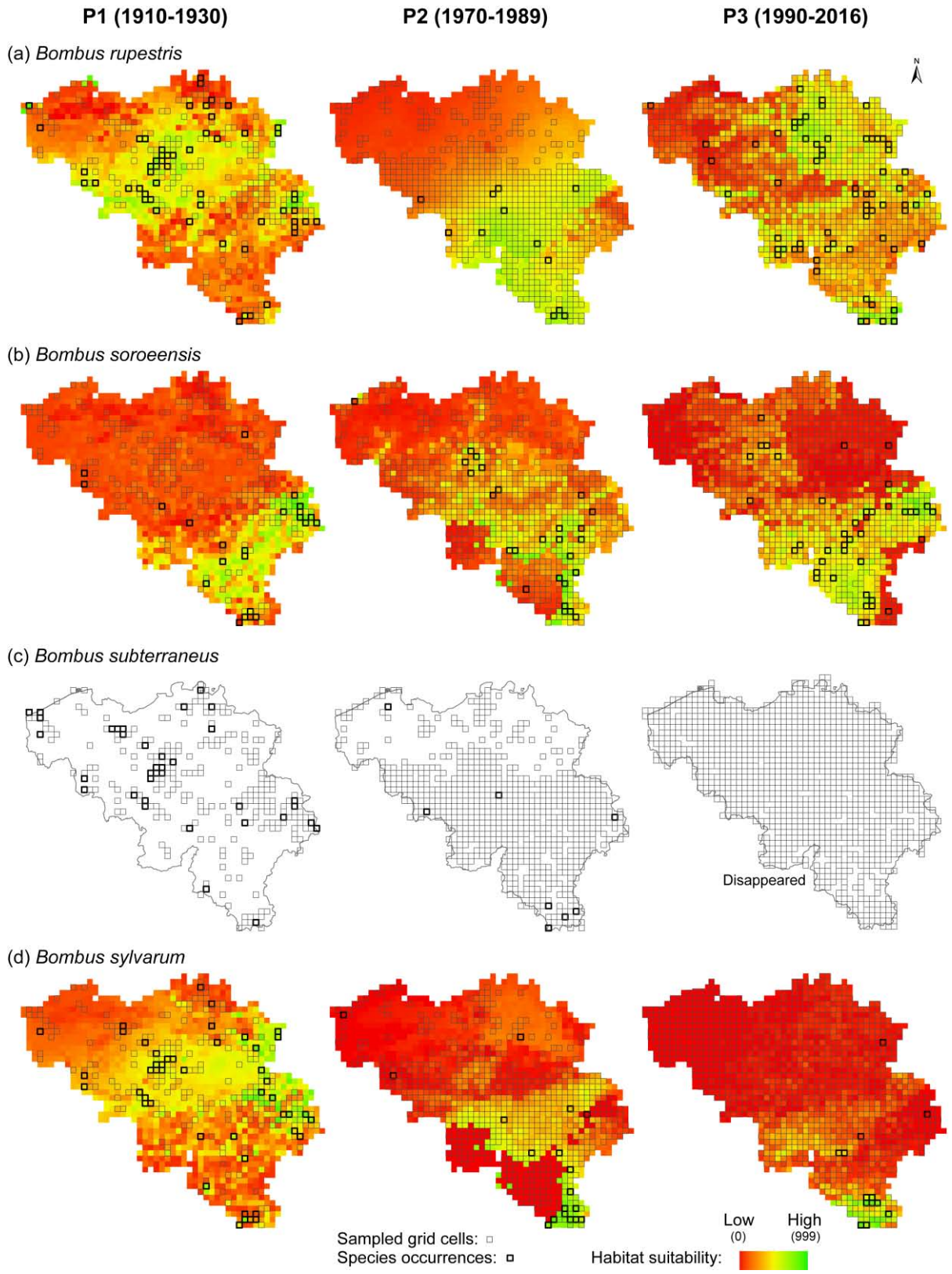

Figure S9. Species distribution maps. When the number of grid cells was inferior to 10 or when the SDM was not significant based on its AUC, only the map of the records is shown.

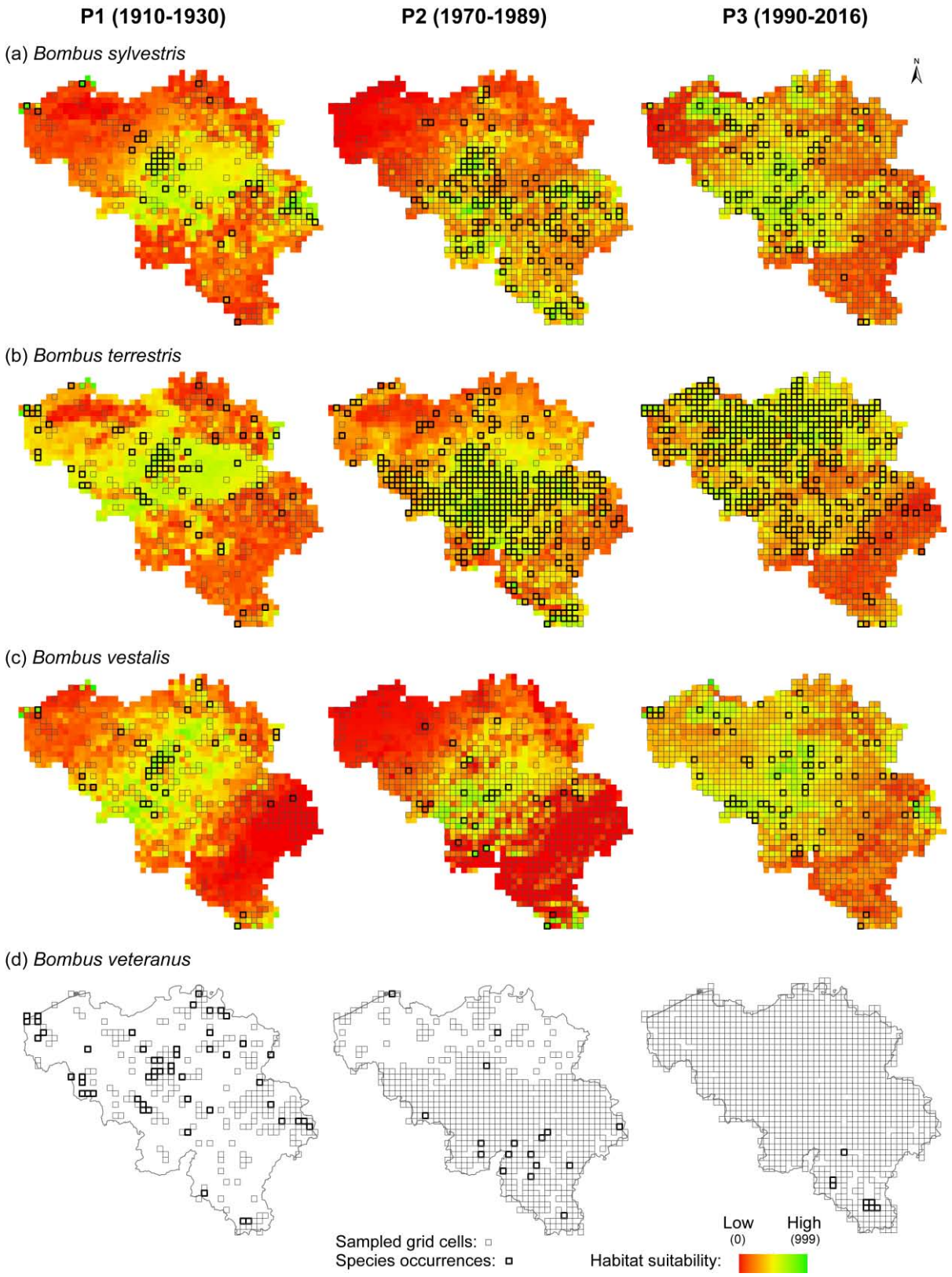

Figure S10. Species distribution maps. When the number of grid cells was inferior to 10 or when the SDM was not significant based on its AUC, only the map of the records is shown.

Table S1. Summary of the two linear mixed-effect models. ":" means interaction between two variables. "SE" = standard error; "DF" = degrees of freedom.

	Value	SE	DF	t-value	p-value
With variable types (AIC = 1795.7)					
(Intercept)	3.182	0.180	445	17.691	0.000
Period [P2]	-0.211	0.258	445	-0.819	0.413
Period [P3]	0.123	0.266	445	0.463	0.643
Variable type [Precipitation]	-0.363	0.312	445	-1.165	0.244
Variable type [Temperature]	-0.531	0.275	445	-1.932	0.054
Period [P2] : Variable type [Precipitation]	0.664	0.447	445	1.486	0.138
Period [P3] : Variable type [Precipitation]	0.157	0.461	445	0.342	0.733
Period [P2] : Variable type [Temperature]	-0.070	0.394	445	-0.178	0.859
Period [P3] : Variable type [Temperature]	-0.405	0.406	445	-0.997	0.319
With variables (AIC = 1748.8)					
(Intercept)	2.194	0.336	427	6.522	0.000
Period [P2]	1.196	0.482	427	2.480	0.014
Period [P3]	1.887	0.497	427	3.793	0.000
Variable [grass]	1.183	0.476	427	2.487	0.013
Variable [sett]	0.488	0.476	427	1.026	0.305
Variable [wood]	2.282	0.476	427	4.797	0.000
Variable [PDQ]	-0.653	0.476	427	-1.372	0.171
Variable [PWQ]	1.903	0.476	427	4.001	0.000
Variable [ΔT]	0.432	0.476	427	0.907	0.365
Variable [MTWM]	0.782	0.476	427	1.644	0.101
Variable [TDQ]	0.159	0.476	427	0.334	0.739
Period [P2]: Variable [grass]	-2.436	0.682	427	-3.572	0.000
Period [P3]: Variable [grass]	-2.411	0.703	427	-3.427	0.001
Period [P2]: Variable [sett]	-0.749	0.682	427	-1.098	0.273
Period [P3]: Variable [sett]	-1.337	0.703	427	-1.901	0.058
Period [P2]: Variable [wood]	-2.443	0.682	427	-3.583	0.000
Period [P3]: Variable [wood]	-3.306	0.703	427	-4.700	0.000
Period [P2]: Variable [PDQ]	1.349	0.682	427	1.979	0.049
Period [P3]: Variable [PDQ]	-0.591	0.703	427	-0.840	0.401
Period [P2]: Variable [PWQ]	-2.836	0.682	427	-4.158	0.000
Period [P3]: Variable [PWQ]	-2.621	0.703	427	-3.726	0.000
Period [P2]: Variable [ΔT]	-1.365	0.682	427	-2.002	0.046
Period [P3]: Variable [ΔT]	-2.111	0.703	427	-3.001	0.003
Period [P2]: Variable [MTWM]	-1.641	0.682	427	-2.406	0.017
Period [P3]: Variable [MTWM]	-2.798	0.703	427	-3.977	0.000
Period [P2]: Variable [TDQ]	-1.425	0.682	427	-2.090	0.037
Period [P3]: Variable [TDQ]	-1.598	0.703	427	-2.271	0.024

Table S2. Results of the post-hoc Tukey tests (pairwise differences of contrast) following the mixed-effect model based on the interaction between time periods and variable types. "SE" = standard error; "df" = degrees of freedom.

	Estimate	SE	df	t-ratio	p-value
Period = P1					
LC - Prec	0.363	0.312	445	1.165	0.475
LC - Temp	0.531	0.275	445	1.932	0.131
Prec - Temp	0.168	0.328	445	0.511	0.866
Period = P2					
LC - Prec	-0.301	0.320	445	-0.939	0.616
LC - Temp	0.601	0.282	445	2.129	0.085
Prec - Temp	0.902	0.337	445	2.672	0.021*
Period = P3					
LC - Prec	0.206	0.339	445	0.606	0.817
LC - Temp	0.936	0.299	445	3.127	0.005**
Prec - Temp	0.731	0.358	445	2.041	0.104
Variable type = LC					
P1 - P2	0.211	0.258	445	0.819	0.691
P1 - P3	-0.123	0.266	445	-0.463	0.888
P2 - P3	-0.335	0.269	445	-1.242	0.429
Variable type = Prec					
P1 - P2	-0.453	0.365	445	-1.241	0.430
P1 - P3	-0.281	0.376	445	-0.746	0.736
P2 - P3	0.172	0.381	445	0.451	0.894
Variable type = Temp					
P1 - P2	0.281	0.298	445	0.945	0.612
P1 - P3	0.282	0.307	445	0.918	0.629
P2 - P3	0.001	0.311	445	0.002	1.000

Table S3. Results of the post-hoc Tukey tests (pairwise differences of contrast) following the mixed-effect model based on the interaction between time periods and variables. "SE" = standard error; "df" = degrees of freedom.

Period = P1	**Estimate**	**SE**	**df**	**t-ratio**	**p-value**
crop - grass	-1.183	0.476	427	-2.487	0.2414
crop - sett	-0.488	0.476	427	-1.026	0.9832
crop - wood	-2.282	0.476	427	-4.797	0.0001***
crop - PDQ	0.653	0.476	427	1.372	0.9078
crop - PWQ	-1.903	0.476	427	-4.001	0.0024**
crop - DT	-0.432	0.476	427	-0.907	0.9925
crop - MTWM	-0.782	0.476	427	-1.644	0.7800
crop - TDQ	-0.159	0.476	427	-0.334	1.0000
grass - sett	0.695	0.476	427	1.460	0.8731
grass - wood	-1.099	0.476	427	-2.311	0.3379
grass - PDQ	1.835	0.476	427	3.859	0.0041**
grass - PWQ	-0.720	0.476	427	-1.514	0.8488
grass - DT	0.751	0.476	427	1.579	0.8157
grass - MTWM	0.401	0.476	427	0.843	0.9955
grass - TDQ	1.024	0.476	427	2.153	0.4386
sett - wood	-1.794	0.476	427	-3.771	0.0057**
sett - PDQ	1.141	0.476	427	2.398	0.2875
sett - PWQ	-1.415	0.476	427	-2.974	0.0750
sett - DT	0.057	0.476	427	0.119	1.0000
sett - MTWM	-0.294	0.476	427	-0.617	0.9995
sett - TDQ	0.329	0.476	427	0.693	0.9989
wood - PDQ	2.934	0.476	427	6.169	<0.0001***
wood - PWQ	0.379	0.476	427	0.797	0.9969
wood - DT	1.850	0.476	427	3.890	0.0037**
wood - MTWM	1.500	0.476	427	3.154	0.0450*
wood - TDQ	2.123	0.476	427	4.464	0.0004***
PDQ - PWQ	-2.555	0.476	427	-5.372	<0.0001***
PDQ - DT	-1.084	0.476	427	-2.279	0.3571
PDQ - MTWM	-1.434	0.476	427	-3.016	0.0669
PDQ - TDQ	-0.811	0.476	427	-1.706	0.7430
PWQ - DT	1.471	0.476	427	3.093	0.0537
PWQ - MTWM	1.121	0.476	427	2.357	0.3108
PWQ - TDQ	1.744	0.476	427	3.667	0.0084**
DT - MTWM	-0.350	0.476	427	-0.736	0.9982
DT - TDQ	0.273	0.476	427	0.574	0.9997
MTWM - TDQ	0.623	0.476	427	1.310	0.9281
Period = P2	**Estimate**	**SE**	**df**	**t-ratio**	**p-value**
crop - grass	1.253	0.489	427	2.564	0.2050
crop - sett	0.260	0.489	427	0.533	0.9998
crop - wood	0.161	0.489	427	0.330	1.0000
crop - PDQ	-0.697	0.489	427	-1.426	0.8874
crop - PWQ	0.933	0.489	427	1.909	0.6077
crop - DT	0.933	0.489	427	1.910	0.6070

crop - MTWM	0.859	0.489	427	1.757	0.7103
crop - TDQ	1.266	0.489	427	2.592	0.1931
grass - sett	-0.993	0.489	427	-2.031	0.5224
grass - wood	-1.092	0.489	427	-2.234	0.3854
grass - PDQ	-1.950	0.489	427	-3.990	0.0025**
grass - PWQ	-0.320	0.489	427	-0.655	0.9992
grass - DT	-0.320	0.489	427	-0.654	0.9993
grass - MTWM	-0.394	0.489	427	-0.807	0.9966
grass - TDQ	0.013	0.489	427	0.027	1.0000
sett - wood	-0.099	0.489	427	-0.203	1.0000
sett - PDQ	-0.957	0.489	427	-1.959	0.5729
sett - PWQ	0.672	0.489	427	1.376	0.9063
sett - DT	0.673	0.489	427	1.377	0.9060
sett - MTWM	0.598	0.489	427	1.224	0.9509
sett - TDQ	1.006	0.489	427	2.059	0.5033
wood - PDQ	-0.858	0.489	427	-1.756	0.7110
wood - PWQ	0.772	0.489	427	1.579	0.8158
wood - DT	0.772	0.489	427	1.580	0.8153
wood - MTWM	0.697	0.489	427	1.427	0.8870
wood - TDQ	1.105	0.489	427	2.262	0.3681
PDQ - PWQ	1.630	0.489	427	3.335	0.0257*
PDQ - DT	1.630	0.489	427	3.336	0.0256*
PDQ - MTWM	1.556	0.489	427	3.183	0.0412*
PDQ - TDQ	1.963	0.489	427	4.018	0.0022**
PWQ - DT	0.000	0.489	427	0.001	1.0000
PWQ - MTWM	-0.074	0.489	427	-0.152	1.0000
PWQ - TDQ	0.334	0.489	427	0.682	0.9990
DT - MTWM	-0.075	0.489	427	-0.153	1.0000
DT - TDQ	0.333	0.489	427	0.681	0.9990
MTWM - TDQ	0.408	0.489	427	0.834	0.9958
Period = P3	**Estimate**	**SE**	**df**	**t-ratio**	**p-value**
crop - grass	1.228	0.518	427	2.369	0.3039
crop - sett	0.849	0.518	427	1.638	0.7830
crop - wood	1.024	0.518	427	1.976	0.5608
crop - PDQ	1.244	0.518	427	2.400	0.2868
crop - PWQ	0.718	0.518	427	1.385	0.9030
crop - DT	1.679	0.518	427	3.240	0.0347*
crop - MTWM	2.016	0.518	427	3.890	0.0037**
crop - TDQ	1.439	0.518	427	2.776	0.1256
grass - sett	-0.379	0.518	427	-0.730	0.9983
grass - wood	-0.203	0.518	427	-0.393	1.0000
grass - PDQ	0.016	0.518	427	0.031	1.0000
grass - PWQ	-0.510	0.518	427	-0.983	0.9872
grass - DT	0.452	0.518	427	0.871	0.9943
grass - MTWM	0.788	0.518	427	1.521	0.8454
grass - TDQ	0.211	0.518	427	0.407	1.0000
sett - wood	0.175	0.518	427	0.338	1.0000
sett - PDQ	0.395	0.518	427	0.761	0.9978

sett - PWQ	-0.131	0.518	427	-0.253	1.0000
sett - DT	0.830	0.518	427	1.602	0.8037
sett - MTWM	1.167	0.518	427	2.251	0.3746
sett - TDQ	0.590	0.518	427	1.138	0.9682
wood - PDQ	0.219	0.518	427	0.423	1.0000
wood - PWQ	-0.306	0.518	427	-0.591	0.9996
wood - DT	0.655	0.518	427	1.264	0.9411
wood - MTWM	0.992	0.518	427	1.913	0.6049
wood - TDQ	0.414	0.518	427	0.800	0.9969
PDQ - PWQ	-0.526	0.518	427	-1.014	0.9844
PDQ - DT	0.436	0.518	427	0.841	0.9955
PDQ - MTWM	0.772	0.518	427	1.490	0.8599
PDQ - TDQ	0.195	0.518	427	0.376	1.0000
PWQ - DT	0.961	0.518	427	1.855	0.6454
PWQ - MTWM	1.298	0.518	427	2.504	0.2328
PWQ - TDQ	0.721	0.518	427	1.390	0.9011
DT - MTWM	0.337	0.518	427	0.650	0.9993
DT - TDQ	-0.241	0.518	427	-0.464	0.9999
MTWM - TDQ	-0.577	0.518	427	-1.114	0.9720
Variable = crop	**Estimate**	**SE**	**df**	**t-ratio**	**p-value**
P1 - P2	-1.196	0.482	427	-2.480	0.0360*
P1 - P3	-1.887	0.497	427	-3.793	0.0005***
P2 - P3	-0.691	0.504	427	-1.372	0.3565
Variable = grass	**Estimate**	**SE**	**df**	**t-ratio**	**p-value**
P1 - P2	1.240	0.482	427	2.572	0.0281*
P1 - P3	0.524	0.497	427	1.053	0.5439
P2 - P3	-0.716	0.504	427	-1.422	0.3303
Variable = sett	**Estimate**	**SE**	**df**	**t-ratio**	**p-value**
P1 - P2	-0.447	0.482	427	-0.927	0.6235
P1 - P3	-0.549	0.497	427	-1.105	0.5118
P2 - P3	-0.102	0.504	427	-0.203	0.9775
Variable = wood	**Estimate**	**SE**	**df**	**t-ratio**	**p-value**
P1 - P2	1.247	0.482	427	2.587	0.0270*
P1 - P3	1.419	0.497	427	2.853	0.0126*
P2 - P3	0.172	0.504	427	0.341	0.9378
Variable = PDQ	**Estimate**	**SE**	**df**	**t-ratio**	**p-value**
P1 - P2	-2.545	0.482	427	-5.278	<0.0001***
P1 - P3	-1.296	0.497	427	-2.605	0.0257*
P2 - P3	1.249	0.504	427	2.481	0.0359*
Variable = PWQ	**Estimate**	**SE**	**df**	**t-ratio**	**p-value**
P1 - P2	1.640	0.482	427	3.401	0.0021**
P1 - P3	0.734	0.497	427	1.476	0.3036
P2 - P3	-0.906	0.504	427	-1.799	0.1713
Variable = ΔT	**Estimate**	**SE**	**df**	**t-ratio**	**p-value**
P1 - P2	0.169	0.482	427	0.351	0.9343
P1 - P3	0.224	0.497	427	0.451	0.8942
P2 - P3	0.055	0.504	427	0.109	0.9935
Variable = MTWM	**Estimate**	**SE**	**df**	**t-ratio**	**p-value**

P1 - P2	0.445	0.482	427	0.923	0.6263
P1 - P3	0.911	0.497	427	1.832	0.1606
P2 - P3	0.466	0.504	427	0.926	0.6244
Variable = TDQ	**Estimate**	**SE**	**df**	**t-ratio**	**p-value**
P1 - P2	0.229	0.482	427	0.476	0.8827
P1 - P3	-0.289	0.497	427	-0.582	0.8300
P2 - P3	-0.519	0.504	427	-1.030	0.5584

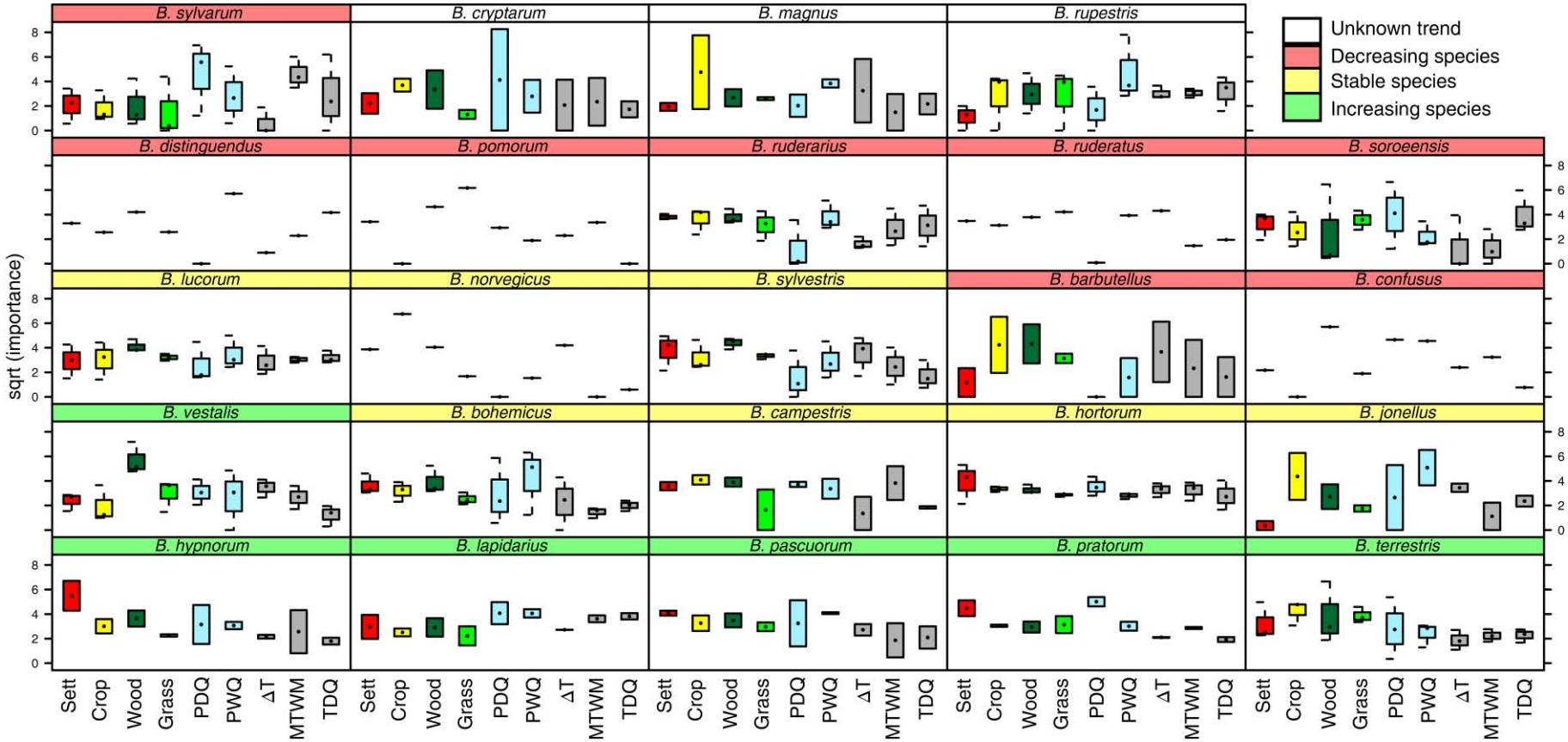

Figure S11. Boxplots showing the median and the quartiles of the influence of environmental variables on the distribution of each species. "Sett": settlement area; "Crop": cropland area; "Wood": woodland area; "Grass": grassland area; "PDQ": precipitation of the driest quarter; "PWQ": precipitation of the warmest quarter; "ΔT": mean diurnal range; "MTWM": maximum temperature of the warmest month, "TDQ": mean temperature of the driest quarter.

Discussion générale

Discussion générale

Dans cette partie, les résultats des différents chapitres sont assemblés et discutés. La discussion va cependant bien souvent au-delà des résultats en eux-mêmes et ouvre le champ d'interprétation. Une série de spéculations sont émises selon les résultats mais aussi selon la littérature scientifique et les connaissances actuelles.

1. Le déclin des bourdons en Belgique

Dans le **Chapitre 1** de cette thèse, nous avons constaté le déclin massif d'une multitude d'espèces de bourdons en Belgique durant le siècle dernier (Figure 1). Parmi les 28 espèces recensées il y a un siècle, quatre ont totalement disparu (*Bombus confusus*, *B. distinguendus*, *B. pomorum* et *B. subterraneus*), 15 sont en régression, une est stable (*B. jonellus*), et huit sont en augmentation en termes d'abondances. En termes de taille de distribution, ces chiffres sont encore plus alarmistes malgré une augmentation du nombre de sites échantillonnés : 17 espèces en régression, trois espèces stables (*B. hortorum*, *B. norvegicus* et *B. terrestris*), et seulement quatre en expansion (*B. hypnorum, B. lapidarius, B. pascuorum*, et *B. pratorum*). Ces tendances confirment celles évoquées par les précédentes études en Belgique (Rasmont & Mersch 1988 ; Rasmont *et al.* 1993), mais aussi en Europe (Kosior *et al.* 2007 ; Carvalheiro *et al.* 2013 ; Nieto *et al.* 2015 ; Rasmont *et al.* 2015b). Nous avons constaté que les assemblages de bourdons de Belgique se sont fortement homogénéisés en un siècle. Un tirage aléatoire de 100 spécimens collectait presque 19 espèces il y a 100 ans, contre seulement 10 aujourd'hui. Plus de 90% des observations sont aujourd'hui représentées par

seulement cinq espèces largement répandues à travers le pays: *B. pascuorum*, *B. lapidarius*, *B. pratorum*, *B. hypnorum* et *B. terrestris*. Au point 3.1.1, nous abordons les différentes hypothèses qui pourraient expliquer le succès relatif de ces espèces.

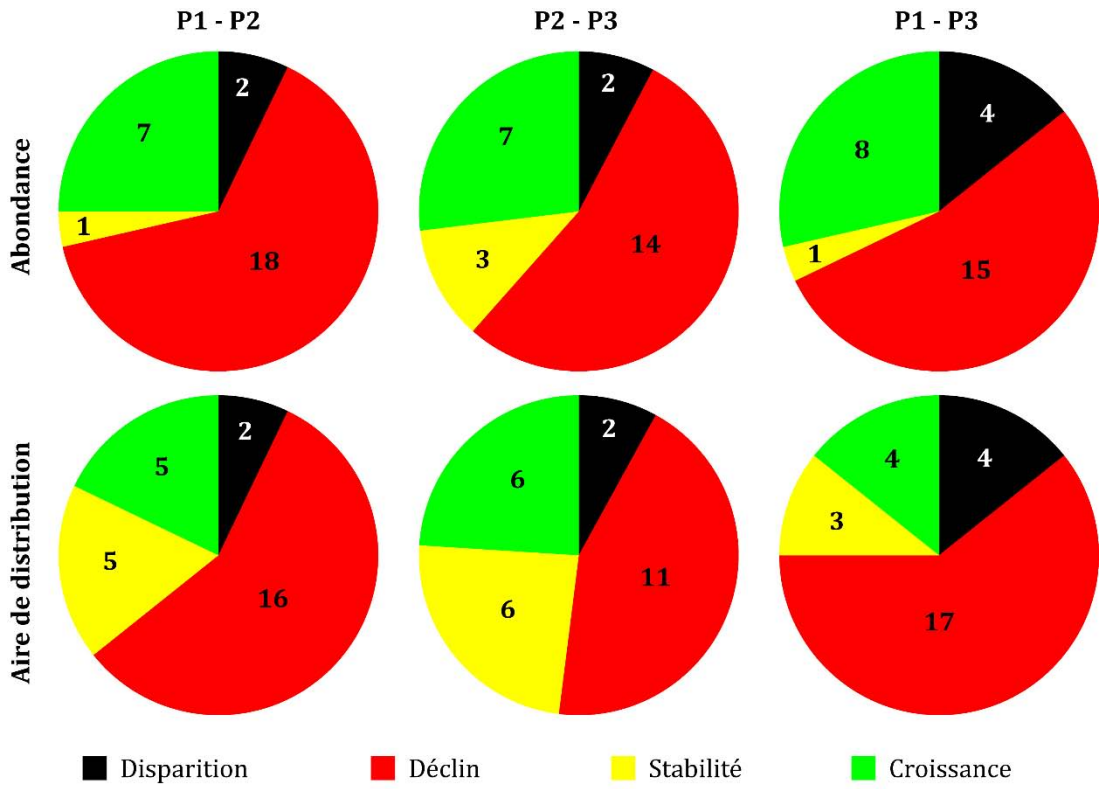

Figure 1. Nombre d'espèces par tendance de population basée sur l'abondance ou sur la taille de l'aire de distribution, entre les trois périodes étudiées deux à deux (P1 = 1910-1930 ; P2 = 1970-1989 ; P3 = 1990-2016).

Nous avons observé que le déclin et l'homogénéisation des assemblages n'étaient pas de la même intensité selon les régions du pays, le sud-est semblant plus épargné. Les analyses d'occupation du sol et du climat des **Chapitres 3 et 4** tentent d'apporter des réponses à ces différences entre régions et sont abordées aux points 2.2 et 2.3. Enfin, nous avons constaté que le déclin semblait plus marqué entre les deux premières périodes étudiées (P1 : 1910-1930 et P2 : 1970-1989) qu'entre les deux dernières (P2 et P3 : 1990-2016 ; Figure 1). Une partie d'explication, abordée au point 5, pourrait provenir de la prise en considération et

de l'application des mesures agro-environnementales à partir des années 1990 (Kleijn & Sutherland 2003 ; Batáry *et al.* 2015), qui auraient éventuellement contribué à améliorer la disponibilité en ressources florales et en sites de nidification pour les bourdons (*e.g*. Dramstad & Fry 1995 ; Carreck & Williams 2002; Carvell *et al.* 2004, 2007; Pywell *et al.* 2005, 2006 ; Kallioniemi *et al.* 2017). Cependant, l'échantillonnage plus intense et plus répandu en P3 qu'en P2 pourrait également avoir contribué à cette impression d'amélioration. De plus, les différences de tendance pourraient également provenir de processus intrinsèques aux espèces de bourdons, comme des changements phénotypiques ou des processus d'adaptation et d'évolution face aux changements environnementaux. Il a ainsi notamment été montré que certaines espèces de bourdons étaient capables de modifier leur diète pour qu'elle corresponde aux fleurs les plus abondantes (Kleijn & Raemakers 2008 ; Roger *et al.* 2016, 2017). Ce point est abordé plus en détail au point 3.

1.1. Comparaison entre les résultats du Chapitre 1 et du Chapitre 4

Dans le **Chapitre 1**, nous avons estimé les tailles relatives des aires de distribution belge de chaque espèce en comptant le nombre de carrés de 5x5km dans les trois périodes de temps. Nous avons également converti les cartes de probabilité de présence de chaque espèce dans chaque période issues du **Chapitre 4** en cartes binaires de présence-absence (voir Cadre méthodologique général). Celles-ci nous ont également permis de compter le nombre de carrés de 5x5 km où l'espèce a été jugée probablement présente. Nous y avons ensuite appliqué les mêmes analyses

qu'au Chapitre 1, à savoir la transformation en aire relative, le calcul du ratio entre les périodes deux à deux, ainsi que l'estimation des tendances de population selon la méthode de Stroot & Depiereux (1989). Nous comparons les résultats obtenus dans le Tableau 1, auquel nous avons ajouté les tendances européennes de population selon l'IUCN (Rasmont *et al*. 2015b) à titre de comparaison. Pour plusieurs espèces, aucune information ne peut être extraite du Chapitre 4, en raison d'un nombre de carrés inférieur à 10 ou d'un modèle non significatif (voir Chapitre 4).

Tableau 1. Comparaison des nombres absolu et relatif de carrés de 5x5km, ainsi que des ratios entre périodes deux à deux et des estimations de tendance de population selon la méthode de Stroot & Depireux (1989) provenant des résultats des Chapitres 1 et 4. Les résultats sont triés par ordre décroissant du nombre relatif de carrés en P1 du Chapitre 1. « EU » = tendance européenne selon l'IUCN. « / » = nombre de carrés < 10 ou SDM non significatif. * = 0,05 ; ** = 0,01 ; *** = 0,001.

Espèce	Chapitre 1 (= données brutes)												Chapitre 4 (= données issues des SDMs)												EU
	Nbre absolu			Nbre relatif			Ratio			Tendance			Nbre absolu			Nbre relatif			Ratio			Tendance			
	P1	P2	P3	P1	P2	P3	2/1	3/2	3/1	1-2	2-3	1-3	P1	P2	P3	P1	P2	P3	2/1	3/2	3/1	1-2	2-3	1-3	
pascuorum	138	573	965	52.9	82.4	83.8	1.56	1.02	1.59	+ ***	=	+ ***	816	882	/	59.8	64.7	/	1.08	/	/	=	/	=	+
hortorum	106	316	438	40.6	45.5	38.1	1.12	0.84	0.94	=	- *	=	710	760	1013	52.1	55.7	74.3	1.07	1.33	1.43	=	+***	=	=
lapidarius	104	297	824	39.8	42.7	71.6	1.07	1.68	1.80	=	+ ***	+ ***	601	761	/	44.1	55.8	/	1.27	/	/	+***	/	+***	+
pratorum	90	473	683	34.5	68.1	59.3	1.97	0.87	1.72	+ ***	- *	+ ***	/	882	1011	/	64.7	74.1	/	1.15	/	/	+**	/	+
distinguendus	73	2	0	28.0	0.3	0.0	0.01	0.00	0.00	- ***	(=)	- ***	613	/	/	44.9	/	/	/	/	/	/	/	/	-
terrestris	70	323	392	26.8	46.5	34.1	1.73	0.73	1.27	+ ***	- ***	=	712	806	833	52.2	59.1	61.1	1.13	1.03	1.17	+*	=	+*	+
ruderarius	70	113	54	26.8	16.3	4.7	0.61	0.29	0.17	- ***	- ***	- ***	607	707	668	44.5	51.8	49.0	1.16	0.94	1.10	+**	=	+**	-
campestris	66	42	167	25.3	6.0	14.5	0.24	2.40	0.57	- ***	+ ***	- ***	/	402	871	/	29.5	63.9	/	2.17	/	/	+***	/	=
ruderatus	63	3	6	24.1	0.4	0.5	0.02	1.21	0.02	- ***	=	- ***	653	/	/	47.9	/	/	/	/	/	/	/	/	-
barbutellus	60	11	4	23.0	1.6	0.3	0.07	0.22	0.02	- ***	- **	- ***	858	118	/	62.9	8.7	/	0.14	/	/	-***	/	-***	-
lucorum	58	238	118	22.2	34.2	10.3	1.54	0.30	0.46	+ **	- ***	- ***	674	853	774	49.4	62.5	56.7	1.27	0.91	1.15	+***	=	+***	=
rupestris	57	12	67	21.8	1.7	5.8	0.08	3.37	0.27	- ***	+ ***	- ***	506	457	687	37.1	33.5	50.4	0.90	1.50	1.36	=	+***	=	?
veteranus	53	17	8	20.3	2.4	0.7	0.12	0.28	0.03	- ***	- **	- ***	/	/	/	/	/	/	/	/	/	/	/	/	-
muscorum	52	5	2	19.9	0.7	0.2	0.04	0.24	0.01	- ***	(=)	- ***	/	/	/	/	/	/	/	/	/	/	/	/	-
sylvarum	48	18	13	18.4	2.6	1.1	0.14	0.44	0.06	- ***	- *	- ***	547	201	53	40.1	14.7	3.9	0.37	0.26	0.10	-***	-***	-***	-
cryptarum	47	78	44	18.0	11.2	3.8	0.62	0.34	0.21	- **	- ***	- ***	/	593	528	/	43.5	38.7	/	0.89	/	/	=	/	?
sylvestris	46	149	115	17.6	21.4	10.0	1.22	0.47	0.57	=	- ***	- ***	373	678	739	27.3	49.7	54.2	1.82	1.09	1.98	+***	=	+***	=
hypnorum	44	263	573	16.9	37.8	49.8	2.24	1.32	2.95	+ ***	+ ***	+ ***	/	968	928	/	71.0	68.0	/	0.96	/	/	=	/	+
magnus	44	6	36	16.9	0.9	3.1	0.05	3.62	0.19	- ***	+ **	- ***	606	/	405	44.4	/	29.7	/	/	0.67	/	/	/	?
humilis	38	9	7	14.6	1.3	0.6	0.09	0.47	0.04	- ***	=	- ***	/	/	/	/	/	/	/	/	/	/	/	/	-
vestalis	37	28	70	14.2	4.0	6.1	0.28	1.51	0.43	- ***	=	- ***	492	438	702	36.1	32.1	51.5	0.89	1.60	1.43	=	+***	=	+
subterraneus	37	8	0	14.2	1.2	0.0	0.08	0.00	0.00	- ***	- ***	- ***	/	/	/	/	/	/	/	/	/	/	/	/	-
bohemicus	34	91	67	13.0	13.1	5.8	1.01	0.44	0.45	=	- ***	- ***	498	493	749	36.5	36.1	54.9	0.99	1.52	1.50	=	+***	=	=
jonellus	33	6	54	12.6	0.9	4.7	0.07	5.43	0.37	- ***	+ ***	- ***	258	/	226	18.9	/	16.6	/	/	0.88	/	/	/	=
soroeensis	24	26	41	9.2	3.7	3.6	0.41	0.95	0.39	- **	=	- ***	677	599	358	49.6	43.9	26.2	0.88	0.60	0.53	-*	-***	-*	-
confusus	20	0	0	7.7	0.0	0.0	0.00	/	0.00	- ***	/	- ***	228	/	/	16.7	/	/	/	/	/	/	/	/	-
pomorum	14	0	0	5.4	0.0	0.0	0.00	/	0.00	- ***	/	- ***	36	/	/	2.6	/	/	/	/	/	/	/	/	-
norvegicus	5	19	18	1.9	2.7	1.6	1.43	0.57	0.82	=	=	=	/	209	/	/	15.3	/	/	/	/	/	/	/	=
Total	261	695	1151										1364	1364	1364										

Comme les prédictions de présence issues des SDMs s'étendent au-delà des données brutes de présence des espèces, la grande différence entre les résultats du Chapitre 1 et du Chapitre 4 est en toute logique le nombre absolu de carrés beaucoup plus grand pour les prédictions des SDMs que pour les données brutes. De ce fait, un grand nombre d'espèces présente une aire relative plus grande dans les résultats issus des SDMs. Les espèces les plus répandues en P1 dans le Chapitre 1 sont pratiquement les mêmes que les plus répandues dans le Chapitre 4, à quelques exceptions près (Tableau 1). Plusieurs espèces présentent une aire relative modélisée beaucoup plus grande que leur aire relative brute en P1, en particulier *B. barbutellus* (qui est même plus répandue que *B. pascuorum*) et *B. soroeensis* (cinquième plus répandue). Les aires relatives modélisées en P2 et en P3 sont également beaucoup plus grandes que les brutes, ce qui a pour conséquence de moins grandes différences entre les périodes, et donc beaucoup moins de ratios inférieurs à 1 (signe d'une contraction d'aire relative) et de tendances de population négatives. Les résultats basés sur les aires modélisées du Chapitre 4 semblent donc bien moins alarmistes que ceux basés sur les résultats bruts du Chapitre 1 (Figure 2). Malgré tout, trois espèces présentent tout de même une tendance au déclin très claire sur base des aires modélisées entre P1 et P3: *B. barbutellus*, *B. soroeensis* et *B. sylvarum*. Cependant, il est à noter que pour une grande partie des espèces rares et en déclin, il n'a pas été possible d'obtenir un SDM significatif (tendances inconnues sur la Figure 2), notamment car la plupart d'entre elles ne présentaient plus qu'un nombre de carrés inférieur à 10. Si ces espèces étaient considérées, les tendances seraient très probablement presque identiques

et donc aussi alarmistes que celles obtenues sur base des nombres bruts du Chapitre 1 (Figure 2).

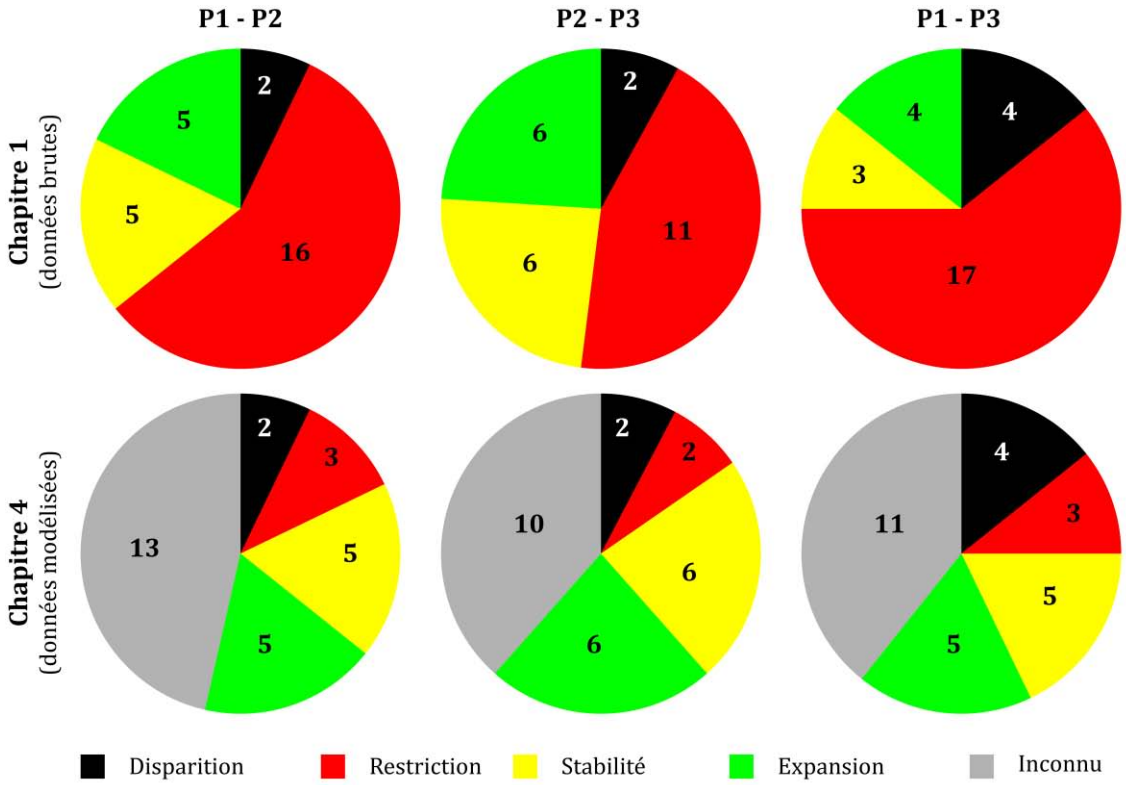

Figure 2. Nombre d'espèces par tendance de population basée sur la taille de l'aire de distribution brute (Chapitre 1) et modélisée (Chapitre 4), entre les trois périodes étudiées deux à deux (P1 = 1910-1930 ; P2 = 1970-1989 ; P3 = 1990-2016). La catégorie « inconnu » représente les espèces pour lesquelles il n'a pas été possible d'obtenir un SDM significatif, notamment à cause d'un nombre de carrés inférieur à 10 dans une (ou deux) des périodes.

2. Les grands facteurs de déclin des bourdons en Belgique

2.1. La diminution en ressources florales

2.1.1. L'importance des légumineuses

Il est largement admis que les légumineuses, c'est-à-dire les plantes appartenant à la famille des Fabaceae, constituent une ressource très importante pour un très grand nombre d'espèces de bourdons en Belgique et en Europe (Rasmont & Mersch 1988 ; Goulson *et al.* 2005, 2008 ; Kleijn & Raemakers 2008). Dans le **Chapitre 2**,

nous avons montré que les Fabaceae représentaient effectivement la première famille de plantes visitées par les femelles de bourdons. Les espèces à longue langue sont particulièrement bien adaptées à collecter le nectar de ces plantes à longue corolle. Il y a cent ans, le système agraire préconisait la rotation de culture, qui comprenait une phase d'enrichissement des terres au travers de la plantation d'une culture de légumineuse fixatrice d'azote. Les paysages agricoles présentaient une grande abondance en trèfles, luzerne, sainfoin, etc. Cependant, l'augmentation considérable de l'usage des engrais chimiques de synthèse à partir des années 1950 conduisit à l'abandon de cette phase de rotation et à la suppression d'une grande partie des cultures de légumineuses. D'environ 160 000 hectares dans les années 1910, la surface des cultures de légumineuses a chuté à 100 000 hectares en 1930, 55 000 en 1950, environ 15 000 en 1970, pour atteindre finalement seulement 5 000 hectares en 2014 (Ministère de l'Agriculture et des Travaux Publics 1913 ; Belgian Federal Government 2017). Selon Rasmont & Mersch (1988), cette régression des cultures de légumineuses est un facteur pouvant expliquer le déclin d'une grande partie des espèces de bourdons. Nos résultats tendent à confirmer ce constat. Comme mentionné dans le **Chapitre 1**, ce sont les espèces à longue langue et les plus spécialisées sur les Fabaceae qui ont le plus décliné. De plus, parmi les quatre communes échantillonnées dans le cadre du **Chapitre 3**, la seule hébergeant encore une grande diversité en bourdons est également la seule à encore présenter des cultures de légumineuses. Même si aucun lien de cause à effet ne peut être établit à partir de ce dernier constat, il est très probable que ces cultures de

légumineuses ont contribué à soutenir les populations de bourdons dans cette commune.

2.1.2. L'importance des Cardueae

Outre l'importance cruciale des légumineuses dans la diète des bourdons, le rôle des autres familles et espèces de plantes a été beaucoup moins investigué (Carvell 2002; Carvell *et al.* 2004; Goulson *et al.* 2008). En effet, la majorité des études portant sur la diète des bourdons s'attardent sur la collecte de pollen, et donc sur les reines et plus majoritairement les ouvrières. Le pollen des Asteraceae étant peu favorable au développement des colonies (Vanderplanck *et al.* 2016), il est, probablement en conséquence, très peu retrouvé dans les pelotes de pollen des ouvrières et des reines (Kleijn & Raemakers 2008; Goulson 2010). Mais qu'en est-il des mâles ? Malgré le fait qu'ils ne participent pas à la vie de la colonie, leur rôle est pourtant primordial dans la prospérité d'une espèce. Le **Chapitre 2** investigue ces différences de préférences florales entre les différentes castes de bourdons en Belgique et dans quelques autres pays d'Europe Occidentale (Vray *et al.* 2017). Nous avons mis en évidence la forte prédominance des Asteraceae, et plus particulièrement des chardons (tribu des Cardueae), dans la diète des mâles. Les genres *Cirsium* spp., *Carduus* spp. et *Centaurea* spp. sont les genres de plantes les plus visités par les mâles, toutes espèces confondues. Chez certaines espèces rares, comme *B. veteranus*, les mâles mais aussi les ouvrières sont presque exclusivement observés sur les Cardueae. La disponibilité en Cardueae comme source de nectar est donc cruciale lors de la période de vol des mâles, en particulier lors de leur

parade nuptiale très consommatrice d'énergie à la fin de l'été (Croxton *et al.* 2002; Pywell *et al.* 2005; Goulson 2010). De plus, les chardons ayant une floraison jusqu'à la fin de l'été, ils représentent parfois une des seules ressources florales encore disponibles à la fin de la saison de vol des bourdons et en particulier des espèces tardives.

Or, les chardons sont considérés comme étant des espèces nuisibles pour l'agriculture et font l'objet de lois obligeant tout propriétaire de terres à « empêcher par tous les moyens la floraison ainsi que le développement et la dissémination des semences de chardons nuisibles » (arrêté royal du 19 novembre 1987 relatif à la lutte contre les organismes nuisibles aux végétaux et aux produits végétaux en Belgique ; Crémer *et al.* 2008), sous peine d'amende. Une telle loi remonte au Code Rural du 7 octobre 1886 en Belgique et existe également dans d'autres pays comme la France, le Royaume-Uni et les Pays-Bas. Nous nous sommes concentrés sur ces quatre pays et sur les quatre espèces ciblées par ces lois (*Carduus crispus*, *Cirsium arvense*, *Cirsium palustre* et *Cirsium vulgare*), et nous avons constaté qu'elles représentaient à elles quatre une part non négligeable des visites de bourdons, incluant des espèces rares (ou même disparues) en Belgique comme *B. norvegicus*, *B. distinguendus*, *B. soroeensis*, ou encore *B. bohemicus*. Cependant, même si seulement quatre espèces de chardons sont légalement concernées, ce sont en réalité tous les chardons qui sont impactés par ces lois. En effet, différencier les espèces de chardons nécessite de solides connaissances botaniques, que la plupart des fermiers et propriétaires de terres ne possèdent pas. Il en résulte une suppression complète de toutes les espèces de chardons et autres plantes à

piquants, dont certaines sont menacées en Belgique (par exemple *Carduus nutans*, *Cirsium dissectum*, *Cirsium eriophorum* et *Cirsium tuberosum*; Crémer *et al.* 2008). De plus, les techniques d'échardonnage, comme une haute fréquence de fauchage ou l'utilisation d'herbicides, impactent aussi d'autres espèces de plantes pérennes (Turner *et al.* 2007; Crémer *et al.* 2008; Andreasen & Andresen 2011), comme les légumineuses. L'existence des lois d'échardonnage pourrait donc représenter une réelle menace pour les populations de bourdons, déjà fortement affaiblies par la régression globale en ressources florales. Mais la suppression des chardons pourrait aussi impacter d'autres espèces de pollinisateurs très observés sur ces plantes (Ricou *et al.* 2014; Vanbergen *et al.* 2014), comme les autres espèces d'abeilles sauvages (Pauly A., com. Pers.), les papillons (Haaland & Gyllin 2010), ainsi que les espèces phytophages (Freese 1994) et celles se nourrissant de leurs graines (McCallum & Kelly 1990). Supprimer les chardons peut donc avoir des répercussions sur l'ensemble des communautés, tant animales que végétales.

Dans le contexte actuel de déclin global de la biodiversité, la persistance d'anciennes lois comme celle de l'échardonnage semble donc obsolète et inappropriée. Même si les chardons peuvent encore, à certains endroits, envahir quelques cultures et pâtures et donc réduire leur productivité, l'obligation légale de détruire les chardons partout et à tout moment devrait être remplacée par des interventions locales et surtout par des techniques de prévention. En effet, maintenir une grande diversité floristique dans les prairies, par exemple en évitant le surpâturage et l'utilisation d'engrais, peut bien souvent suffire à empêcher la monopolisation des chardons, tout en leur laissant une place légitime (Andreasen

& Andresen 2011; Nicholls & Altieri 2013). De plus, l'obligation de détruire les chardons ailleurs que dans les prairies et cultures semble encore plus inconcevable. Permettre de laisser une place aux chardons sur les bords des routes et chemins ainsi que dans les jardins pourrait grandement augmenter leur capacité de refuge pour les bourdons et les autres pollinisateurs, particulièrement dans les paysages urbains et d'agriculture intensive (*e.g*. Pywell *et al.* 2005 ; McFrederick & LeBuhn 2006 ; Carvell *et al.* 2007 ; Ahrné *et al.* 2009; Samnegård *et al.* 2011).

2.2. Les modifications de l'occupation du sol et du paysage

2.2.1. A l'échelle de la Belgique

Les changements d'occupation du sol à l'échelle de la Belgique sont abordés dans le **Chapitre 4**, entre les trois mêmes périodes que celles étudiées au Chapitre 1 : 1910-1930, 1970-1989 et 1990-2016. Entre les deux premières périodes, nous observons une diminution de la surface des cultures contre une augmentation des surfaces urbanisées, des prairies et des forêts. Ensuite, entre les deux dernières périodes, les prairies ont légèrement régressé et les cultures légèrement augmenté. Ces résultats confirment les tendances de changements d'occupation du sol en Europe et dans le monde, qui se sont principalement produits durant la première moitié du 20ème siècle (Hougton 1994 ; Steffen *et al.* 2011) suite à deux phénomènes distincts : l'urbanisation et l'étalement urbain (Antrop 2004 ; European Environment Agency 2016), ainsi que la profonde réforme de l'agriculture (Meuus 1993 ; Christians 1998 ; Robinson & Sutherland 2002 ; Mazoyer & Roudart 2006). Même si la surface des cultures a drastiquement diminué en Belgique, leur rendement à l'hectare a

triplé en 100 ans suite à l'intensification agricole (Belgian Federal Government 2017). Les pratiques modernes de l'agriculture promeuvent un mode de gestion nuisible pour la biodiversité : abandon de la rotation des cultures suite à la généralisation des engrais chimiques dans les années 1950, simplification du paysage (suppression du bocage, des talus, etc.) et transformation des polycultures en larges monocultures suite à la mécanisation agricole, régression des zones humides suite au drainage des terres, arrachage des vergers, etc. (Christians 1998). Les paysages agricoles d'il y a cent ans ne ressemblent donc plus aux paysages agricoles modernes. Même si nos variables ne prennent en compte que la surface relative des cultures et non l'utilisation de celles-ci, le fait que la variable « cultures » ne soit plus la même entre la période actuelle et il y a cent ans se reflète dans nos résultats. En effet, l'influence des cultures sur la distribution des bourdons a profondément changé depuis un siècle. Il y a cent ans, cette variable était l'occupation du sol qui influençait le moins la distribution des bourdons. Malgré que la surface relative des cultures ait fortement diminué, son influence s'est drastiquement accrue pour devenir actuellement la variable la plus importante parmi toutes les variables, même climatiques. Mais surtout, en plus d'augmenter, son influence sur la distribution des bourdons est passée d'un effet positif à un effet largement négatif pour la majorité des espèces. Dans la discussion du Chapitre 4 et dans la partie « perspectives et recommandations pour les recherches futures », nous mettons en garde contre l'utilisation de cette variable dans les modélisations de distribution d'espèces à travers le temps.

Nos résultats montrent que l'urbanisation et le maintien de l'agriculture se sont principalement passés dans le nord du pays, alors que le sud a conservé une grande densité de forêts et de prairies. La majorité des espèces de bourdons semblaient être aussi bien présentes dans le nord que dans le sud du pays il y a cent ans, et même dans les plaines agricoles et à proximité des grandes villes comme Bruxelles. Aujourd'hui, les espèces devenues rares en Belgique semblent avoir déserté les régions hautement urbanisées et agricoles du nord du pays, où seules les espèces les plus communes sont encore bien présentes (voir plus loin pour les détails entre espèces). Ces résultats tendent à confirmer les effets néfastes de l'urbanisation et de l'intensification de l'agriculture sur les assemblages de bourdons et sur la biodiversité en général présentés par les études précédentes (*e.g*. Burel *et al.* 1998 ; Robinson & Sutherland 2002 ; Tscharntke *et al.* 2005 ; Hendrickx *et al.* 2007 ; Henle *et al.* 2008 ; Ahrné *et al.* 2009 ; Le Féon *et al.* 2010 ; Geslin *et al.* 2013 ; Ollerton *et al.* 2014). Les zones fortement urbanisées et en agriculture intensive peuvent notamment jouer le rôle de barrières en limitant les flux de gènes chez les bourdons (Jha & Kremen 2013 ; Jha 2015), même s'il a été montré que les jardins pouvaient parfois leur servir de refuge dans ces régions hautement anthropiques (McFrederick & LeBuhn 2006 ; Samnegård *et al.* 2011).

2.2.2. A l'échelle du paysage

Résultats généraux

Dans le **Chapitre 3**, nous avons réalisé une analyse détaillée de la composition et de la structure du paysage de quatre anciennes communes de Belgique (Moorsel,

Trivières, Francorchamps et Torgny) parmi les plus échantillonnées par la collection de Ball datant de plus d'un siècle (Ball 1914, 1920), qui couvre la période 1910-1930. Ces quatre localités ont fait l'objet de nouveaux inventaires de bourdons durant trois années consécutives entre 2013 et 2015. Les modifications observées dans les assemblages de bourdons entre ces deux périodes sont globalement similaires à celles observées à l'échelle de la Belgique (**Chapitre 1**). La richesse spécifique a chuté de 28 à 19 espèces, avec un déclin beaucoup plus intense dans les deux localités du nord-ouest du pays (Moorsel et Trivières) que dans celles du sud-est (Francorchamps et Torgny). Les changements dans les assemblages de bourdons ont été d'une telle ampleur que ces derniers sont plus différents entre les deux périodes au sein d'une même localité qu'entre les quatre localités au sein d'une même période, à l'exception de Torgny. Dans nos analyses multivariées, nous avons pu faire ressortir l'importance significative des surfaces urbanisées (bâtiments, routes, etc.) ainsi que des terres arables pour expliquer les différences de composition des assemblages de bourdons entre les quatre localités et les deux périodes de temps. Ces deux types d'occupation du sol sont ceux qui distinguent le plus les quatre localités en termes de composition paysagère. L'urbanisation a été la plus intense dans les localités où les modifications des assemblages ont été les plus fortes (*i.e.* Trivières et Moorsel). De plus, comme à l'échelle de la Belgique, nous avons vu que la superficie des terres arables a drastiquement diminué durant le siècle dernier, mais reste cependant la plus importante à Trivières et Moorsel. Les statistiques agricoles que nous avons pu collecter ont montré que l'utilisation des cultures s'est fortement modifiée et intensifiée, avec l'abandon des cultures de

légumineuses au profit de cultures industrielles (pomme de terre, betterave sucrière, maïs…) et le triplement des rendements à l'hectare. Ces résultats tendent à réaffirmer l'importance de l'urbanisation et des changements de l'agriculture sur les changements observés chez les espèces de bourdons comme ceux mentionnés à l'échelle de la Belgique (**Chapitre 4**), et sur les changements sur la biodiversité en général abordés par d'autres études (*e.g*. Burel *et al.* 1998 ; Robinson & Sutherland 2002 ; Tscharntke *et al.* 2005 ; Hendrickx *et al.* 2007 ; Henle *et al.* 2008 ; Ahrné *et al.* 2009 ; Le Féon *et al.* 2010 ; Geslin *et al.* 2013 ; Ollerton *et al.* 2014).

Détails par localité

Nous avons vu que les modifications des assemblages de bourdons diffèrent grandement entre les quatre localités. Moorsel et Trivières sont celles qui présentent le déclin le plus drastique. Il y a cent ans, ces deux anciennes communes accueillaient une grande diversité d'espèces de bourdons : 25 espèces pour Moorsel et 26 pour Trivières. Des espèces devenues très rares aujourd'hui en Belgique y étaient parmi les plus abondantes (notamment *B. veteranus* à Moorsel, ainsi que *B. ruderatus* et *B. humilis* à Trivières), mais n'y ont plus été retrouvées durant les inventaires de 2013-2015. Aujourd'hui, leurs assemblages de bourdons sont bien plus pauvres en espèces, avec une perte de 17 espèces pour Moorsel et 15 espèces pour Trivières. Un inventaire de 100 spécimens permettait à l'époque de collecter environ 17 espèces à Moorsel et 14 à Trivières, contre un maximum de seulement 7 espèces aujourd'hui. Les assemblages sont actuellement largement dominés par *B. pascuorum* (61% des observations à Moorsel et 47% à Trivières), *B. terrestris*, *B.*

lapidarius, *B. pratorum* et *B. hypnorum*, c'est-à-dire les cinq espèces les plus abondantes à l'échelle de la Belgique (**Chapitre 1**) qui accumulent 95% des observations à Moorsel et 96% à Trivières. Ces deux anciennes communes représentent le phénomène typique d'urbanisation des périphéries urbaines de Belgique et d'Europe Occidentale (Antrop 2004 ; European Environment Agency 2016): Moorsel est en périphérie d'Alost en Flandres, et Trivières en périphérie de La Louvière en Wallonie. Le paysage de ces localités était autrefois largement agricole et dominé par des cultures de céréales, de betteraves sucrières et de légumineuses (majoritairement *Trifolium incarnatum* ; Ministère de l'Agriculture et des Travaux Publics 1913). En cent ans, la croissance de la population et le phénomène d'étalement urbain ont remplacé les champs par des routes, des bâtiments et des résidences avec jardins. La densité de population est passée de 8 à 11 habitants par hectare à Moorsel, et de 11 à 13 à Trivières (Belgian Federal Government 2017). Le devenir des parcelles agricoles restantes a suivi deux chemins : l'exploitation en cultures à haut rendement (majoritairement du blé, du maïs, des betteraves sucrières et des pommes de terre ; Belgian Federal Government 2017) ou la transformation en prairies. Même si la superficie des prairies a augmenté, elles sont pour la plupart devenues des prés en fauchage précoce ou des pâturages supportant une lourde charge de bétail, et ne présentant pratiquement aucune ressource florale pour les bourdons. Nous avons en effet constaté durant nos observations de terrain que les seuls milieux présentant des ressources florales et des bourdons étaient dans les sous-bois au printemps, ainsi que dans quelques jardins fleuris, quelques terrains en friche et talus de gravats

couverts de végétation rudérale, quelques petites cultures maraichères et vergers basse-tige (à Moorsel), et le long de certains champs et chemins. Ces derniers étaient cependant régulièrement fauchés, hautement eutrophes (grande densité d'orties), et parfois envahis par des espèces invasives (comme *Reynoutria japonica* à Moorsel). A Trivières, les vergers couvraient jadis la même superficie que les bois et ont pratiquement disparus aujourd'hui, probablement en conséquence du Plan Mansholt dans les années 1970 qui a fortement encouragé l'abattage des vergers haute-tige (Christians 1998).

Cependant, une grande différence distingue le paysage de Trivières des autres localités : la présence de terrils. Comme dans tout le bassin industriel du sillon Sambre-Meuse, le développement des mines à charbon durant le 19ème et le début du 20ème siècles (fin de l'exploitation en 1973 à Trivières ; Seblebillysien 2014) a profondément remodelé le paysage en produisant plusieurs terrils dans la région. Les terrils sont connus pour héberger une riche faune d'abeilles sauvages grâce à leur grande diversité de fleurs (Rasmont & Barbier 1990, 2000; Tropek *et al.* 2013; Hendrychová & Bogusch 2016). Les terrils laissés en succession spontanée tendent à héberger une plus grande diversité et des espèces plus rares que les terrils aménagés par exemple par la plantation d'arbres (Hendrychová & Bogusch 2016). A Trivières, un terril de chaque type est encore présent aujourd'hui : un entièrement boisé et un restant largement découvert et présentant un développement de végétation rudérale spontanée. Durant nos inventaires de 2013-2015, ce terril en succession spontanée présentait une grande diversité et abondance de fleurs tout au long de la saison de vol des bourdons (avec par

exemple *Ballota nigra*, *Carduus* spp., *Cirsium* spp., *Dipsacus fullonum*, *Echium vulgare*, *Epilobium* spp., *Lathyrus* spp., *Lotus corniculatus*, *Medicago* spp., *Prunus* spp., *Rubus* spp., *Salix* spp., *Trifolium* spp., *Vicia* spp.). Il constituait le site d'échantillonnage le plus riche en diversité et en abondance de bourdons parmi tous les sites échantillonnés à Trivières. Une espèce rare et en déclin à l'échelle de la Belgique (**Chapitre 1**) et de l'Europe (Rasmont *et al.* 2015b) y a même été trouvée: *B. barbutellus*. Le deuxième site le plus riche en espèces de bourdons et de plantes était un site écologiquement similaire à ce terril : un terrain vague en pente couvert de gravats et de végétation rudérale. Ceci suggère que ces milieux rudéraux en succession naturelle agissent comme un refuge pour la flore et pour la faune de bourdons dans cette région, ce qui confirme l'importance majeure des écosystèmes en développement spontané riches en végétation pionnière pour la conservation de la nature (Prach *et al.* 2011; Tropek *et al.* 2013; Hendrychová & Bogusch 2016), et ce encore plus dans les régions hautement anthropiques comme Trivières et ses alentours.

Les deux localités de l'est du pays présentent des dynamiques d'occupation du sol très différentes de Moorsel et Trivières. Francorchamps est un exemple typique du paysage Ardennais, avec il y a cent ans une large domination de forêts, principalement de conifères, entrecoupées par quelques landes et quelques parcelles agricoles (prairies et cultures de céréales). Un siècle plus tard, l'étendue des forêts s'est accrue pour occuper la moitié de la superficie des landes, alors que toutes les cultures ont été transformées en prairies, en quelques zones résidentielles, et en un circuit de course automobile avec ses dépendances (ce

dernier n'étant bien sûr pas typique de l'Ardenne). Parmi les 26 espèces trouvées il y a un siècle, seulement 14 ont été retrouvées aujourd'hui, et une y est apparue : *B. norvegicus*. Cette espèce inquiline de *B. hypnorum* et de *B. jonellus* affectionne particulièrement les habitats de forêt et de lisière (Pittioni & Schmidt 1942 ; Rasmont 1988). Une autre espèce ayant une préférence marquée pour ces types d'habitat (Rasmont 1988 ; Svensson *et al.* 2000 ; Rasmont *et al.* 2015a), déjà présente dans le passé, a particulièrement augmenté en abondance avec un accroissement de 23% : *B. lucorum*. Probablement en conséquence, son parasite, *B. bohemicus*, a également augmenté en abondance. Les espèces connues pour être inféodées aux landes, comme *B. cryptarum*, *B. jonellus* et *B. magnus* (Alford 1975 ; Moquet *et al.* 2017b), étaient beaucoup plus abondantes à Francorchamps que dans les autres localités il y a cent ans. Cependant, deux d'entre elles (*B. jonellus* et *B. magnus*) n'ont plus été retrouvées lors des récents inventaires, et ce même à Francorchamps. *B. jonellus* est parmi les espèces les plus spécialisées sur les Ericaceae trouvées dans les landes (Kleijn & Raemakers 2008; Moquet *et al.* 2017b). Le peu de landes restantes à Francorchamps n'a peut-être pas suffi à maintenir une population viable de cette espèce, qui est pourtant encore retrouvée dans cette région du pays (**Chapitre 1**). Par contre, *B. cryptarum* est encore relativement abondant à Francorchamps, alors qu'il a disparu dans les autres localités (à l'exception d'un spécimen à Torgny) et qu'il est en régression à l'échelle de la Belgique (**Chapitre 1**). Les landes, et plus particulièrement les Ericaceae, sont connues pour être importantes pour plusieurs espèces de bourdons, surtout au début du printemps et à la fin de l'été quand les autres ressources florales sont

rares (Moquet *et al.* 2017a). Cependant, comme à Francorchamps, elles ont fortement régressé en Europe suite aux changements de gestion des terres. Le pâturage extensif des landes principalement par des moutons durant les 18ème et 19ème siècles, qui permettait de limiter la recolonisation par les arbres, a été remplacé par un assèchement et un enrichissement du sol avec des engrais pour les rendre propices à l'agriculture ou à la plantation de conifères durant le 20ème siècle (Aerts & Heil 1993; Webb 1998). A Francorchamps, ce sont les plantations de conifères qui dominent les paysages. Leur sous-bois étant peu favorable au développement de la flore herbacée, très peu de bourdons y ont été recensés durant les inventaires, et exclusivement sur *Vaccinium* spp. au printemps le long des chemins et clairières plus ensoleillées. Comme d'autres études, nous avons constaté qu'une forte densité de forêts autour des sites d'échantillonnage ne favorise en général pas une grande diversité de bourdons (Winfree *et al.* 2007; Diaz-Forero *et al.* 2013), probablement parce que la plupart des espèces affectionnent les paysages plus ouverts et plus riches en fleurs comme les prairies et les landes (Pittioni & Schmidt 1942, Reinig 1972, Rasmont 1988). Les jardins des zones urbanisées dans les régions à forte densité forestière apparaissent même plus favorables aux bourdons que la forêt elle-même (Winfree *et al.* 2007; Diaz-Forero *et al.* 2013), ce que nous avons également observé à Francorchamps. Dans les Pyrénées, nous avons montré que l'augmentation des surfaces de forêt au détriment des habitats ouverts résultant de la déprise agricole (abandon des pratiques agro-pastorales) pourrait conduire à une perte de diversité en bourdons (Iserbyt *et al.* 2015 en **Annexe II**). Si un paysage d'agriculture intensive

entièrement ouvert (*i.e.* sans haie ni bois) n'est en général pas favorable aux bourdons, l'extrême opposé (*i.e.* prédominance de la forêt au détriment des paysages ouverts) ne semble pas l'être non plus. Une petite proportion de bois autour des habitats ouverts semble le plus favorable car ils fournissent en général de bons sites de nidification et d'hibernation pour les bourdons (Bols 1939 ; Svensson *et al.* 2000 ; Diaz-Forero *et al.* 2011, 2013).

Enfin, l'ancienne commune gaumaise de Torgny est aujourd'hui la localité la plus riche parmi les quatre échantillonnées, et figure parmi les plus riches régions de toutes celles échantillonnées en Belgique (**Chapitre 1**). Des 21 espèces inventoriées il y a cent ans, 18 sont encore trouvées aujourd'hui. Des espèces devenues rares en Belgique et même en Europe y sont encore observées alors qu'elles ont disparu des autres localités, comme *B. sylvarum* et *B. rupestris* qui sont parmi les plus abondantes dans les récents inventaires, ainsi que *B. ruderarius* et *B. humilis* (Rasmont *et al.* 2015b). Cette diversité conservée et la présence d'espèces rares à Torgny pourraient être en partie expliquées par sa composition paysagère restée relativement stable et favorable entre les deux périodes de temps investiguées. Le paysage de Torgny était dominé il y a cent ans par les cultures (principalement de céréales et de légumineuses, Ministère de l'Agriculture et des Travaux Publics 1913), les prairies et les forêts. Ces proportions sont restées relativement similaires aujourd'hui, avec une régression des cultures majoritairement remplacées par des prairies, des bois et quelques résidences avec jardin. Les cultures restantes consistent principalement en maïs et céréales. Cependant, contrairement aux autres localités, les cultures de légumineuses n'ont

pas été totalement abandonnées et couvrent encore 2% de la superficie de la commune (*Trifolium arvense* et *Medicago sativa*, Belgian Federal Government 2017). De plus, les vergers haute-tige y sont encore bien représentés. Le bocage est bien développé et encadre les pairies. Or, les haies fournissent de bons sites de nidification pour bon nombre d'espèces et facilitent l'orientation et les déplacements des bourdons dans le paysage (Svensson *et al.* 2000 ; Cranmer *et al.* 2012 ; Morandin & Kremen 2013). Beaucoup de bords de route sont en fauchage tardif et arborent une grande diversité floristique, y compris de nombreuses plantes appréciées des bourdons (*e.g*. *Trifolium pratense*, *Origanum vulgare*, *Knautia* spp., *Onobrychis viciifolia*). Une réserve naturelle de 6,4 hectares y a été créée en 1942 sur les restes d'une ancienne carrière et est majoritairement composée de pelouses sèches calcaires et de fourrés, où beaucoup d'espèces rares de bourdons ont été recensées (DGARNE 2016). La grande diversité d'habitats favorables connectés entre eux par le bocage ainsi que la disponibilité en ressources florales diversifiées et en sites de nidification permettent sans doute de maintenir la remarquable diversité de bourdons observée aujourd'hui.

En résumé, nous avons montré que le déclin des bourdons a été le plus drastique dans les localités qui ont subi la plus forte urbanisation et qui présentent encore une grande surface dédiée aux terres agricoles, mais où cette surface a le plus diminué et où son utilisation s'est la plus intensifiée (selon les quelques statistiques agricoles que nous avons pu collecter). Nous attirons donc l'attention sur le fait que la surface en terres agricoles seule ne peut rendre compte de l'effet

de l'agriculture sur les assemblages de bourdons, et doit être accompagnée d'informations relatives à l'intensité d'utilisation du sol et aux pratiques agricoles. Les rendements à l'hectare, les types de cultures, l'intensité de pâturage, l'épandage d'engrais et de pesticides…, sont autant de caractéristiques qui peuvent plus affecter les assemblages de bourdons que la surface de cultures elle-même. Similairement, nous avons constaté lors de nos observations de terrains plusieurs grandes différences entre les localités qui ne pouvaient pas être résumées par la composition du paysage seule. Il s'agit notamment de la présente de bocage et d'éléments paysagers favorables aux bourdons (talus, bandes de terres en fauchage tardif, etc.), ainsi que de la disponibilité en ressources florales dans les champs, les prairies, le long des chemins ou dans des types d'habitats plus particuliers. Nous avons notamment souligné l'importance pour les bourdons des terrils et autres milieux rudéraux en succession spontanée, des landes, ou encore des bords de routes et des talus en fauchage tardif riches en fleurs. Ajouter ces informations plus détaillées concernant l'utilisation du sol et la structure du paysage en plus de l'occupation du sol pourrait grandement améliorer les études d'écologie du paysage relatives aux bourdons.

2.3. Les changements climatiques

L'impact des changements climatiques sur les bourdons n'est étudié que depuis récemment (Rasmont & Iserbyt 2012 ; Kerr *et al.* 2015 ; Martinet *et al.* 2015a ; Rasmont *et al.* 2015a). Des changements d'aire de distribution des espèces de bourdons sont observés à travers le monde suite aux changements climatiques

(Kerr *et al.* 2015). Dans l'Hémisphère Nord, alors que les espèces peinent à suivre leur préférendum climatique vers le nord, leur aire de distribution s'est fortement réduite dans les zones où la température est la plus élevée (Kerr *et al.* 2015). Dans le **Chapitre 4**, nous avons considéré des variables bioclimatiques représentant des moyennes de températures et de précipitations, mais aussi des variables actant de phénomènes climatiques extrêmes, connus pour affecter les communautés animales et végétales (Walther *et al.* 2002 ; Hegland *et al.* 2009 ; Iserbyt & Rasmont 2012 ; Rasmont & Iserbyt 2012). Nous avons donc utilisé cinq variables bioclimatiques pour modéliser la distribution des espèces de bourdons en Belgique : trois variables de températures et deux de précipitations. Les plus grands changements climatiques ont eu lieu entre les deux dernières périodes étudiées (1970-1989 et 1990-2016), avec principalement une augmentation des températures (de 1.07°C pour la température maximale du mois le plus chaud et de 2.11°C pour la température moyenne du trimestre le plus sec). Il a été montré que les évènements extrêmes de hausse de température (*i.e.* vagues de chaleur) provoquent de lourdes pertes chez les populations de bourdons (Rasmont & Iserbyt 2012), probablement suite à leurs capacités limitées de thermorégulation hyperthermique (Sunday *et al.* 2014 ; Martinet *et al.* 2015a). Malgré l'inclusion d'une variable reflétant les hautes températures extrêmes (température maximale du mois le plus chaud), les températures influencent significativement moins la distribution des bourdons que les précipitations et l'occupation du sol toutes périodes confondues. Leur influence est inférieure à celles des précipitations particulièrement durant la période 1970-1989, pour laquelle la quantité de

précipitations du trimestre le plus sec est de loin la variable climatique la plus influente. Ceci pourrait s'expliquer en partie par le biais d'échantillonnage largement en faveur du sud du pays durant cette période, où les précipitations sont les plus fortes. Cependant, il est à noter que cette période a connu une sécheresse intense suite à un déficit important de précipitations de décembre 1975 jusqu'à un pic d'intensité à la fin de l'été 1976 (Klein 2009). Les précipitations du trimestre le plus chaud sont d'ailleurs plus faibles pour cette période que pour les deux autres. L'intensité du déficit en précipitations a cependant très fortement varié entre les régions de Belgique, avec un gradient d'intensité allant du plus faible dans l'est au plus fort dans l'ouest (Klein 2009). Cette tendance de gradient de précipitations se retrouve dans nos trois périodes étudiées, avec toujours de plus fortes précipitations dans le sud-est que dans le nord-ouest du pays. La sécheresse de 1976 a provoqué d'importants dégâts sur la végétation (Brochet 1977). De par leur impact négatif sur la flore, les épisodes de sécheresse peuvent fortement affecter les populations de pollinisateurs en diminuant la disponibilité en ressources florales (Oliver *et al.* 2013, 2015 ; Burkle & Runyon 2016 ; Thompson 2016). En plus de l'impact important que semblent avoir les évènements extrêmes de hausse de température (*i.e.* canicules) sur les bourdons (Rasmont & Iserbyt 2012 ; Martinet *et al.* 2015a), il semblerait donc que les épisodes de sécheresse pourraient également avoir une grande influence sur leurs populations. Comme ces deux phénomènes sont souvent associés, leurs effets pourraient agir en synergie et entrainer de plus lourdes conséquences sur les populations de bourdons qu'en action isolée. C'est notamment ce qui a été observé dans les Pyrénées Orientales

(Iserbyt & Rasmont 2012), où les conditions chaudes et sèches durant l'été étaient corrélées à une plus faible abondance de bourdons l'année suivante, et où les plus fortes abondances étaient observées durant et suivant les conditions les plus pluvieuses. Outre l'augmentation des températures moyennes et extrêmes à l'échelle du globe (Meehl & Tebaldi 2004), les épisodes de sécheresse ont également augmenté depuis les années 1970 (IPCC 2014). Il est fort probable que ces évènements climatiques extrêmes soient de plus en plus fréquents et intenses dans le futur (Meehl & Tebaldi 2004 ; IPCC 2014) et provoquent donc de lourds impacts sur les populations de bourdons, aussi bien à l'échelle locale qu'à travers le globe.

2.4. Interaction entre changements de l'occupation du sol et changements climatiques

Nous avons vu dans les **Chapitres 3 et 4** que les changements d'occupation du sol et les changements climatiques sont liés au déclin des populations de bourdons en Belgique. Dans la majorité des études, l'impact de ces deux changements environnementaux sur la biodiversité a principalement été étudié isolément. Or, ces deux facteurs ne sont pas indépendants et leurs effets sur les individus, populations et écosystèmes peuvent interagir, parfois de manière antagoniste, parfois en synergie (*e.g*. Travis 2003 ; Brook *et al.* 2008 ; Rose & Burton 2009 ; Schweiger *et al.* 2010 ; Oliver *et al.* 2014, 2015 ; Scherber 2015). La réponse des populations et communautés à ces changements environnementaux ne peut donc être extrapolée à partir de leur réponse à chaque facteur séparément. C'est ce que

nous avons notamment démontré chez les bourdons en Europe, où les distributions issues de modèles prédictifs basés à la fois sur le climat et sur l'occupation du sol se sont avérées plus performantes que celles issues de modèles basés seulement sur le climat (Marshall *et al.* 2017 en **Annexe IV**). Dans le **Chapitre 4**, nous avons vu à quel point les variables climatiques pouvaient être corrélées aux variables d'occupation du sol en Belgique. En effet, le nord de la Belgique présente une plus forte densité de cultures et de zones urbanisées et une plus faible densité de forêts que le sud, mais il présente aussi de plus hautes températures et de plus faibles précipitations. Le fait que la majorité des espèces en déclin ne soient actuellement plus retrouvées que dans le sud du pays provient probablement en partie de l'association de ces changements globaux, dont les effets sur les bourdons sont difficilement dissociables même si quelques variations sont observées entre l'importance des différentes variables environnementales. L'étude de l'impact des changements d'occupation du sol ne peut donc se faire sans la prise en compte du changement climatique, que ce soit à l'échelle du paysage ou à l'échelle de la distribution d'une espèce. En effet, les conditions climatiques peuvent influencer la dispersion des individus et donc la connectivité fonctionnelle des paysages, notamment chez les insectes dont le développement et la capacité de vol dépendent de la température (*e.g.* Corbet *et al.* 1993 ; Berwaerts *et al.* 2008 ; Cormont *et al.* 2011 ; Delattre *et al.* 2013). Inversement, l'effet du changement climatique sur une espèce est sans aucun doute dépendant de l'occupation du sol. A l'échelle du paysage, il a par exemple été montré que la résilience des populations d'une espèce de papillon aux sécheresses est influencée par la taille et l'état de fragmentation de

son habitat (Oliver *et al.* 2013). De plus, les modifications de l'occupation du sol peuvent influencer le microclimat, notamment en modifiant l'énergie solaire reçue au sol. Ainsi, l'abattage d'une forêt pour en faire un milieu ouvert (champs, prairie, surface bétonnée, etc.) provoque généralement une augmentation de la température et une diminution de l'humidité (Vitousek 1994). Les changements microclimatiques dus aux modifications de l'occupation du sol s'ajoutent donc aux changements climatiques globaux. A l'échelle de l'aire de distribution des espèces, les individus ne seront pas capables de suivre les déplacements latitudinaux et altitudinaux de leur niche climatique si leur habitat est trop fragmenté et empêche leur dispersion (Walther *et al.* 2002 ; Best *et al.* 2007 ; Brooker *et al.* 2007). La plupart des espèces de bourdons échouent d'ailleurs à suivre le réchauffement climatique dans les limites nordiques de leur aire de distribution, qui diminue drastiquement dans leurs limites sud (Kerr *et al.* 2015). Ces considérations sont malheureusement très peu souvent prises en compte dans les projections des aires de distribution futures des espèces de bourdons (voir cependant Marshall *et al.* 2017 en **Annexe IV**), les rendant de la sorte très peu réalistes (Rasmont *et al*. 2015a). Ignorer ces relations intimes entre les effets du climat et de l'occupation du sol sur les espèces ne peut que diminuer la confiance que nous pourrions avoir dans les prédictions et les conclusions réalisées à partir de ces études dans le cadre de la conservation de la biodiversité (Titeux *et al*. 2016a,b ; Titeux *et al*. 2017).

3. Des destins différents selon les caractéristiques écologiques des espèces

Les espèces qui présentent des traits écologiques différents réagissent différemment aux changements environnementaux (*e.g*. Rasmont & Mersch 1988 ; Goulson *et al.* 2005, 2008 ; Williams *et al.* 2007, 2009, 2010 ; Bommarco *et al.* 2010 ; De Palma *et al.* 2015 ; Aguirre-Gutiérrez *et al.* 2016). Dans le **Chapitre 1**, nous avons pu mettre en évidence des différences de tendances selon les traits écologiques des espèces. Ces différences touchent tant les préférences d'habitat et de diète que la phénologie ou encore la rareté de l'espèce à l'échelle de l'Europe. Comme d'autres études (*e.g*. Goulson *et al.* 2005, 2008 ; Williams *et al.* 2009 ; De Palma *et al.* 2015 ; Aguirre-Gutiérrez *et al.* 2016), nos résultats montrent que les espèces qui ont le plus tendance à décliner sont les plus spécialisées en termes de diète et d'habitat. Plus une espèce est présente dans un nombre restreint de types d'habitats et visite un nombre restreint de genres de plantes, plus elle est sensible aux perturbations environnementales (Williams *et al.* 2010). Il s'agit en particulier des espèces avec une plus longue langue, donc plus associées aux plantes à longue corolle comme les Fabaceae et Lamiaceae, et qui préfèrent les habitats ouverts comme les pelouses et prairies. Les espèces qui fondent leur colonie dans un nid en surface (« *carder bee* »), par exemple dans une touffe d'herbe, plutôt que dans une cavité préexistante (« *renter bee* ») tendent également à décliner plus que les autres. Cette tendance à nicher en surface les rend inévitablement plus sensibles aux perturbations comme le pâturage ou le fauchage (Kells & Goulson 2003 ; Goulson 2010). De plus, contrairement aux espèces nichant dans le sol qui

bénéficient de son inertie thermique, les espèces nichant en surface pourraient être plus vulnérables aux évènements extrêmes de température comme les canicules et donc aux changements climatiques. Enfin, les espèces qui émergent plus tard dans la saison et qui développent des colonies plus petites sont également plus sujettes au déclin. Une plus petite colonie implique une plus faible force ouvrière et donc un approvisionnement en ressources plus faible, alors qu'une émergence tardive rend les colonies plus dépendantes des ressources florales estivales (dont les chardons) que printanières. Comme les usages agricoles modernes privilégient les quelques cultures florales printanières et réduisent fortement la disponibilité en ressources florales en fin de saison (fauchage précoce des prés, échardonnage, etc.), il semble logique que ces espèces soient les plus directement impactées par ces changements de pratiques agricoles.

De plus, nous avons vu dans le **Chapitre 4** que l'influence des variables environnementales sur la distribution des espèces variait entre les espèces. Une grande partie des espèces semble plus influencée par l'occupation du sol (*e.g. B. terrestris, B. norvegicus, B. ruderarius, B. ruderatus, B. pomorum*), d'autres par les précipitations (*e.g. B. lapidarius, B. jonellus*), et certaines semblent influencées équitablement par l'occupation du sol, les précipitations et les températures (*e.g. B. hortorum*). La majorité des espèces en régression, comme *B. barbutellus, B. humilis, B. soroeensis, B. ruderarius, B. sylvarum* et *B. veteranus*, sont aujourd'hui principalement restreintes au sud du pays. Or, nous avons vu que le sud de la Belgique présente de plus fortes précipitations, une plus grande densité de forêts et une plus faible surface de cultures et de zones urbanisées. Les espèces en déclin

semblent donc parvenir à maintenir leurs populations seulement dans les régions présentant un haut apport hydrique et de faibles niveaux d'urbanisation et d'agriculture. Elles seraient donc potentiellement plus sensibles au déficit hydrique (et donc aux sécheresses), à l'urbanisation et à la forte densité de cultures que les autres espèces. Deux autres espèces affichent des distributions très restreintes également, mais cette fois-ci dans l'est du pays : *B. jonellus* et *B. magnus*. Elles sont principalement présentes en Campine et, surtout pour *B. jonellus*, dans les Hautes Fagnes. Ces régions sont celles qui présentent encore de nos jours le plus de surfaces de landes, dont dépendent en grande partie ces espèces (Alford 1975 ; Moquet *et al.* 2017). La tendance de population européenne est stable pour *B. jonellus* et inconnue pour *B. magnus*. A l'échelle de la Belgique, leur aire de distribution a cependant significativement diminué entre 1910-1930 et 1990-2016.

Pourquoi certaines espèces sont-elles restées communes ?

Comme nous l'avons vu dans le **Chapitre 1**, toutes les espèces ne régressent pas et certaines espèces sont restées dans le « top 5 » des espèces les plus étendues et abondantes en Belgique (*B. pascuorum, B. terrestris, B. lapidarius, B. pratorum* et *B. hypnorum*), ce qui est également observé à l'échelle de l'Europe (Rasmont *et al.* 2015a). Contrairement aux espèces en déclin, nous avons vu dans le **Chapitre 4** que leur distribution est encore actuellement très répandue dans tout le pays. Elles sont encore largement présentes dans le nord, où l'urbanisation a été la plus forte au cours du siècle dernier et où les terres agricoles représentent encore une très

grande surface. Comme constaté dans d'autres études, ces espèces apparaissent souvent dans les jardins et les parcs des zones fortement urbanisées ou agricoles, où elles dominent les assemblages (McFrederick & LeBuhn 2006; Ahrné *et al.* 2009). Elles semblent donc bien adaptées aux environnements urbains et ne semblent pas souffrir de l'urbanisation et de l'intensification de l'agriculture, c'est-à-dire des deux changements d'utilisation du sol les plus drastiques qui ont eu lieu en Belgique et en Europe Occidentale durant ce dernier demi-siècle. Le même constat a été établi en Grande Bretagne, où les espèces qui subsistent dans les régions d'agriculture intensive ne sont plus que les espèces communes et généralistes (Robinson & Sutherland 2002). Une part d'explication proviendrait donc de leur plus faible spécialisation en termes d'habitat et de ressources florales (*e.g*. Robinson & Sutherland 2002 ; Goulson *et al.* 2005 ; Williams *et al.* 2009). Comme nous l'avons vu dans le **Chapitre 1**, confirmant les résultats de Kleijn & Raemakers (2008), les espèces qui ont le moins décliné durant les 100 dernières années sont celles qui présentent une diète plus large que les autres, c'est-à-dire qui collectent du pollen sur une plus grande diversité de fleurs. L'espèce considérée comme la plus polylectique par Kleijn & Raemakers (2008) est *B. terrestris*, qui a une langue relativement courte mais qui est capable de « voler » le nectar des fleurs à longue corolle en les perçant à leur base. Les espèces les plus abondantes (en particulier *B. lapidarius* et *B. terrestris*) ont tendance à butiner sur les plantes plus communes ou en expansion (Roger *et al.* 2017), comme par exemple le trèfle blanc (*Trifolium repens*) que nous avons beaucoup rencontré dans les pelouses et prairies fréquemment tondues ou fauchées. De plus, leur diète peut même s'étendre aux

fleurs non-indigènes (Kleijn & Raemakers 2008 ; Roger *et al.* 2016, 2017), comme *B. pascuorum* sur *Impatiens glandulifera* (Roger *et al.* 2016) ou *B. terrestris* sur *Buddleia davidii*, cette dernière ayant été fréquemment rencontrée dans les paysages les plus urbanisés que nous avons échantillonnés (*i.e.* Moorsel et Trivières). Le fait que ces espèces soient capables de choisir les ressources florales les plus abondantes, qui varient entre les régions et les périodes, reflète une grande capacité d'adaptation à de nouvelles ressources.

De plus, les espèces de plus grande taille comme *B. terrestris* et *B. lapidarius* ont une plus grande aire de butinage (Knight *et al.* 2005 ; Greenleaf *et al.* 2007 ; Osborne *et al.* 2008a ; Wolf & Moritz 2008). Cette grande capacité de déplacement les rend plus résistantes à la fragmentation des parcelles fleuries et donc plus adaptées aux paysages agricoles modernes où elles subsistent en grand nombre (Goulson *et al.* 2006; Osborne *et al.* 2008a). Il semblerait également que les espèces plus abondantes aient une plus grande capacité de dispersion que les espèces plus rares (*e.g*. Bommarco *et al.* 2010 ; Darvill *et al.* 2010 ; Lepais *et al.* 2010). Plus la capacité de dispersion d'une espèce est grande, plus elle est en mesure de coloniser de nouvelles régions et de suivre les déplacements de son aire climatique (Watkinson & Gill 2002 ; Best *et al.* 2007 ; Brooker *et al.* 2007). *B. hypnorum* a rapidement colonisé le Royaume-Uni à partir de 2001 probablement après avoir traversé la Manche en provenance de l'Europe continentale (Goulson & Williams 2001). Les distributions de *B. terrestris* et de *B. lapidarius* tendent à se déplacer vers le nord et même à dépasser le Cercle Arctique depuis quelques années (Martinet *et al.* 2015b ; Rasmont *et al.* 2015a). Chez *B. terrestris*, il semblerait que la dispersion

des individus se limiterait généralement à 10 kilomètres (Rasmont 1983 ; Stout & Goulson 2000). Les analyses génétiques permettant d'estimer les flux de gènes entre les populations de *B. terrestris* et de *B. pascuorum* ont montré une très faible structure de population à travers l'Europe, ce qui suggère une dispersion fréquente des individus entre populations sur le continent (Estoup *et al.* 1996 ; Pirounakis *et al.* 1998 ; Widmer *et al.* 1998 ; Widmer & Schmid-Hempel 1999). Par contre, les espèces rares que sont *B. muscorum*, *B. sylvarum* et *B. distinguendus* présentent une structuration de population significative (Darvill *et al.* 2006 ; Ellis *et al.* 2006 ; Bourke & Hammond 2002). Chez *B. muscorum*, une distance de 3 à 10 km suffit pour séparer deux populations (Darvill *et al.* 2006, 2010). Dans notre étude sur la diversité génétique des espèces de bourdons dans les mêmes localités que celles étudiées dans le **Chapitre 3** (Maebe *et al.* 2016 en **Annexe III**), nous avons montré que les populations des espèces répandues (*B. pascuorum*, *B. hortorum*, *B. pratorum* et *B. lapidarius*) présentaient en général une plus grande diversité génétique (*i.e.* richesse allélique et hétérozygotie) que les espèces plus rares (*B. ruderarius*, *B. sylvarum*, *B. humilis* et *B. soroeensis*). Ceci suggère que les espèces plus répandues présentent des flux de gènes plus fréquents et donc un risque plus faible de dérive génétique et de dépression de consanguinité, ce qui les rend plus résistants aux perturbations de l'environnement.

Enfin, une hypothèse complémentaire pour expliquer l'abondance de ces cinq espèces proviendrait de leurs exigences en termes de conditions climatiques. Pour la plupart, la Belgique tend à se situer plus au centre qu'à la limite de leur niche climatique (Rasmont *et al.* 2015a), par ailleurs beaucoup plus large que celle des

espèces en déclin. Ceci pourrait les rendre capables de persister dans des habitats moins favorables et en faire des espèces plus compétitrices que les espèces en limite d'aire. C'est du moins ce qui est observé au Royaume-Uni, où les espèces en limite de leur aire climatique (*B. subterraneus* et *B. distinguendus*) ont été beaucoup plus affectées par la perte en habitats que *B. terrestris* qui se trouve au centre de sa niche climatique et qui occupe même les habitats de moindre qualité (Goulson 2010). La grande capacité d'adaptation de *B. terrestris* (Rasmont *et al.* 2008) lui permet même de coloniser de nouveaux environnements et de devenir une espèce envahissante là où elle a été introduite (*e.g.* Tasmanie, Japon, Amérique du Sud ; Stout & Goulson 2000 ; Matsumura *et al.* 2004 ; Schmid-Hempel *et al.* 2014). Il s'agirait notamment de la seule espèce de bourdon européenne capable de modifier sa phénologie selon les conditions climatiques saisonnières, étant capable d'éviter l'hibernation et de produire une à trois générations par an selon les régions (Rasmont *et al*. 2008 ; Rasmont *et al.* 2015a).

En résumé, leur faible spécialisation et leurs probables plus grandes capacités de dispersion et possibilités adaptatives en termes de ressources alimentaires, d'habitat et de conditions climatiques pourraient expliquer le succès de ces cinq espèces encore très abondantes en Belgique et en Europe.

4. Les conséquences probables du déclin des bourdons

Le service écosystémique de pollinisation est actuellement sous la pression des changements environnementaux globaux qui pèsent sur les insectes pollinisateurs (Kremen *et al.* 2002 ; Gallai *et al.* 2009 ; Vanbergen & Garratt 2013 ; Bartomeus *et*

al. 2014 ; Ollerton *et al.* 2014). Comme dit précédemment, le service de pollinisation fourni par les bourdons est considérable, souvent plus important que celui fourni par l'abeille mellifère (*e.g*. Woodcock *et al.* 2013 ; Mallinger & Gratton 2015) tant pour les écosystèmes naturels que pour les productions agricoles et horticoles (Ollerton *et al.* 2011). Une abondance réduite en bourdons entraine inévitablement une baisse de productivité des plantes, leur production de graines dépendant directement de l'effort de pollinisation (*e.g*. Ågren 1996 ; Steffan-Dewenter & Tscharntke 1999). Puisque les bourdons sont des pollinisateurs relativement généralistes, les changements dans leurs assemblages et dans leurs abondances peuvent lourdement affecter les communautés végétales (Corbet *et al.* 1991) et donc indirectement les communautés animales qui en dépendent. Des simulations ont démontré que le fait d'extraire les bourdons d'un réseau de pollinisation provoquait les plus haut taux de déclin en diversité floristique, comparée au fait de retirer les espèces plus polylectiques (Memmott *et al.* 2004). Pour cette raison, les bourdons pourraient être considérés comme des espèces « parapluie » ou « clés de voute » car leur conservation requiert des mesures qui privilégient aussi un grand nombre d'autres espèces au sein des communautés, qu'elles soient au même niveau trophique (*i.e.* les autres espèces de pollinisateurs) ou non (*e.g*. les espèces de plantes pollinisées et toutes celles qui en dépendent, dont l'espèce humaine).

Nous avons vu que les localités (**Chapitre 3**) et les régions (**Chapitres 1 et 4**) de Belgique où la richesse spécifique en bourdons avait le plus souffert au cours du siècle dernier et qui ne comptaient plus que les espèces les plus communes

aujourd'hui étaient notamment celles où l'agriculture était la plus développée. Or, le service de pollinisation fourni notamment par les bourdons est crucial pour de nombreuses cultures en Belgique (Jacquemin *et al.* 2017). Rendre les paysages agricoles plus favorables à l'établissement et au maintien des populations de bourdons apparait donc indispensable pour conserver un service de pollinisation efficace dans ces écosystèmes (Goulson 2003 ; Földesi *et al.* 2016 ; Kovács-Hostyánszki *et al.* 2017).

5. Quelles pistes pour la conservation des bourdons ?

Comme nous l'avons vu, les paysages agricoles et urbains sont actuellement les plus défavorables pour les bourdons. Dans les premiers, l'intensification agricole a fortement diminué la disponibilité en ressources florales (légumineuses et adventices) et en sites de nidifications (haies, hautes herbes, talus, etc.). Dans les seconds, seuls les jardins, les parcs, les bords de route et autres surfaces non bétonnées peuvent encore potentiellement accueillir des colonies de bourdons (McFrederick & LeBuhn 2006). L'impact négatif des zones urbanisées est cependant beaucoup moins important lorsqu'une grande proportion d'habitats naturels est maintenue dans leurs alentours (Winfree *et al.* 2007).

Depuis les années 1990, plusieurs mesures agro-environnementales ont permis d'encourager la conservation de la nature dans les paysages agricoles d'Europe (Kleijn & Sutherland 2003 ; Batáry *et al.* 2015 ; EUROPA 2017). Celles-ci incluent notamment la reconstruction d'éléments du bocage à travers la plantation de haies, la plantation de bandes fleuries aux abords des champs, ou encore la restauration

de prairies oligotrophes riches en fleurs (Kleijn *et al.* 1998 ; Kleijn & Sutherland 2003 ; Batáry *et al.* 2015). Toutes les mesures qui visent à augmenter la biodiversité globale semblent généralement bénéfiques pour les populations locales de bourdons (Dramstad & Fry 1995 ; Carreck & Williams 2002; Carvell *et al.* 2004, 2007; Pywell *et al.* 2005, 2006 ; Kallioniemi *et al.* 2017). Le fait que moins d'espèces de bourdons soient en régression entre les périodes 1970-1989 et 1990-2016 qu'entre les périodes 1910-1930 et 1970-1989 dans nos résultats pourrait en partie provenir de ces mesures de conservation, même si aucune relation de cause à effet ne peut être démontrée. Cependant, il serait intéressant de prévoir des mesures qui ciblent plus particulièrement l'écologie des bourdons (*e.g*. Carvell *et al.* 2007). Par exemple, il a été montré que les semis de plantes pérennes ou bisannuelles étaient préférées aux plantes annuelles par les bourdons, probablement car elles tendent à produire plus de nectar (Fussell & Corbet 1992; Dramstad & Fry 1995 ; Carreck & Williams 2002). Plus précisément, nous recommandons de privilégier une plus haute densité de fleurs à longues corolles comme les légumineuses (dans les bandes fleuries, dans les prairies ou même en cultures à part entière), et de laisser une place aux chardons aux abords des champs et dans les prairies (tout en limitant l'apport d'engrais qui pourraient provoquer leur surabondance). La présence de chardons dans les bords de champs laissés en régénération naturelle augmente fortement la biodiversité et l'abondance en bourdons, au moins autant que les semis de plantes sauvages (Carvell *et al.* 2004 ; Pywell *et al.* 2005). Notre **Chapitre 2** ne laisse aucun doute à l'importance capitale

des chardons pour les bourdons et plaide en faveur de la suppression des lois d'échardonnage et de leur retour légitime dans nos paysages.

Outre l'amélioration de la disponibilité en ressources florales, plusieurs mesures peuvent augmenter la quantité de sites de nidification et d'hibernation. La plantation de haies ou le maintien de talus et de zones non fauchées aux abords des prairies et des champs permettent d'attirer les populations de micromammifères, dont les terriers une fois abandonnés sont utilisés par bon nombre d'espèces de bourdons (Svensson *et al.* 2000). La restauration du bocage, en plus de fournir des sites de nidification aux bourdons ou même une ressource florale (*e.g*. présence de saules, de ronces ou d'aubépines), peut faciliter leurs déplacements à travers le paysage et donc réduire son état de fragmentation (Svensson *et al.* 2000 ; Cranmer *et al.* 2012 ; Morandin & Kremen 2013).

Par ailleurs, même s'ils sont relativement peu abordés dans cette dissertation, deux autres phénomènes de l'agriculture intensive viennent s'ajouter aux facteurs de déclin précédents et devraient également être pris en considération dans tout plan de conservation. Le premier est l'usage massif des engrais azotés de synthèse, qui provoquent une homogénéisation de la flore par la domination des espèces nitrophiles (*e.g*. *Taraxacum officinale*, *Urtica diocia*) au détriment d'autres espèces comme les légumineuses. Le deuxième est l'utilisation de pesticides, dont les effets néfastes sur les populations de bourdons sont de mieux en mieux connus (voir un aperçu dans l'introduction générale). Etablir des plans de conservation favorisant les plantes nourricières et les habitats des bourdons sans limiter l'apport d'engrais et de pesticides dans les prairies et champs environnants semble incohérent.

Ignorer ces deux aspects de l'agriculture intensive dans les mesures agro-environnementales affaiblit très probablement leurs effets positifs sur les populations de bourdons. Une conservation efficace ne peut donc se limiter à ces seules mesures et devrait remettre en cause l'entièreté du système agricole conventionnel actuellement basé sur l'apport d'intrants chimiques. Une solution pourrait provenir de l'agroécologie (Francis *et al*. 2003 ; Wezel *et al*. 2011), qui propose un système de production basé sur les services écosystémiques et non sur l'apport d'intrants. Ce système, qui offre une logique de gestion « d'écosystèmes cultivés » à la place d'une logique d'exploitation du sol et des ressources naturelles, pourrait être une solution durable pour la conservation des bourdons et de la biodiversité en général dans les paysages agricoles.

Le même principe peut s'appliquer dans les zones urbaines. Les espaces verts, qu'ils soient publics (parcs) ou privés (jardins), peuvent représenter un réel refuge pour plusieurs espèces de bourdons dans les régions fortement urbanisées ou les paysages d'agriculture intensive (Goulson *et al.* 2002; McFrederick & LeBuhn 2006; Osborne *et al.* 2008b). Cependant, ces types d'habitat pourraient être bien plus favorables pour les bourdons si leur gestion permettait de mieux prendre en compte leur écologie et plus largement la conservation de la nature. Une gestion efficace et favorable pour les bourdons privilégierait les plantes à fleurs indigènes à la place des variétés horticoles modernes qui ne fournissent parfois que très peu ou pas du tout de pollen et de nectar accessibles (Corbet *et al.* 2001), laisserait une place aux éléments du paysage favorables aux bourdons (haie, végétation haute, buissons, etc.), et limiterait fortement l'emploi des herbicides et insecticides. En

plus des jardins et parcs, l'aménagement de petites zones favorables (plantations de fleurs et autres éléments cités plus haut) disséminées à travers les espaces urbains permettrait de fournir des étapes (« *stepping stones* ») aux individus en déplacement et donc d'améliorer la connectivité dans ces types de paysage, connus pour limiter les flux de gènes chez les bourdons (Jha & Kremen 2013 ; Jha 2015).

Enfin, nous avons montré qu'il est également important de conserver les habitats plus spécifiques comme les terrils en succession spontanée et autres milieux rudéraux, ainsi que les landes, qui permettent le maintien d'un plus grand nombre d'espèces et d'espèces plus spécialisées.

Toutes ces mesures ne peuvent être effectives que si elles couvrent toute l'aire de distribution des espèces, et ce afin de former ce qui est couramment appelé le maillage ou réseau écologique (Opdam *et al.* 2006). En effet, si les milieux rendus plus favorables grâce aux mesures de conservation ne sont pas connectés entre eux par des zones de liaisons favorables (corridors et « *stepping stones* »), ces mesures ne peuvent assurer la dispersion des individus entre les populations et donc la pérennité des espèces (Correa Ayram *et al.* 2016 ; Hanski 1999). Par exemple, une partie de bord de route en fauchage tardif ne pourra être réellement efficace pour soutenir une population locale ou pour jouer le rôle de corridor si aucun autre habitat favorable n'est présent dans le rayon de déplacement des individus.

6. Perspectives et recommandations pour les recherches futures

Avant de pouvoir étudier et prédire de manière réaliste l'effet des changements d'occupation du sol et des changements climatiques sur les populations des espèces

de bourdons, il semble indispensable de prendre en compte leurs différentes caractéristiques écologiques. En particulier, afin de déterminer si une espèce est menacée par la fragmentation de son habitat, deux critères doivent être considérés : la taille minimale de parcelle d'habitat favorable nécessaire au maintien d'une population viable et les capacités de déplacement (*i.e.* dispersion) des individus reproducteurs entre les parcelles (Bowler & Benton 2005 ; Kokko & Lopez-Sepulcre 2006). Comment savoir si l'habitat d'une espèce est trop fragmenté si on ne sait pas quelle taille minimale d'habitat est nécessaire et quelle étendue d'habitat non favorable peut séparer deux populations ? Il en va de même pour les études de décalages prédictifs des aires de distribution d'espèces, qui ne peuvent être réalistes si l'espèce n'est pas capable de suivre les déplacements de sa niche écoclimatique, à cause d'une dispersion limitée naturellement ou par la présence de barrières écologiques constituées d'habitat non favorable (*e.g*. Kokko & Lopez-Sepulcre 2006 ; Best *et al.* 2007 ; Brooker *et al.* 2007). La capacité de dispersion des bourdons commence à être investiguée depuis peu (voir une revue dans Woodard *et al.* 2015), mais les études ne concernent pour l'instant que quelques espèces dans des milieux particuliers (majoritairement au Royaume-Uni, *e.g*. Ellis *et al.* 2006 ; Charman *et al.* 2010 ; Darvill *et al.* 2010 ; Lepais *et al.* 2010 ; Goulson *et al.* 2011 ; Dreier *et al.* 2014), ce qui ne permet pas encore de généraliser aux autres espèces, régions et conditions environnementales. La même constatation de lacunes peut être faite sur la connaissance des interactions entre les espèces de bourdons au sein des assemblages et avec les autres espèces au sein des communautés, qui peuvent pourtant aussi influencer la réponse des espèces aux changements

environnementaux (*e.g*. Brooker *et al.* 2007). Nous avons notamment abordé le cas des espèces inquilines (*Psithyrus*), dont l'écologie et la fidélité aux espèces hôtes sont encore peu connues (mais voir par exemple Løken 1984 ; Williams 2008 ; Lhomme 2009). Or, la distribution et la viabilité des populations de ces espèces parasites dépendent de celles de leurs espèces hôtes. En résumé, les études ciblant les caractéristiques écologiques des espèces de bourdons à travers plusieurs régions pourraient ajouter de précieuses informations sur les impacts qu'ont eu et qu'auront les changements environnementaux globaux sur les assemblages de bourdons.

A notre connaissance, toutes les études prenant en compte l'abondance des espèces de bourdons se basent sur l'effectif total des espèces, c'est-à-dire incluant les trois castes (*e.g*. Rasmont & Mersch et Rasmont *et al.* 1993 en Belgique). Cependant, comme chez tous les insectes sociaux, il serait plus judicieux de considérer la taille effective de population (*i.e.* le nombre d'individus reproducteurs) plutôt que l'effectif global des espèces de bourdons lors des analyses de tendance de populations. En effet, les ouvrières ne participant pas à la reproduction, la taille effective des populations de bourdons peut être largement inférieure à l'effectif total car ne comprenant que les mâles et les reines. Une population peut apparaître abondante en nombre d'ouvrières mais présenter un très faible effectif reproducteur, ce qui entraine une plus grande sensibilité à la fragmentation des habitats et aux événements stochastiques (Gilpin & Soulé 1986 ; Hanski *et al.* 1996 ; Zayed & Packer 2005). De plus, le nombre d'ouvrières produites par colonie varie considérablement entre les espèces, allant par exemple de 20 à

100 chez les espèces du sous-genre *Thoracobombus* et de 100 à 400 chez *B. terrestris* et *B. lapidarius* (Alford 1975 ; von Hagen & Aichhorn 2014), ou même aucune pour les espèces inquilines du sous-genre *Psithyrus* (Løken 1984). Par ailleurs, comme l'identification visuelle des ouvrières de bourdons peut être parfois extrêmement difficile pour certaines espèces (par exemple les quatre espèces du sous-genre *Bombus* : *B. cryptarum*, *B. lucorum*, *B. magnus* et *B. terrestris*), les erreurs peuvent être fréquentes et ainsi biaiser les résultats. Comme l'effectif reproducteur dépend du nombre de colonies, l'estimation de la taille théorique de parcelle d'habitat pour héberger une taille minimale viable de population pourrait prendre en compte la densité de nids. Malheureusement, peu d'information sont disponibles car les nids sont rarement détectables. Plusieurs études anglaises ont suggéré une densité de nids allant de 0.2 à 2 nids par hectare pour les espèces communes (Darvill *et al.* 2004; Knight *et al.* 2005; Osborne *et al.* 2008b). Selon les calculs de Goulson (2010), il faudrait 333 ha d'habitat favorable pour héberger une population de 100 individus reproducteurs, en considérant une densité de 0.2 nids par hectare. Dans le **Chapitre 1**, nous avons réalisé les analyses de tendance basées sur les abondances en analysant d'une part l'effectif global (incluant les ouvrières) et d'autre part l'effectif reproducteur (excluant les ouvrières). Dans l'ensemble, les résultats sont similaires entre les deux analyses, à deux exceptions près. Comme les abondances sont bien inférieures lorsque les ouvrières ne sont pas considérées, plus d'espèces ne montrent pas de différence significative entre les effectifs de P2 et de P3 par rapport aux analyses qui incluent les ouvrières. De plus, comme les espèces inquilines ne produisent pas d'ouvrières, leurs abondances relatives sont

plus élevées lorsque les ouvrières des autres espèces ne sont pas considérées. Par conséquent, celles-ci présentent plus de tendances positives que dans les analyses incluant les ouvrières. Dans le **Chapitre 3**, nous n'avions malheureusement pas assez de données récentes pour pouvoir ne considérer que l'effectif reproducteur. Il serait dès lors très intéressant d'acquérir plus de données par un plus grand nombre d'inventaires afin d'estimer la taille effective des populations de bourdons dans les localités étudiées. Une autre approche pourrait être d'estimer le nombre de colonies en comptant la proportion d'ouvrières sœurs au sein d'un inventaire. Ceci reviendrait à identifier les relations de parenté entre les ouvrières sur base d'analyses génétiques *via* des techniques dites de « *half-* » ou « *full-sibship reconstruction* » qui permettent d'identifier les individus apparentés respectivement par un ou deux parents (*e.g*. Painter 1997 ; Thomas & Hill 2002). Ce type de méthode a notamment été utilisé sur les bourdons par Lepais *et al*. (2010) pour estimer les distances de dispersion des reines.

En ce qui concerne l'occupation du sol, nos résultats suggèrent, tant à l'échelle locale (**Chapitre 3**) qu'à l'échelle de la Belgique (**Chapitre 4**), que l'influence et l'effet des terres agricoles ont profondément changé depuis cent ans. Nous avons notamment lié cela au phénomène d'intensification agricole, qui a pour conséquence que les cultures d'aujourd'hui ne ressemblent plus en rien aux cultures d'il y a un siècle. La surface en terres agricoles seule ne peut donc rendre compte de l'effet de l'agriculture sur les bourdons, et devrait impérativement être accompagnée d'informations relatives à l'utilisation du sol, que ce soit dans les études locales ou pour les modélisations de distribution à plus large échelle. Par

ailleurs, nous avons vu que l'influence des terres agricoles mais aussi des autres types d'occupation du sol sur la distribution des bourdons varient au cours du temps (**Chapitre 4**). Afin de rendre plus vraisemblables les projections futures de distribution d'espèces, il semble dès lors essentiel d'intégrer, en plus des scénarios climatiques, les scénarios de changements d'occupation du sol (mais aussi d'utilisation du sol) aux modèles de distribution, à la place des modèles basés uniquement sur le climat ou sur les changements d'occupation du sol induits par les changements climatiques (Titeux *et al*. 2016a,b ; Marshall *et al*. 2017 ; Titeux *et al*. 2017).

Enfin, cette thèse s'est principalement penchée sur l'importance des ressources florales (en particulier des chardons), du climat, de l'occupation et (en partie) de l'utilisation du sol pour les bourdons. Il est évident que d'autres changements environnementaux ont une influence sur les assemblages de bourdons, notamment les pesticides, les pathogènes et parasites, ou encore les espèces invasives (*e.g*. Brittain *et al.* 2010 ; Schweiger *et al.* 2010 ; Thompson 2011 ; Cameron *et al.* 2016). Ces autres facteurs non développés dans cette thèse se combinent au mélange nuisible des changements environnementaux globaux et méritent d'être ajoutés aux études futures ayant pour but d'assurer une conservation efficace des bourdons. Il serait ainsi intéressant de réaliser une étude multifactorielle incluant tous ces facteurs (en plus de ceux abordés dans cette dissertation) afin d'extraire l'effet respectif de chacun d'entre eux et de leurs interactions sur les populations de bourdons. Une telle analyse nécessiterait d'acquérir les données relatives à tous ces facteurs pour les mêmes échelles et résolutions à la fois spatiales et temporelles.

Ces données pourraient alors être analysées dans un même modèle multivarié ou *via* une méthode de partition de variance comme celle utilisée dans le Chapitre 3 (*i.e.* analyse partielle en coordonnées principales sous contraintes (pCAP) ou analyse canonique de redondance partielle (pRDA)).

7. Conclusion générale

Dans ce travail, nous avons constaté le déclin drastique d'une grande partie des espèces de bourdons en Belgique depuis un siècle. Nos résultats indiquent de grandes différences de tendances de populations entre les espèces ayant des traits écologiques différents, impliquant des changements de composition dans les assemblages. Les espèces les plus spécialisées en termes de ressources florales et d'habitat, nichant en surface, émergeant plus tard dans la saison et produisant de plus petites colonies sont les plus enclines au déclin. Nous avons révélé l'importance des chardons pour les bourdons et suggérons l'abandon des lois d'échardonnage. Nous avons abordé l'influence de l'occupation du sol et du climat sur les espèces et les assemblages de bourdons en Belgique, à l'échelle du paysage et à l'échelle du pays. En particulier, nos résultats tendent à confirmer les répercussions négatives de l'urbanisation et de l'intensification agricole sur les assemblages de bourdons. En plus de l'impact des températures élevées investigué par d'autres études, nos résultats montrent que les précipitations ont également un rôle prédominant dans la définition de l'aire de distribution des bourdons en Belgique, et suggèrent un impact négatif non négligeable des épisodes de sécheresse. Enfin, à la vue de ces résultats et de la littérature, nous avons proposé

un ensemble de mesures permettant d'améliorer la conservation des populations de bourdons en Belgique et en Europe. Nous avons également émis des conseils pour les recherches futures, en mettant particulièrement l'accent sur l'importance des études investiguant davantage les caractéristiques écologiques des espèces (*e.g*. capacités de dispersion, préférences d'habitat, interactions entre espèces) et sur l'importance d'intégrer tous les facteurs environnementaux plutôt qu'isolément. Enfin, ce travail met en évidence le grand intérêt du monitoring à long terme des populations et des changements environnementaux pour pouvoir dresser un tableau de l'état actuel des espèces, prévoir les changements à venir, et établir des bases auxquelles les études futures pourront se référer.

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Glossaire

Glossaire

Ici sont repris les termes techniques les plus souvent cités dans cette dissertation. Chaque entrée du glossaire (en gras) est suivie de sa traduction anglaise (en italique) lorsque nécessaire. Lorsqu'il y a lieu, une référence est citée et/ou un renvoi est indiqué vers le chapitre qui cite ce terme.

Aire de butinage (« *foraging range* »). Rayon ou distance jusqu'où un individu peut se déplacer pour chercher une ressource alimentaire.

Aire de dispersion (« *dispersal range* ») ou capacité de dispersion. Rayon ou distance jusqu'où un individu reproducteur peut se déplacer pour atteindre un site de reproduction. Voir « dispersion ».

Aire de distribution géographique (« *geographic range* »). Etendue géographique d'une espèce. Aussi plus simplement appelée aire de distribution ou distribution géographique. Voir les Chapitres 1 et 4.

Allèle (« *allele* »). Une des formes alternatives prises par un gène, c.-à-d. une des variantes de la séquence de l'ADN d'un locus d'un chromosome.

Allélopathie (« *allelopathy* »). Effet nocif d'un composé chimique produit par une espèce de plante qui agit sur les plantes voisines d'autres espèces. Voir le Chapitre 2.

Analyse Canonique en Coordonnées Principales (CAP pour « *Constrained Analysis of Principal Coordinates* »). Aussi appelée db-RDA (« *Distance-Based Redundancy Analysis* »). Méthode d'ordination sous contrainte, dite supervisée, qui associe une Analyse en Coordonnées Principales à une régression linéaire. Voir le point 4.6. dans le Cadre Méthodologique Général.

Analyse en Coordonnées Principales (PCoA pour « *Principal Coordinate Analysis* »). Aussi appelée MDS (« *Metric Multidimensional Scaling* »). Méthode d'ordination non-supervisée basée sur une matrice de distance laissée au choix de l'utilisateur. Voir le point 4.5.2. dans le Cadre Méthodologique Général.

Analyse Factorielle des Correspondances (AFC ou « *CA* » pour « *Correspondance Analysis* »). Méthode d'ordination non-supervisée basée sur la distance du Chi-carré. Voir le point 4.5.1. dans le Cadre Méthodologique Général et le Chapitre 2.

AUC (« *Area Under the Curve* », abréviation de AUROC pour « *Area Under the Receiver Operator Curve* »). Surface sous la courbe ROC (« *Receiver Operating Characteristic* »). Le ROC est une mesure de la performance d'un classificateur binaire lorsque le seuil de discrimination varie. L'AUC est notamment utilisé pour mesurer la performance (la fiabilité) d'un modèle de distribution d'espèce. Voir le point 4.7. dans le Cadre Méthodologique Général et le Chapitre 4.

Biodiversité. Diversité des espèces, types d'organismes, habitats ou écosystèmes de la planète ou d'un lieu donné.

« *Carder bees* ». Espèces de bourdons qui nichent directement à la surface du sol, sous la végétation (le plus souvent dans les touffes d'herbe), dans un nid qu'elles aménagent elles-mêmes à l'aide de matériel végétal déchiqueté (Sladen 1912). Ce mode de nidification s'oppose à celui des « *Renter bees* ». Voir le point 4.4.2. de l'Introduction générale et le Chapitre 1.

Caste. Sous-groupe d'individus au sein d'un groupe social qui ont la même forme spécialisée ou le même comportement. Chez les bourdons, trois castes existent : les reines et les mâles (castes reproductrices), ainsi que les ouvrières (caste non reproductrice).

Communauté (« *community* »). Association des populations d'espèces en interaction qui vivent dans un même lieu.

Condition abiotique. Attribut physique ou chimique de l'environnement qui influence les processus biologiques et la survie des populations d'espèces sans pour autant faire l'objet d'une consommation. Exemples : température, acidité de l'eau ou du sol… A comparer au terme « ressource ».

Corridor (« *corridor* »). Connection entre deux parcelles d'habitat qui permet les déplacements et la dispersion d'individus entre ces deux parcelles.

Dérive génétique (« *genetic drift* »). Perte de diversité génétique et changements dans les fréquences alléliques issus d'évènements aléatoires intervenant dans les populations de petits effectifs.

Dette d'extinction (« *extinction debt* »). Future extinction d'espèces causée par les changements environnementaux actuels.

Dispersion (« *dispersal* »). Mouvement spatial d'individus d'un site de départ (de naissance ou de première reproduction) à un site de reproduction qui a des conséquences potentielles sur les flux génétiques à travers l'espace (Ronce, 2007).

Endothermie facultative. Capacité, chez un animal ectotherme, d'augmenter sa température corporelle par certains processus physiologiques.

Espèce parapluie. Espèce dont la protection induit de fait la protection d'autres espèces.

Eusocialité. Organisation d'une société en différentes castes, dominées par une reine pondeuse aidée par certains de ses jeunes qui ne se reproduisent pas. Cas des termites, des fourmis, de l'abeille mellifère… Chez les bourdons, on parle d'eusocialité « primitive » car la reine passe un stade de sa vie en phase solitaire.

Flux de gènes. Transfert d'allèles (et donc de combinaisons génétiques) entre populations résultant de la dispersion des individus, gamètes ou spores.

Généraliste. Espèce qui a une grande étendue de diète alimentaire ou de larges préférences en termes d'habitat. Opposé à « spécialiste ».

Habitat. L'habitat d'une espèce est l'ensemble des ressources et conditions biophysiques nécessaires à l'accomplissement de son cycle de vie, y compris sa reproduction (Hall *et al*. 1997 ; Dennis *et al*. 2003).

Haplodiploïdie. Mécanisme de déterminisme du sexe dans lequel les femelles sont issues d'œufs fécondés et sont donc diploïdes (deux jeux de chromosomes) et les mâles sont issus d'œufs non fécondés et sont donc haploïdes (un jeu de chromosomes). C'est notamment le cas des bourdons.

Inquilinisme (« *inquilinism* ») **– espèce inquiline** (ou espèce « coucou », « *cuckoo species* »)). Forme de parasitisme où l'espèce parasite le nid d'une colonie d'une autre espèce, appelée espèce hôte. L'espèce pond ses œufs dans le nid de la colonie qui s'occupe alors d'élever ses jeunes à sa place. Chez les bourdons, les espèces inquilines appartiennent (en majorité) au sous-genre *Psithyrus*. Voir le point 4.2. de l'Introduction générale pour plus de détails.

IPBES (« *Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services* »). Plateforme scientifique et politique intergouvernementale sur la biodiversité et les services écosystémiques. Groupe international de scientifiques créé en 2012 pour étudier les services écosystémiques et leur dynamique dans le contexte des changements environnementaux globaux. Similaire à l'IPCC pour le climat. Voir le point 3 de l'Introduction générale et le site « www.ipbes.net ».

IPCC (« *Intergovernmental Panel on Climate Change* ») ou GIEC pour « Groupe intergouvernemental d'étude du climat ». Groupe international de scientifiques créé en 1988 pour étudier les implications et les conséquences des activités humaines sur les changements climatiques. Voir le site « www.ipcc.ch ».

IUCN (« *International Union for Conservation of Nature* »). Union internationale pour la conservation de la nature. Organisation internationale créée en 1948 et regroupant plus de 10 000 experts qui fournit des informations sur l'état de la nature et des ressources naturelles dans le monde et sur les mesures pour les préserver. Voir le site « www.iucn.org ».

Maxent. Algorithme utilisé pour la modélisation de distribution d'espèce qui utilise la méthode d'entropie maximum (« *Maximum Entropy* »). Voir une explication détaillée dans Elith *et al.* (2011) et une explication du logiciel Maxent sur le site « www.biodiversityinformatics.amnh.org/open_source/maxent/ » (Phillips *et al*. 2006).

Métapopulation (« *metapopulation* »). Ensemble des populations séparées spatialement et qui interagissent entre elles *via* la dispersion (Hanski & Gilpin 1991).

Modèle de distribution d'espèce (SDM pour « *species distribution models* »). Modèle qui permet de projeter la distribution d'une espèce dans des environnements où les données d'occurrence de l'espèce manquent, en reliant les données d'occurrence disponibles à des prédicteurs environnementaux (Elith *et al.* 2011). Voir le point 4.7. dans le Cadre Méthodologique Général.

Occupation du sol (« *land cover* »). Couverture biophysique du sol. Exemples : forêt, prairie, culture… Voir les Chapitres 3 et 4.

Parcelle (« *patch* »). Aire d'habitat (ou de type d'occupation/utilisation du sol) de structure homogène, avec des frontières discernables. Voir le Chapitre 3.

Phénologie (« *phenology* »). Saisonnalité et chronologie des évènements périodiques rythmant le cycle de vie d'une espèce, comme par exemple l'hibernation, la reproduction, la floraison, etc.

Phénotype (« *phenotype* »). Caractéristiques physiques, physiologiques, biochimiques et comportementales d'un individu qui résultent de l'expression de son génotype (c.-à-d. de ses gènes) dans un environnement donné.

Phéromone (« *pheromone* »). Composé chimique produit par un organisme qui induit la réponse (comportementale, physiologique…) d'un autre organisme de la même espèce, notamment utilisé pour la communication entre individus.

Population (« *population* »). Groupe d'individus de la même espèce qui partagent le même espace au même moment, et qui ont donc une plus forte probabilité de se reproduire entre eux qu'avec les autres individus de la même espèce.

« ***Renter bees*** ». Espèces de bourdon qui nichent dans des cavités préexistantes, sous le sol ou en surface. Il peut s'agir d'un nid souterrain accessible par un petit tunnel, comme un ancien nid de rongeur, ou de cavités dans les arbres, des vieux nids d'oiseaux ou même des nichoirs artificiels (Sladen 1912). Ce mode de nidification est le plus courant et est opposé aux « *Carder bees* ». Voir le point 4.4.2. de l'Introduction et le Chapitre 1.

Réseau écologique (« *ecological network* »). Ensemble de parcelles d'habitat dans un paysage entre lesquels les échanges d'individus sont possibles, et donc pouvant abriter une métapopulation.

Ressource. Substance ou objet consommé ou utilisé par un organisme nécessaire à l'accomplissement de son cycle de vie, y compris sa reproduction. Exemples : eau, nutriment, site de nidification…

Richesse spécifique (« *species richness* »). Nombre d'espèces recensées dans un lieu donné à un moment donné.

Rudéral (« *ruderal* »). Un milieu rudéral est un terrain vague en friche, avec souvent une grande proportion de sol nu, qui peut s'être formé naturellement (*e.g*. glissement de terrain, éboulement, fonte d'un glacier) ou suite à une perturbation anthropique (*e.g*. bords de route, voies de chemin de fer, anciens sites d'extraction, terrils et autres friches industrielles). Similairement, une flore rudérale est une communauté de plantes pionnières, introduites ou indigènes, qui se développent dans les milieux rudéraux.

Services écosystémiques (« *ecosystem services* »). Ensemble des bénéfices matériels et immatériels fournis par les écosystèmes aux populations humaines. Il peut s'agir de services culturels, d'approvisionnement direct de biens, ou de régulation (comme la pollinisation) (TEEB 2010).

Spécialiste. Espèce qui présente une utilisation ou des préférences restreintes en termes de ressources et d'habitat. Opposé à « généraliste ».

Stochasticité. Variation due à des effets aléatoires.

- **Stochasticité démographique :** variations aléatoires endogènes (internes à la biologie d'une espèce) des taux de natalité, de mortalité et de dispersion, constituant une source potentielle de déclin des petites populations.
- **Stochasticité environnementale :** variations aléatoires exogènes (externes à la biologie de l'espèce) des conditions biotiques et abiotiques de l'environnement, qui peuvent entrainer des fluctuations des taux de survie des individus au sein des populations.
- **Stochasticité génétique :** variations aléatoires endogènes (internes à la biologie d'une espèce) des fréquences alléliques et de la diversité génétique au sein des populations. Voir dérive génétique.

Succession écologique. Processus graduel de changement de la composition spécifique et des caractéristiques d'un écosystème au cours du temps, à la suite d'une perturbation d'origine naturelle ou anthropique, jusqu'à atteindre un état stable.

Synergie (« *synergy* »). Interaction de deux facteurs (ex : climat et occupation du sol) qui entraine un effet total plus grand que la somme des effets de ces deux facteurs s'ils agissaient séparément.

Taille effective de population (« *effective population size* »). Taille d'une population exprimée en termes de nombre d'individus participant à la reproduction, en général plus petite que la taille réelle de la population (nombre total d'individus).

Utilisation du sol (« *land use* »). Type d'usage (ou de non-usage) et de gestion anthropiques du sol. Exemples : plantation d'épicéas, pâture, pré de fauche, culture permanente, culture annuelle… Voir le Chapitre 3.

Vortex d'extinction (« *extinction vortex* »). Enchainement de processus démographiques et génétiques entrainant les petites populations vers l'extinction.

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Annexe I

Vray S., Lecocq T., Roberts S.P.M., Rasmont P. 2017.

Endangered by laws: potential consequences of regulations against thistles on bumblebee conservation.

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Endangered by laws: potential consequences of regulations against thistles on bumblebee conservation

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Summary. Many bumblebee (*Bombus*) species are undergoing a strong decline in Europe due to, amongst other things, a decrease of food resources. While leguminous plants (Fabaceae) are considered to be one of the main pollen sources of bumblebees, thistles (Asteraceae tribe Cardueae) have been suggested to be important for male diet. Yet, several European countries apply strict regulations against thistles since they are considered to be one of the principal weeds in agricultural landscapes. Such regulations could impact bumblebee conservation through disruption of male diet and ecology. Here, we assess the male-depending importance of thistles for bumblebee species based on field observations across countries where a legal regulation against thistles is in effect. We ultimately aim to evaluate the potential consequences of these regulations on bumblebee conservation. Our results confirm that most floral visit observations of males occur on thistles (mainly *Cirsium* spp. and *Carduus* spp.) and some species are almost exclusively observed on them. Thistle removal is thus most likely a threat for bumblebees. Therefore, we advocate repealing the thistle removal acts to make way for alternative thistle regulations which reconcile biodiversity conservation and agricultural requirements.

Résumé. Menacés par des lois : les conséquences potentielles des législations d'échardonnage sur la conservation des bourdons. Les bourdons sont en régression en Europe, en partie à cause d'une diminution de leurs ressources alimentaires. Alors que les légumineuses sont considérées comme étant une des principales sources de pollen pour les bourdons, les chardons (Asteraceae, tribu des Cardueae) ont été suggérés comme étant importants pour l'alimentation des mâles. Pourtant, plusieurs pays européens appliquent des règles législatives strictes contre les chardons car ils sont considérés comme étant des « mauvaises herbes » majeures dans le paysage agricole. De telles régulations pourraient avoir un impact sur la conservation des bourdons à travers la perturbation de l'alimentation et de l'écologie des mâles. Ici, nous estimons l'importance des chardons pour les mâles d'espèces de bourdons en se basant sur des observations sur le terrain dans les différents pays où un règlement d'échardonnage est en vigueur. Notre objectif est d'évaluer les conséquences potentielles de ces règlements sur la conservation des bourdons. Nos résultats confirment que la plupart des visites florales des mâles sont observées sur les chardons (principalement *Cirsium* spp. et *Carduus* spp.) et que certaines espèces sont presque exclusivement observées sur ces plantes. La suppression des chardons constitue donc très probablement une menace pour les bourdons. Par conséquent, nous préconisons d'abroger les lois d'échardonnage pour faire place à des réglementations alternatives qui concilient la conservation de la biodiversité et les besoins agricoles.

Keywords: Bombus; Cardueae; Carduus; Cirsium; floral resources; pollinator conservation; weed control legislation

Almost 90% of all flowering plant species are pollinated by animals (Ollerton et al. 2011). In cold and temperate regions of the Northern Hemisphere, the social species group of bumblebees (*Bombus* spp.) is the main pollinating agent (Heinrich 1979). However, many species are experiencing a strong population decline and range contraction fostered by landscape modifications and fragmentation, intensive use of agrochemicals, pathogen infections, competition with alien species, climate change, reduction in floral resources, and interactions between all these factors (Goulson et al. 2005). Conservation actions have been developed to ensure bumblebee survival and to avoid a major disruption of pollination service. One strategy aims to mitigate the current qualitative and quantitative reduction in floral resources (e.g. Carvell et al. 2007; Kleijn & Raemakers 2008; Scheper et al. 2014) because bumblebees require a long-term flowering and a large diversity in plant species (Persson & Smith 2013; Rundlöf et al. 2014). Determining the most important plants for bumblebee diet and ecology is therefore essential to plan efficient conservation management.

Several studies based on field observations have pointed out that leguminous plants (Fabaceae) are among the main pollen sources for bumblebees, especially for scarcer

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Table 1. National regulations requiring the control of thistles in the European Union.

Countries	Period	Species concerned	Regulation's name /source
Belgium	1987 to present	*Carduus crispus* *Cirsium arvense* *Cirsium palustre* *Cirsium vulgare*	"*Arrêté royal du 19 novembre 1987 relatif à la lutte contre les organismes nuisibles aux végétaux et aux produits végétaux*." (Crémer et al. 2008)
France	1994 to present	*Cirsium arvense*	*"Article L383 du Code Civil"*; and subsequent local legislations (i.e. " *arrêtés préfectoraux/municipaux"*)
Netherlands (only provinces Friesland, Utrecht, Noord-Holland, Zeeland, Zuid-Holland, and Noord-Brabant)	1950 to present	Varies per province, usually: *Cirsium arvense* *Cirsium palustre* *Cirsium vulgare*	Local legislations (i.e. "*Distelverordening (bestrijdingsplicht)*" and "*Algemene Plaatselijke Verordening (APV)*")
United Kingdom	1959 to present	*Cirsium arvense* *Cirsium vulgare*	"Weeds Act 1959"

species (Rasmont & Mersch 1988; Goulson et al. 2005 2008). However, most of these assessments consider only the worker caste. Since workers are often the most abundant caste during field studies, floral preferences of other castes could be blurred. Yet, other caste requirements are thought to be different (Goulson 2010). For instance, some studies have suggested the importance of thistles (Asteraceae tribe Cardueae; Tree of Life Web Project 2009) for male diet at regional scales (e.g. Croxton et al. 2002; Pywell et al. 2005; Carvell et al. 2006). Yet, several European countries apply strict legal regulations against thistles, aimed at curbing flowering, development and seed dispersal since they are considered to be noxious weeds in the agricultural landscape (Table 1). Under the assumption of the importance of thistles for bumblebees, such regulations could impact bumblebee conservation through disruption of male diet and ecology.

Here, we assess the importance of thistles for bumblebee males across countries with regulations against thistles by using field observations. We ultimately aim to evaluate the potential consequences of regulations against thistles on bumblebee conservation.

Methods

Plant systematics and definition of "thistles"

We defined thistles as all species included in Cardueae (synonym: Cynareae); a monophyletic worldwide tribe in the Asteraceae family. It contains 2400 species in 73 genera (Barres et al. 2013). For historic reasons, we followed the broad interpretation of Scrophulariaceae s.l. as used in *Flora Europaea* Vol. 3 (Tutin et al. 1972).

Database and description of the dataset

We used opportunistic data consisting of 88,974 field observations of bumblebee (only observations of individuals feeding on flowers) which are recorded in the database *Banque de Données Fauniques de Gembloux et Mons* (Rasmont & Iserbyt 2013; Rasmont et al. 2015). For each observation, we looked at: (i) the plant species visited; (ii) the location; (iii) the date; and (iv) the visiting bumblebee species. All observations were performed in countries with a regulation against thistles (Table 1), with a proportion of 61% in France, 28% in Belgium, 11% in UK and 0.05% in the Netherlands. Observations were not fully distributed evenly across countries but were gathered in several regions (see the spatial distribution map in Figure 1, created using ArcGIS 10 software, ESRI, Redlands, CA, USA, www.esri.com.). Observations were carried out from 1878 to 2015 and more than three-quarters (78%) were in summer (11% in June, 49% in July, and 18% in August).

Assessing the importance of thistles for bumblebees

We assessed the importance of thistles at *Bombus* generic and specific levels.

At the generic level, we compared for each caste the number of observations on flower at three plant taxonomic levels: between (i) plant families (n = 88,974 observations); (ii) Asteraceae tribes (n = 15,746 observations); and (iii) Cardueae genera (n = 14,110 observations). Visit preferences between castes to different plant families were characterized using correspondence analysis (CA; 'FactoMineR' package) on the contingency table [34 plant families × 3 castes], only considering the plant families for which we had at least 50 observations (all castes combined). Moreover, since our data did not follow a normal distribution (Shapiro–Wilk test), differences in floral preferences between plant taxonomic groups were examined with nonparametric tests (two-sample Wilcoxon sign-rank tests) for each caste. All statistical analyses were done using R version 3.3.1 (R Development Core Team 2016).

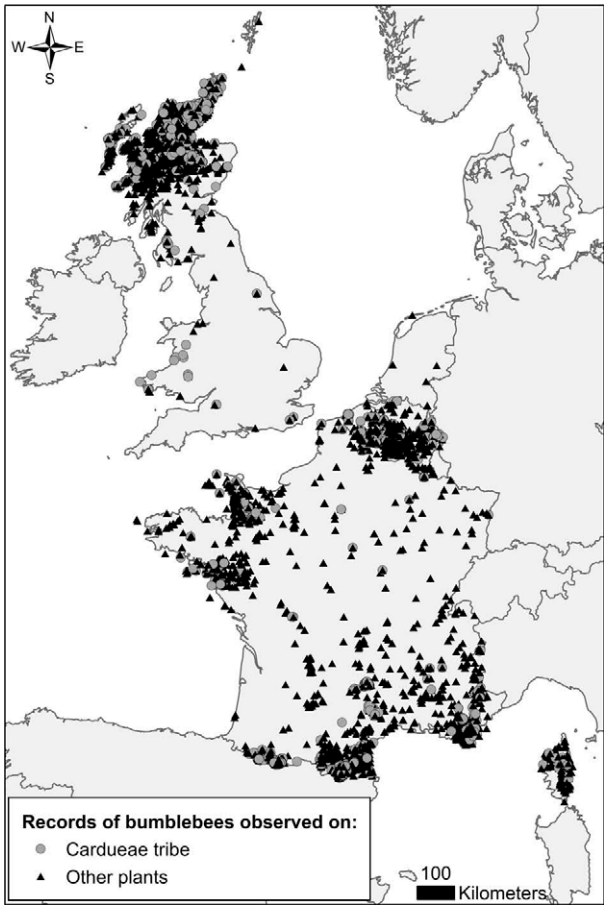

Figure 1. Spatial distribution of the bumblebee observations feeding on flower in the four countries affected by thistle regulations (Belgium, France, the Netherlands and UK).

At the *Bombus* species level (20,567 queen observations, 15,007 male observations and 38,892 worker observations), we compared for each caste the relative number of observations on the Cardueae tribe.

Thereafter, we assessed more specifically the consequences of national regulations against thistles by comparing the number of individuals from each *Bombus* species feeding on the four thistle species affected by national regulations ($n = 3016$ observations): *Carduus crispus* L., *Cirsium arvense* (L.) Scop. (Figure 2(a)), *Cirsium palustre* (L.) Scop. (Figure 2 (b)), and *Cirsium vulgare* (Savi) Ten. (Table 1).

Results

Bumblebees' floral preferences and importance of thistles

Our CA showed differences in floral visit preferences between castes (Figure 3). The total inertia contained in the contingency table [34 plant families × 3 castes] was fully retained in two dimensions (respectively 79.93% for Axis 1 and 20.07% for Axis 2). The first dimension (Axis 1) was mainly defined by three plant families: Solanaceae (39.79%), Asteraceae (16.89%), and Brassicaceae (15.48%), and by two castes: queens (68.45%) and males (21.98%). The second dimension (Axis 2) was mainly explained by Fabaceae (28.59%), Asteraceae (26.81%) and Scrophulariaceae (11.36%), and for the castes by males (57.86%) and workers (38.14%). The global pattern presented by the CA showed that, among the five most visited plant families, Asteraceae was highly associated with male observations (Figure 3). Focus on percentages of observations (Figure 4(a)) and Wilcoxon sign-rank tests confirmed that males were significantly more observed on Asteraceae than on other families

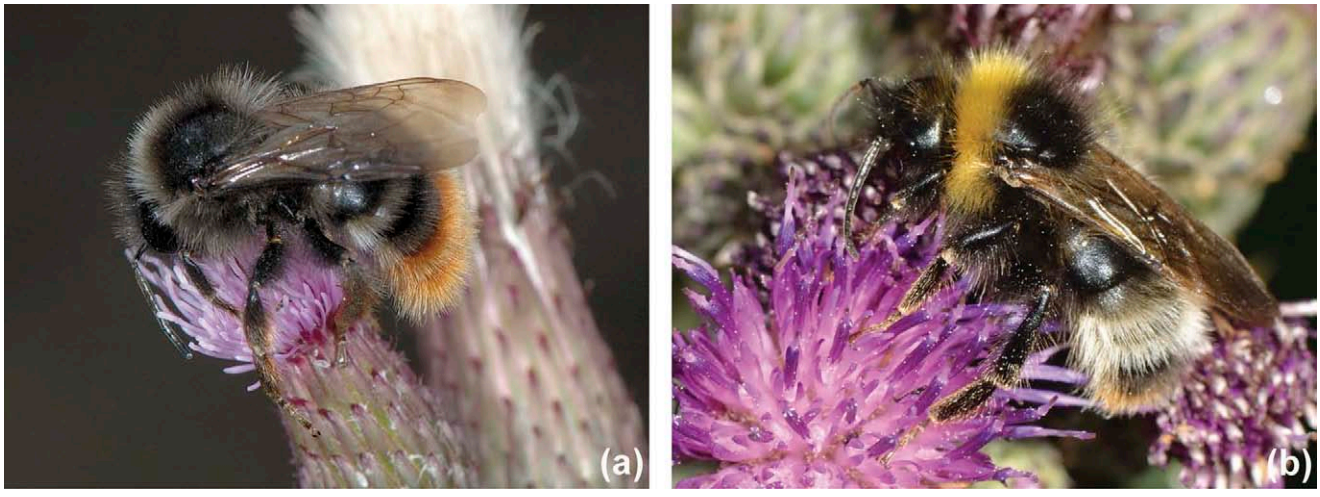

Figure 2. (a) *Bombus rupestris* male on *Cirsium arvense*, and (b) *Bombus sylvestris* male on *Cirsium palustre* (Photos: J.-M. Michalowski).

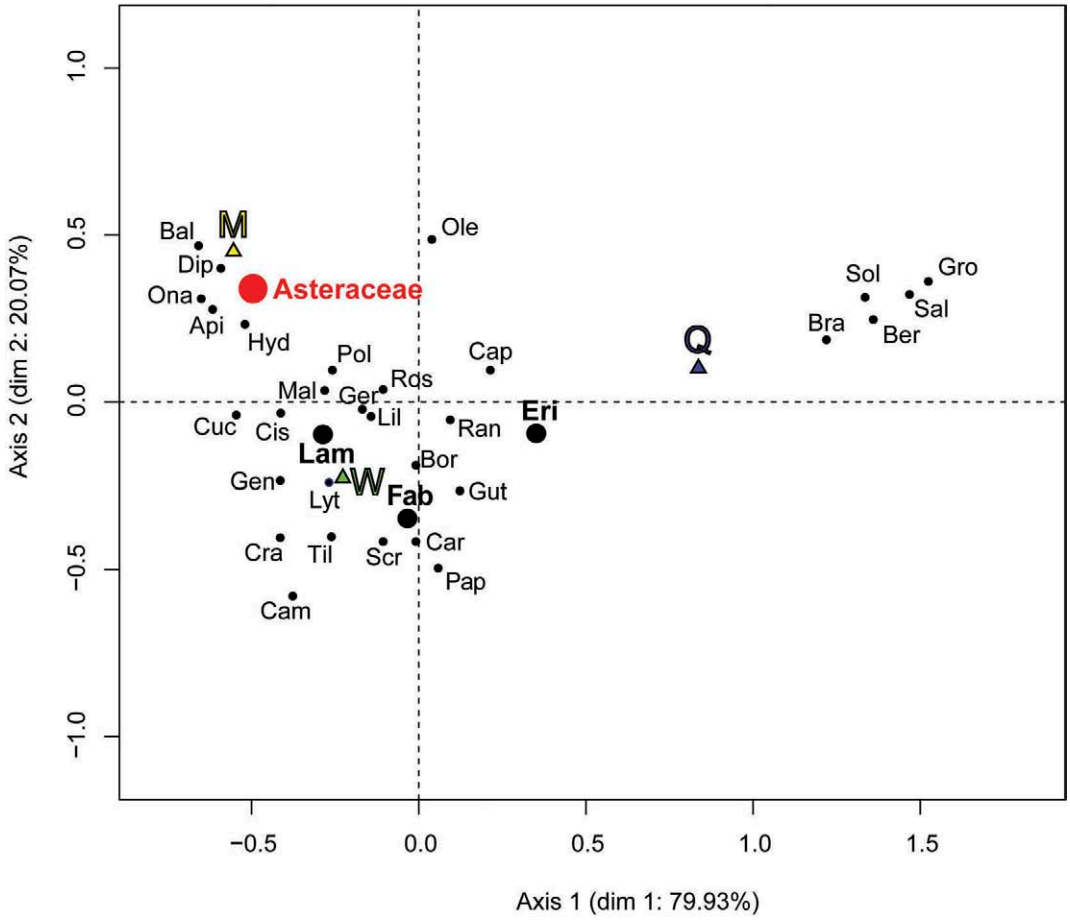

Figure 3. Correspondence analysis factor map obtained from the contingency table of the three castes (triangles) and the 34 plant families for which we had more than 50 observations (points). Bigger points are the four plant families with more than 9000 observations, Asteraceae being the most visited family with 15,015 observations. M = males (14,874 obs.); Q = queens (20,326 obs.); W = workers (38,579 obs.); Api = Apiaceae (1493 obs.); Bal = Balsaminaceae (52 obs.); Ber = Berberidaceae (74 obs.); Bor = Boraginaceae (2642 obs.); Bra = Brassicaceae (2163 obs.); Cam = Campanulaceae (747 obs.); Cap = Caprifoliaceae (74 obs.); Car = Caryophyllaceae (114 obs.); Cis = Cistaceae (184 obs.); Cra = Crassulaceae (113 obs.); Cuc = Cucurbitaceae (320 obs.); Dip = Dipsacaceae (1223 obs.); **Eri = Ericaceae (9570 obs.); Fab = Fabaceae (12,556 obs.)**; Gen = Gentianaceae (436 obs.); Ger = Geraniaceae (227 obs.); Gro = Grossulariaceae (485 obs.); Gut = Guttiferae (176 obs.); Hyd = Hydrophyllaceae (443 obs.); **Lam = Lamiaceae (9624 obs.)**; Lil = Liliaceae (618 obs.); Lyt = Lythraceae (142 obs.); Mal = Malvaceae (121 obs.); Ole = Oleaceae (64 obs.); Ona = Onagraceae (1533 obs.); Pap = Papaveraceae (149 obs.); Pol = Polygonaceae (52 obs.); Ran = Ranunculaceae (2430 obs.); Ros = Rosaceae (2386 obs.); Sal = Salicaceae (359 obs.); Scr = Scrophulariaceae (3434 obs.); Sol = Solanaceae (4643 obs.); Til = Tiliaceae (117 obs.).

(41% of the 15,007 male observations; Wilcoxon sign-rank test, W = 1,324,400, p-value < 0.01). Queens were mainly observed on Solanaceae, Ericaceae, and Fabaceae families (respectively 20, 19 and 14% of the 20,567 queen observations; only 7% on Asteraceae) and workers on Fabaceae, Asteraceae and Lamiaceae families (respectively 22, 19 and 14% of the 38,892 worker observations). The surprisingly high percentage of queens recorded on Solanaceae is explained by observations of *Bombus terrestris* queens on *Salpichroa origanifolia* (Lam.) Baill. during September and October (4082 of the 4123 queens' observations on Solanaceae). Among observations on Asteraceae, the Cardueae tribe was significantly more visited than other tribes by males (90% of the 5947 males' observations, Wilcoxon sign-rank test, W = 84,052, p-value < 0.001) and by workers (92% of the 7413 workers' observations, Wilcoxon sign-rank test, W = 70,105, p-value < 0.001), and recorded 79% of the 1446 queens (Figure 4(b)). Among observations on Cardueae, 61% of the 1112 queens were observed on *Cirsium* spp., 22% on *Centaurea* spp. and 13% on *Carduus* spp. The 4722 males' observations and the 6738 workers' observations were quite equitably distributed among the three genera (respectively 36 and 35% for *Carduus*, 30 and 32% for *Centaurea*, and 29% both for *Cirsium*; Figure 4(c)).

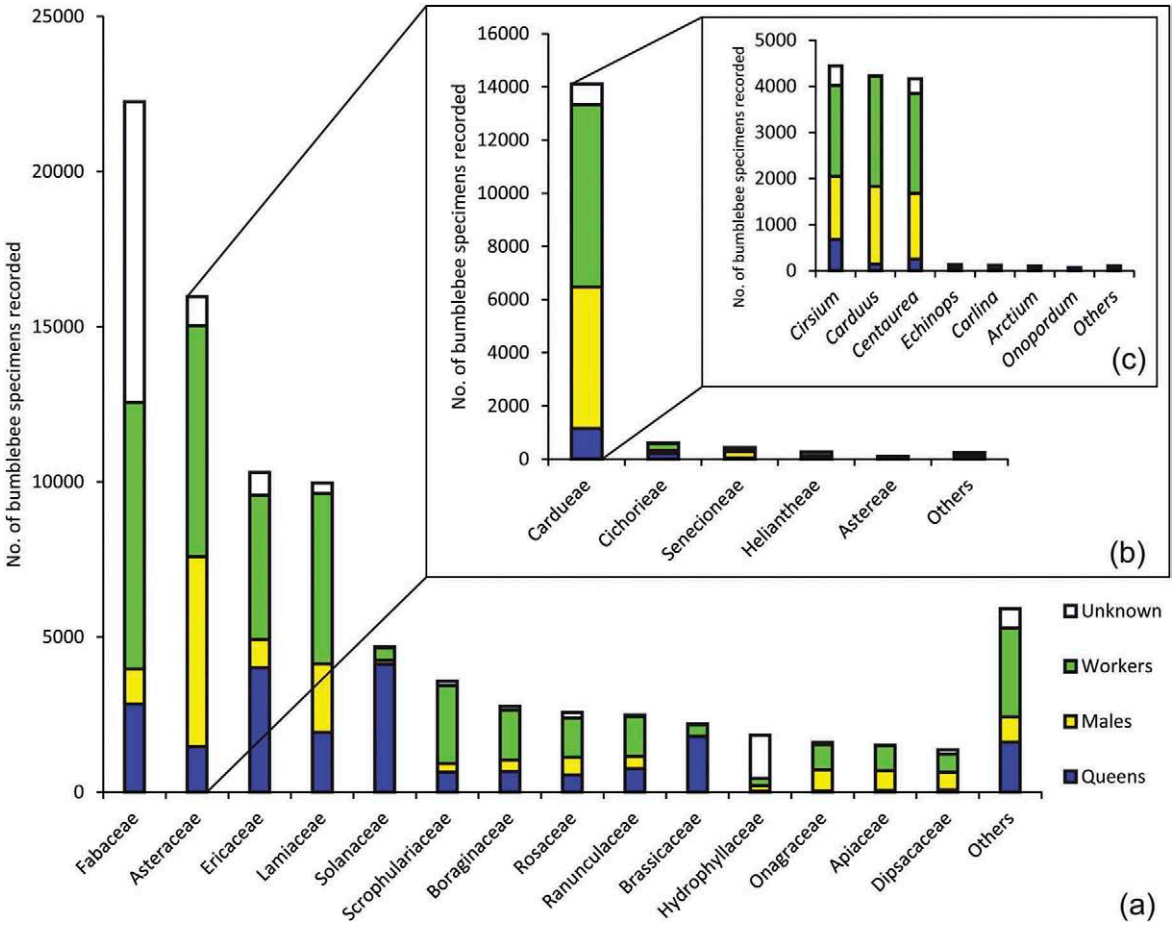

Figure 4. Number of bumblebee specimens recorded on different (a) plant families (all bumblebee species combined; n = 88,974 specimens), (b) tribes of the Asteraceae family (all bumblebee species combined; n = 15,746 specimens), and (c) genera of the Cardueae tribe (all bumblebee species combined; n = 13,353 specimens). "Others" are (a) 79 plant families with less than 800 observations each totalling 5912 observations, (b) 10 tribes of Asteraceae with less than 70 observations each totalling 240 observations, and (c) eight genera of Cardueae with less than 40 observations each, totalling 100 observations.

Bumblebee species most observed on thistles

Regarding the bumblebee specific richness, a total of 43 species was recorded on Cardueae (of the 45 species, all plant species included). For several species, more than half of recorded visits were to this tribe. (Table 2, Figure 5(a)). The largest numbers, in descending order were: *Bombus veteranus* (95% of the observations), *B. quadricolor* (61%), *B. mucidus* (59%), *B. mesomelas* (56%) and *B. norvegicus* (52%; Figure 5(a)).

Moreover, comparative analyses between castes show that many species have one or two castes which are very often observed on Cardueae. *Bombus veteranus* males and workers were almost exclusively recorded on this tribe (respectively 98% and 96% of the observations). Males of *B. mucidus* (87%), *B. mesomelas* (86%), *B. confusus* (85%), *B. mendax* (81%), *B. lapidarius* (73%), *B. quadricolor* (70%) and *B. sylvarum* (70%) were also commonly seen on Cardueae (Figure 5(a)).

Importance of the four thistle species included in the regulations

Thirty-six bumblebee species were observed on the four Cardueae species included in the regulations (see Table S1 in Supporting Information), with the two most visited being *Cirsium palustre* and *C. vulgare*. The most frequently observed bumblebee species on these four plants were *B. norvegicus, B. distinguendus, B. campestris, B. quadricolor, B. soroeensis, B. bohemicus* and *B. sylvestris* (respectively 19, 17, 16, 15, 12, 11 and 10% of the observations; Figure 5(b)). 16% of all the *B. norvegicus* specimens, 13% of all the *B. quadricolor* specimens and 11% of all the *B. soroeensis* specimens were observed on the single species *Cirsium palustre*, while

Table 2. Total number of specimens recorded on all plant species and number of specimens recorded on a plant species of the Cardueae tribe. Q = queen; M = male; W = worker; U = unknown. *Bombus* sp. are *Bombus* unspecified specimens in the database.

	Total number of specimens					Number of specimens observed on a Cardueae				
Bombus species	Q	M	W	U	Total	Q	M	W	U	Total
All *Bombus* species	20,567	15,007	38,892	14,509	88,974	1145	5334	6851	783	14,113
Bombus sp.	38	53	185	152	428	2	9	72	9	92
B. alpinus (L.)	4	3	11	3	21	–	–	–	–	–
B. argillaceus (Scopoli)	12	23	89	1	125	–	2	25	–	27
B. barbutellus (Kirby)	26	118	–	2	146	4	25	–	–	29
B. bohemicus Seild	249	1164	–	25	1438	20	546	–	8	574
B. brodmannicus Vogt	36	–	29	2	67	–	–	1	–	1
B. campestris (Panzer)	31	164	–	2	197	7	74	–	–	81
B. confusus Schenk	21	81	92	–	194	3	69	18	–	90
B. cryptarum (Fabricius)	528	98	235	1	862	2	56	16	–	74
B. cullumanus (Kirby)	14	7	34	–	55	3	1	15	–	19
B. distinguendus Morawitz	226	77	265	1564	2132	30	34	70	556	690
B. flavidus Eversmann	5	122	–	1	128	4	45	–	1	50
B. gerstaeckeri Morawitz	239	55	112	3	409	–	–	–	–	–
B. hortorum (L.)	1051	661	1573	1357	4642	31	103	144	30	308
B. humilis Illiger	306	148	1300	1	1755	10	77	263	–	350
B. hypnorum (L.)	90	88	274	100	552	4	21	18	4	47
B. jonellus (Kirby)	766	104	319	334	1523	16	16	13	2	47
B. lapidarius (L.)	1056	1237	3204	477	5974	115	907	905	24	1951
B. lucorum (L.)	1939	1722	3270	284	7215	31	219	215	17	482
B. magnus Vogt	431	72	263	76	842	19	–	20	13	52
B. mendax Gerstaecker	44	42	359	1	446	4	34	22	–	60
B. mesomelas Gerstaecker	121	121	1058	17	1317	30	104	608	2	744
B. mocsaryi Kriechbaumer	4	–	13	–	17	2	–	13	–	15
B. monticola Smith	179	566	2357	213	3315	2	72	58	–	132
B. mucidus Gerstaecker	33	47	182	2	264	3	41	113	–	157
B. muscorum (L.)	204	81	239	70	594	27	44	101	20	192
B. norvegicus (Sparre Schneider)	2	73	–	–	75	1	38	–	–	39
B. pascuorum (Scopoli)	2695	984	4611	2343	10,633	320	266	468	33	1087
B. perezi (Schulthess-Rechberg)	3	114	–	–	117	–	30	–	–	30
B. pereziellus (Skorikov)	19	6	32	–	57	–	–	1	–	1
B. pomorum (Panzer)	41	16	119	–	176	12	8	56	–	76
B. pratorum (L.)	886	718	1432	216	3252	7	105	131	17	260
B. pyrenaeus Pérez	33	162	833	12	1040	4	82	114	1	201
B. quadricolor (Lepeletier)	31	90	–	1	122	11	63	–	–	74
B. ruderarius (Müller)	450	628	1608	24	2710	22	297	303	7	629
B. ruderatus (Fabricius)	309	513	981	2	1805	4	36	33	–	73
B. rupestris (Fabricius)	79	166	–	6	251	16	92	–	1	109
B. sichelii Radoszkowski	40	75	940	14	1069	7	14	196	2	219
B. soroeensis (Fabricius)	729	445	3671	65	4910	93	157	1321	4	1575
B. subterraneus (L.)	94	155	385	1	635	4	60	50	–	114
B. sylvarum (L.)	220	146	2400	20	2786	25	102	522	8	657
B. sylvestris (Lepeletier)	118	306	–	27	451	15	140	–	4	159
B. terrestris (L.)	6876	2447	4628	7052	21,003	212	448	235	18	913
B. vestalis (Fourcroy)	45	109	–	20	174	1	44	–	2	47
B. veteranus (Fabricius)	69	848	705	–	1622	22	833	678	–	1533
B. wurflenii Radoszkowski	175	151	1074	28	1428	–	20	32	1	53

14% of all the *B. distinguendus* specimens and 10% of all the *B. campestris* were observed on *Cirsium vulgare*.

Discussion

Importance of thistles for bumblebee males

We confirm at a larger geographic scale previous results (e.g. Carvell 2002; Carvell et al. 2006; Goulson et al. 2008) highlighting the large number of bumblebee male floral visits on Cardueae (mainly *Carduus* spp., *Centaurea* spp. and *Cirsium* spp.). This strongly suggests an important role of Cardueae as a nectar resource during late summer when males perform their energy-consuming courtship behaviour (e.g. Croxton et al. 2002; Pywell et al. 2005; Goulson 2010). The lower number of workers and queens foraging on Cardueae could be explained by their need to collect pollen

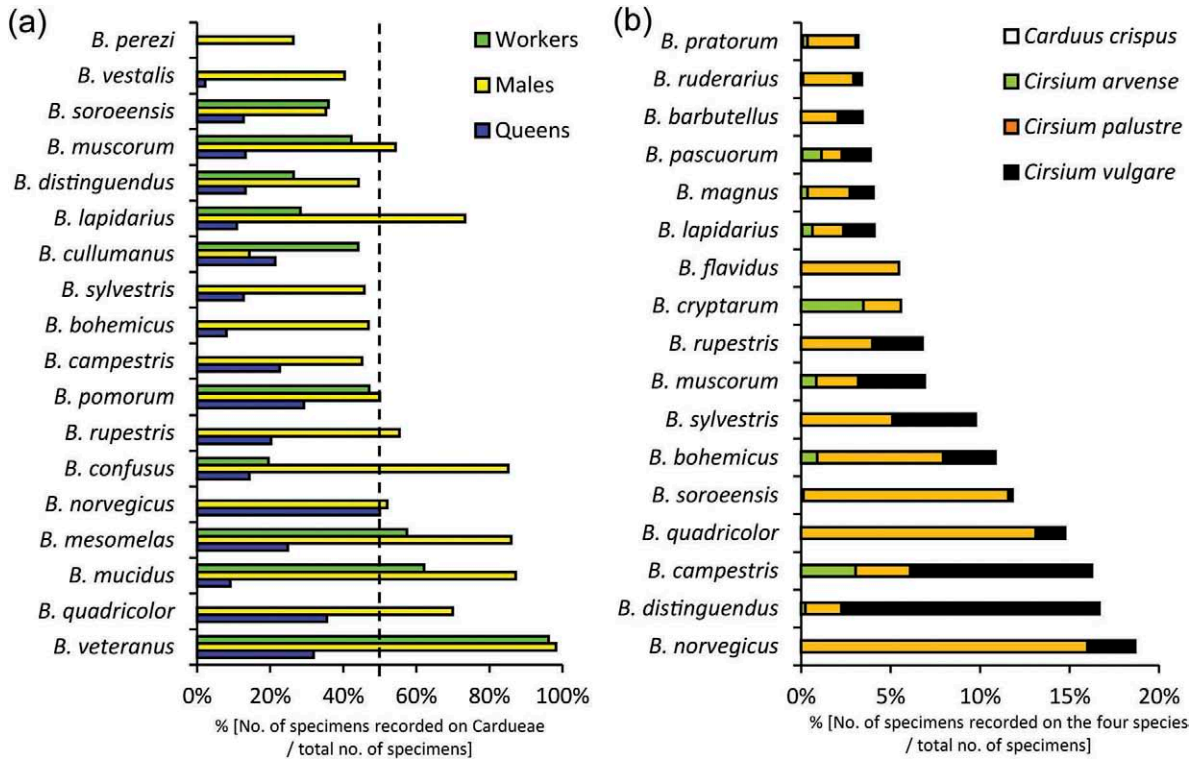

Figure 5. Proportion of bumblebee specimens observed on (a) Cardueae and (b) the four thistle species concerned by the regulations (number on Cardueae = 14,113 specimens; number on the four species = 3016 specimens; total number = 88,974 specimens). Only species with a percentage higher than (a) 25% and (b) 3% (for all castes combined) are presented.

in addition to nectar for the colony development, unlike males which only feed on nectar. Asteraceae pollen is rarely found in queens' and workers' pollen loads (Goulson 2010), probably because it may represent a non-optimal diet for bumblebee colonies. This has recently been shown with the relative unsuitability of *Cirsium* pollen for the colony development of *B. terrestris* (Vanderplanck et al. 2016). It would have been of great interest to perform a comparison between time-periods of the importance of thistles in the foraging choice of bumblebees. However, our dataset does not allow us to perform such a detailed study, because of the very different sampling efforts along time.

Importance of Cardueae has also been pointed out for other insect pollinators (Ricou et al. 2014; Vanbergen et al. 2014). For instance, in the greenways and sown wildflower strips of southern Sweden, *Cirsium* spp. and *Centaurea* spp. were the most visited plant species by eight bumblebee species, but also by 18 butterfly species (Haaland & Gyllin 2010). Cardueae are especially important in areas with intensive agriculture, where pollinators in general have a narrower spectrum and abundance of flowering plants (e.g. *Cirsium* species are a major foraging source where other wildflower species are absent; Haaland & Gyllin 2010). Beside flower visiting insects, many phytophagous species are associated with Cardueae (Freese 1994), while thistle seeds provide food resource for some beetles, birds and rodents (McCallum & Kelly 1990).

Potential consequences of regulations against thistles on bumblebee conservation

The four thistle species affected by regulations against thistles in Belgium, France, the Netherlands and UK (i.e. *Carduus crispus, Cirsium arvense, Cirsium palustre* and *Cirsium vulgare*, Table 1) are commonly visited by male bumblebees (Figure 5(b) and Table S1 in Supplementary Information). The regulations could thus (i) greatly reduce the availability of food resources for, especially, bumblebee males during mating period; and (ii) consequently decrease offspring production, reinforcing the bumblebee decline through the population size decrease and the "extinction vortex" (Gilpin & Soulé 1986; Zayed & Packer 2005). Such a phenomenon is most likely in "Threatened" or "Near Threatened" (Cederberg et al. 2013) species commonly visiting thistle flowers (e.g. *B. confusus, B. distinguendus, B. mucidus, B. veteranus*; Rasmont et al. 2015). Moreover, thistle removal acts may affect several other plant species. Indeed, the correct identification of the species that are legislated against is not always easy for farmers and landowners. For the general public, "thistle" refers to all

flowering plants characterized by leaves with sharp prickles on the margins. This usually implies that all thistles and thistle-like plants are wiped out leading to the decline of other Cardueae species (e.g. in Belgium, *Cirsium dissectum, Cirsium tuberosum, Carduus nutans* and *Cirsium eriophorum*; Crémer et al. 2008). Furthermore, many measures taken to eradicate thistles (e.g. repeated mowing, fertilization and lime to ensure the maintenance of an herbaceous cover, or herbicides) also affect non-Cardueae plants (e.g. Turner et al. 2007; Crémer et al. 2008; Andreasen & Andresen 2011), including Fabaceae, the main food resources for bumblebee females (Figure 4(a)).

Guidelines for policy-makers and conservation organizations

Because of the importance of thistles for bumblebee males (and also for other species ensuring the pollination ecosystem service) there is a premium on repealing the thistle removal acts but also on limiting Cardueae suppression in regions where thistle removal is not mandatory but is still carried out by most farmers (Bond & Grundy 2001; Turner et al. 2007; Melander et al. 2012). Moreover, bumblebees can act as umbrella species for the other organisms associated with thistle and thistle-like plants (butterflies, other bees and hoverflies).

Despite the obvious benefit of minimizing the removal of Cardueae for many pollinators, agricultural policy requires the control of thistles in order to maintain farmland productivity. Indeed, some thistles may weaken pasture and crops due to their allelopathy (i.e. releasing soluble inhibitors that discourage the growth of pasture grasses and legumes; Wardle et al. 1993), their highly effective seed dispersal and their clonal spreading ability (leading to large area monopolization; Kelemen et al. 2014). In grasslands, thistle control can be achieved by prevention techniques that maintain a large floral diversity (e.g. avoiding over-fertilizing, over- or under-grazing, and bare soils) coupled with more specific methods (e.g. alternating mowing and grazing). In crops, thistle control without the use of pesticides is possible by using crop rotation and stubble techniques (Andreasen & Andresen 2011; Nicholls & Altieri 2013). When such actions in croplands and grasslands are not possible, maintaining tolerable densities of thistles in fields could help to support pollinator populations (Nicholls & Altieri 2013). Moreover, thistle removal in other areas should not be promoted because they are very rarely a problem outside farmlands. Therefore, gardens can act as refuges for bees and bumblebees in urban environments and intensively managed farmlands, as already suggested by previous studies (e.g. Ahrné et al. 2009; Samnegård et al. 2011).

In summary, thistle removal regulations are obsolete because they are based on an old agricultural context, and methods of weed control have evolved since their establishment (Andreasen & Andresen 2011). These regulations should therefore be re-evaluated, taking into account the increasing consideration of biodiversity in agricultural landscapes (Nicholls & Altieri 2013) and particularly the high priority given to the wild pollinator conservation (IPBES 2016).

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Supplemental data

Supplemental data for this article can be accessed here.

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Annexe II

Iserbyt S., Vray S., Dendoncker N., Viart S., Rasmont P. 2015.

High-resolution distribution of bumblebees (*Bombus* spp.) in a mountain area marked by agricultural decline.

Annales de la Société Entomologique de France (N.S.) 51:375–391.

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High-resolution distribution of bumblebees (*Bombus* spp.) in a mountain area marked by agricultural decline

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Summary. Since the 1980s, bumblebee species have declined in Europe, partly because of agricultural intensification. Yet little is known about the potential consequences of agricultural decline on bumblebees. In most mountainous areas, agricultural decline from rural exodus is acute and alters landscapes as much as intensive farming. Our study aims at providing a quantitative assessment of agricultural decline through its impact on landscapes, and at characterising bumblebee assemblages associated with land-use types of mountain regions. The studied area (6.2 km^2) belongs to the Eyne's valley in the French Pyrenees, known to host the exceptional number of 33 bumblebee species of the 45 found in continental France. We compare aerial photographs from 1953 and 2000 to quantify agricultural decline. We cross a bumblebee database (2849 observations) with land-use types interpreted from aerial photographs from 2000. Comparison of land-use maps from 1953 and 2000 reveals a strong progression of woodland and urbanised areas, and a decline of agricultural land (pastures and crops), except for hayfields. Spatial correlations between low altitude agro-pastoral structure and the occurrence of bumblebee species shows that bumblebee specific richness is highest in agro-pastoral land-uses (pastures and hayfields) and in the ski area, and poorest in woodland and urbanised areas. Urbanisation and agricultural decline, through increased woodland areas, could lead to a loss of bumblebee diversity in the future. To preserve high bumblebee richness, it is crucial to design measures to maintain open land habitats and the landscape's spatial heterogeneity through agro-pastoral practices.

Résumé. Distribution à haute résolution des bourdons (*Bombus* spp.) dans une zone montagnarde affectée par la déprise agricole. Depuis les années '80, les espèces de bourdons régressent en Europe, en partie à cause de l'intensification agricole. Par contre, on en sait peu sur les conséquences potentielles de la déprise agricole sur les bourdons. Dans la plupart des régions montagnardes, la déprise agricole suite à l'exode rural est aiguë et modifie les paysages autant que l'agriculture intensive. Notre étude vise à fournir une évaluation quantitative de la déprise agricole à travers son impact sur les paysages, et à caractériser les assemblages de bourdons associés aux types d'utilisation du sol des régions montagnardes. La zone étudiée (6.2km^2) appartient à la vallée d'Eyne dans les Pyrénées françaises, connue pour héberger le nombre exceptionnel de 33 espèces de bourdons sur les 45 présentes en France continentale. Nous comparons les photographies aériennes de 1953 et 2000 pour quantifier la déprise agricole. Nous croisons une base de données de bourdons (2849 observations) avec les types d'utilisation du sol interprétés à partir de la photographie aérienne de 2000. La comparaison des cartes d'utilisation du sol de 1953 et 2000 révèle une forte progression des bois et des zones urbanisées, ainsi qu'un recul des terres agricoles (cultures et pâtures) à l'exception des prés de fauche. Les corrélations spatiales entre la structure agro-pastorale de basse altitude et l'occurrence des espèces de bourdons montre que la richesse spécifique des bourdons est plus élevée dans les types d'utilisation du sol agro-pastoraux (pâtures et prés de fauche) et au développement de la station de ski, et plus pauvres dans les bois et les zones urbanisées. L'urbanisation et la déprise agricole, à travers l'augmentation des zones boisées, pourraient conduire à une perte de la diversité des bourdons à l'avenir. Afin de préserver la haute richesse en bourdons, il est crucial de concevoir des mesures visant à maintenir les habitats ouverts et l'hétérogénéité spatiale du paysage à travers des pratiques agro-pastorales.

Keywords: Pyrenees; habitat preference; land use changes; mountain environment; agro-pastoral practices

For several decades, numerous bumblebee species (*Bombus* spp.) have been regressing in Europe (e.g. Rasmont et al. 2005; Williams 2005; Biesmeijer et al. 2006; Williams & Osborne 2009). This decline results among other things from major changes in land use brought about by intensive farming (Burel et al. 1998). Habitat fragmentation through loss of wilderness areas (breeding, nesting, and foraging sites) and loss of their connectivity further contributes to the decline of pollinating insects, including bumblebees (Osborne et al. 1991; Steffan-Dewenter et al. 2006; Le Féon et al. 2010), and the decline of biodiversity as a

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whole (Dauber et al. 2003; Fahrig 2003). Changes in land cover and land use adversely affect bumblebee communities by degrading either nesting or foraging sites, or flower resources (Rasmont & Mersch 1988; Kevan 1999). The first cause of land use change evoked to explain the bumblebee decline is agricultural intensification (e.g. Burel et al. 1998; Carvell 2002; Carvell et al. 2006; Le Féon et al. 2010), resulting in a loss of diversity with a rise in the number of generalist species at the expense of rarer species (Burel et al. 1998).

However, little is known about the potential consequences of agricultural decline on the bumblebee fauna. Some studies have estimated the impact of this phenomenon on plants (Dullinger et al. 2003; Uematsu et al. 2010), beetles (Tocco et al. 2012), butterflies and orthopterans (Uchida & Ushimaru 2014), snails (Labaune & Magnin 2002) and birds (Laiolo et al. 2004; Nikolov 2009; Radović et al. 2013), but to our knowledge no quantitative research has been published on the matter for bumblebees. The concept of agricultural decline describes any cessation of the activity of crop or livestock in lands that are no longer profitable for agriculture, which are sometimes converted into more profitable uses (e.g. forestry, recreation; MacDonald et al. 2000) and sometimes abandoned (land abandonment). In mountain environments of Europe, agricultural decline induced by rural exodus began during the First World War and was then intensified after the Second World War (MacDonald et al. 2000). Arguably, this alters landscapes in a similar way to intensive farming. Consequences of land abandonment include landscape disruption by spontaneous reforestation of formerly open land habitats. Grasslands progressively turns into shrub and, finally, to forest. This leads to a decrease in open land habitats and reduces habitat heterogeneity, which therefore constitutes a threat for biodiversity (Burel 1999; MacDonald et al. 2000; Dirnböck et al. 2003; Laiolo et al. 2004; Bolliger et al. 2007).

The municipality of Eyne, located within the Eastern Pyrenean region, is a particularly interesting spot. It has been known since the eighteenth century for its faunistic and floristic diversity, but it deserves special attention for its bumblebee diversity and species richness (Iserbyt et al. 2008). Many bumblebee species are well represented in Eyne whereas they are regressing in the rest of Europe (for instance *Bombus confusus* Schenck, *B. cullumanus* (Kirby), *B. gerstaeckeri* Morawitz, *B. humilis* Illiger, *B. ruderatus* (Scopoli), *B. subterraneus* (L.) and *B. sylvarum* (L.) (Rasmont et al. 2005; Iserbyt et al. 2008). After a long period of agriculture (37 farms holdings before the First World War), Eyne experienced a long process of agro-pastoral abandonment (Davasse & Galop 1997), with a massive decrease in farm numbers (only three farms left in 2008; Discussion with R. Staats; unreferenced). The utilised agricultural area (UAA) in the municipality of Eyne felt from 235 ha in 2000 to 99 ha in 2010 (AGRESTE-DRAAF Languedoc-Roussillon 2011). Is the agricultural decline a possible explanation for the remarkable species richness observed in Eyne, or is it rather a threat?

This study aims firstly at providing a quantitative assessment of the agricultural decline phenomenon through its impact on landscapes, and secondly at characterising the bumblebee assemblages associated with the different land use types found in Eyne. This will allow evaluation of the potential impact that future agricultural decline could have on the bumblebee fauna.

Material and methods

Studied area

The study was carried out in the low altitude area of Eyne's territory (42°24′36″–42°29′36″N, 2°04′16″–2°08′53″E; Figure 1). It encompasses 6.2 km^2 and ranges in altitude from 1450 m in the vicinity of the village to 1876 m.

The landscape of this zone is currently shaped by three economic activity sectors: forestry, farming and tourism (CRNC 2002; Staats & Mendez 2012). For the last 60 years there has been a steady recolonisation of the bottom of the valley by an increasingly thicker forest, and a resumption of logging in the south-eastern part of the town (ONF 1996; Davasse & Galop 1997). In addition to the agricultural decline resulting in land abandonment, many other reallocations of agricultural land have occurred. The surfaces that were cropped until the second half of the nineteenth century are now grazing lands or hayfields. As for tourism, a ski resort with several ski slopes was set up in 1970. The development of these three economic sectors strongly influences land use dynamics within the studied area (Staats & Mendez 2012).

Land use data

This study is partially based on the aerial photography (1 m ground resolution) missions carried out in July–August 1953 and 2000 by the French Institut Géographique National (IGN) (IGN 1953 F 2250 1/25,000 – P – Partielle (97%); IGN 2000 FD 66 2000 1/25,000). After digitisation and georeferencing of the aerial photographs, we selected an identical study area for 1953 and 2000 (Figure 2a and b). Comparing the aerial photo interpretation of the low altitude area in 1953 and 2000 enables us to assess the vegetation and land use dynamics. As both photographs were taken during the same season, we could distinguish, with aerial photograph interpretation, the urbanised areas and the various ecological systems (cf. land use types in Results) based mainly on the characteristics of the vegetation mat, and delineated them as polygons. In addition, the quality of the results obtained through photo interpretation was assessed and improved with field validation. Field measurements and landscape survey with local farmers and local authorities took place in July 2002. Each measurement was pinpointed using a GPS locator (Magellan SporTrak Pro, Thales Navigation, San Dimas, CA, USA) with an accuracy of 1–10 m.

Faunistic data

Data origin. We extracted most data of Eyne's bumblebees from the *Banque de Données Fauniques de Gembloux et de Mons* (see Rasmont & Iserbyt 2014). Those data result mostly from sample collection carried out by the Laboratory of Zoology of the

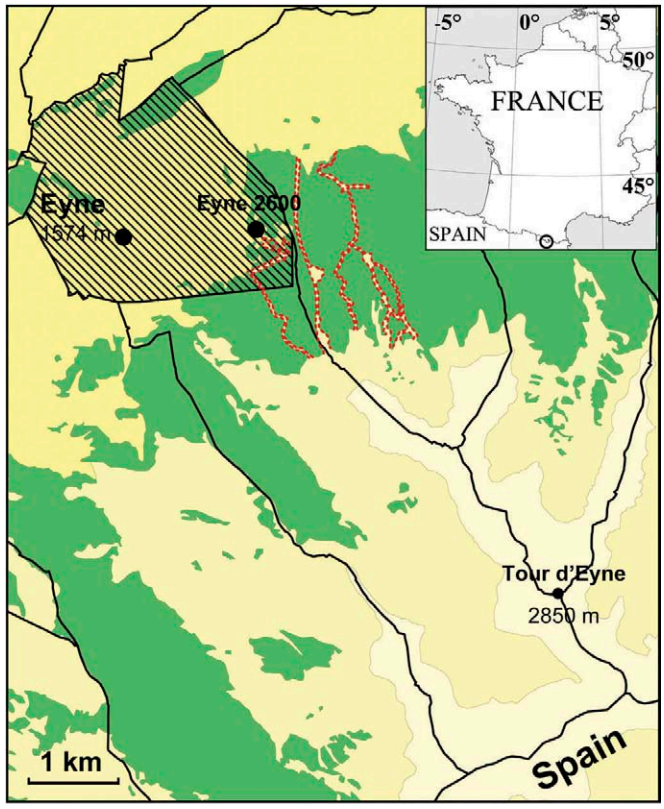

Figure 1. The municipality of Eyne. The shaded zone is the studied area; the dark yellow zone shows the hayfield, crop and pasture area; the green zone is the forested area; the yellow zone shows the subalpine grassland and heathland, and the pale yellow zone the alpine grassland. The red dotted line shows the ski slopes. The village itself is indicated by the black dot on the top left (WGS84, 42° 28′13″N, 02°05′06″E; from IGN 1997; Iserbyt et al. 2008).

University of Mons as part of a faunistic study project (Iserbyt et al. 2008). For analyses of the diversity and richness of bumblebees in each land use type, we only considered GPS-localised data. These were collected during the sampling operations from 1998 to 2008 in the studied area (Figure 1). A total of 2849 observations concerning 29 species and 217 stations were considered.

Data management. Data were managed using the Data-Fauna-Flora software (Barbier et al. 2002). We associated each land use type with its bumblebee species composition by setting up spatially explicit species distribution maps.

Data analysis. Based on CORINE Land Cover data (SOeS – European Union 2011), we assumed that land use did not change between 1998 and 2008 in the municipality of Eyne. We crossed bumblebee information of the period 1998–2008 from the database with land use distribution in 2000. In this way, we obtained a measure of bumblebee diversity and specific richness for each land use type. The presence and abundance of species are related to each land use type.

We computed mathematical indices for diversity and specific richness (Hurlbert expectancy and cumulative rarity index) in order to assess diversity and specific originality respectively. Hurlbert expectancy is the mathematical index used to quantify the specific diversity of a land use type (Hurlbert 1971; Legendre & Legendre 1998). The formula for Hurlbert expectancy used in this work is the simplified version proposed by Rasmont et al. (1990):

$$Es = \Sigma_i \left[1 - ((N - N_i)/N)^S \right] \quad (1)$$

where N_i = number of specimens of the ith species, N = total number of individuals within the station, and S = number of specimens in a random sample. It expresses the expected number of species in a random sample of S specimens (here S = 40, corresponding to the rounded minimum number of specimens observed in the sampled stations).

The originality of the land use type is assessed by the cumulative rarity index proposed by Rasmont et al. (1990):

$$R = \Sigma_i (1/N_i) \quad (2)$$

where N_i = number of individuals of the ith species observed in a reference territory. This index measures (in specimens^{-1}) the richness of each land use type in rare or endemic species compared to a reference territory, here the whole of continental France without the Eyne municipality (Iserbyt 2009).

We compared specific compositions of the various land use types using a nonparametric statistical test: the two-tailed Kolmogorov test (Siegel & Castellan 1988). For each station, we confronted the bumblebee observations (matrix 217 stations × 22 bumblebee species) to the observed land use (matrix 217 stations × seven land use types). In order to measure and describe the relationship between land use types and species' assemblages, we used a linear classification

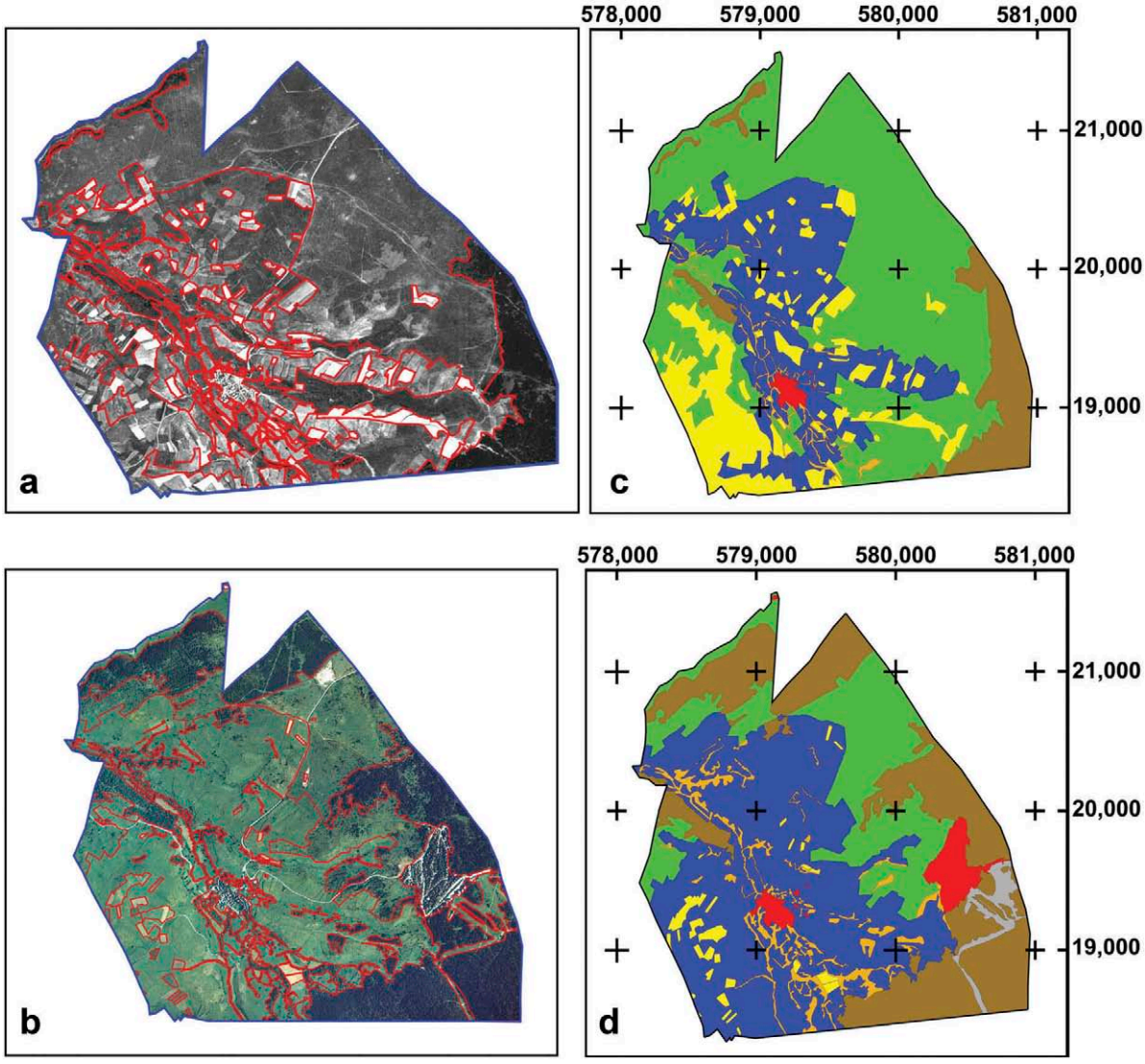

Figure 2. Delimitation of the working zone and land use types in 1953 (a) and 2000 (b). The zone in blue is the studied area, the delimitation of the land use types is shown in red. Identification of the land use types in 1953 (c) and 2000 (d). Yellow corresponds to "crops"; orange colour corresponds to "hedges and banks"; red to "dwellings"; green to "pastures"; blue to "hayfields"; brown to "woodland"; and grey to "pseudo-alpine" land use. The ticks indicate the kilometric grid in the Lambert 3 (south of France) coordinate system (from IGN 1953 and IGN 2000).

technique: canonical correspondence analysis (CCA; Ter Braak & Smilauer 2002). This analysis enables us to calculate the proportion of the bumblebee distribution variance due to land use types. We thus identified the most relevant land use type from a faunistic viewpoint and the relationship between bumblebee species and land use types. CCA enables us to visualise and simplify interactions between the specific distributions and land use types that characterise the stations. The relationship between species and land use types can be represented by projecting species and land use factors onto the canonical axes. Only those species represented by at least five specimens were retained in order to reduce statistical noise in the dataset. CANOCO 4.5 (Ter Braak & Smilauer 2002) was used for this analysis.

Results

Land use classification

By photo interpretation of aerial photographs and field survey, we identified a total of seven simple, consistent land use types: woodlands, crop fields, dwelling areas, hedges and banks, pastures, hayfields, and pseudo-alpine grasslands (Figure 3). The "woodland" type includes reforested zones as well as natural stands and forest edges. The "pseudo-alpine" land cover type is caused directly by human intervention through deforestation of

the subalpine forest to make room for ski slopes (Figure 1). The "crop" type includes all cultivation varieties (e.g. rye, potatoes, and turnips). There is no distinction between "true cropped" land and the field edges, where most of our samplings for this type were done. The "hedges and banks" type includes hedges, bushes, shrubs, isolated trees, banks and embankments. The "pastures" type refers to grasslands grazed by livestock,

Figure 3. Land use types. From left to right and from top to bottom: (a) crops, rye cultivation (1620 m). (b) Hayfields (1750 m). (c) Pastures, grassland (1560 m). (d) Dwellings, the village of Eyne (1570 m). (e) Hedges and banks, near the village (1600 m). (f) Woodland, pinewood of *Pinus uncinata* Ramond ex DC at the entrance to the Eyne valley (1820 m). (g) Pseudo-alpine, ski slopes (1800 m).

whereas the "hayfields" type to grasslands that are mown for hay.

Land use dynamics

Comparing land cover maps drawn in 1953 and 2000 enables us to understand its dynamics (Figure 2c and d). This comparison reveals the following features (Table 1):

- Strong increase of surfaces covered by woody vegetation. The area covered in woodland rose from 54.1 ha in 1953 to 162.0 ha in 2000.
- Strong increase of surfaces devoted to urbanisation and ski area ("dwellings" and "pseudo-alpine"). The dwelled area rose from 3.9 ha in 1953 to 19.7 ha in 2000; the "pseudo-alpine" area reached 10.6 ha in 2000.
- Increase of surfaces covered by shrubby vegetation. "Hedges and banks" trebled their floor space between 1953 (10.6 ha) and 2000 (32.6 ha) especially downslope from the village (Figure 2b and d).
- Decrease of surfaces devoted to agriculture; the "pastures" fell from 310.6 ha in 1953 to 121.2 ha in 2000 and the "crops" fell from 93.4 to 11.4 ha; except for hayfields that increased from 148.8 ha to 264.4 ha.

Diversity and bumblebee richness per land use type

The specific composition of bumblebees remained almost the same before and after 1998 (Table 2). However, data location before 1998 (before the use of GPS) is not sufficient to allow precise linking of bumblebee data with land use data. This association could only be carried out from 1998 and is presented in this paper.

Table 1. Land use types in 1953 and 2000.

Land use types	Surfaces (ha) in 1953	Surfaces (ha) in 2000	Variation (ha)	Tendency
Woodland*	54.1	162.0	+ 107.9	++
Crops**	93.4	11.4	− 82.0	−
Dwellings	3.9	19.7	+ 15.8	+
Hedges and banks	10.6	32.6	+ 22.0	+
Pastures	310.6	121.2	− 189.4	−
Hayfields	148.8	264.4	+ 115.6	++
Pseudo-alpine	—	10.6	+ 10.6	+

Note: The percentage error when calculating the surface areas is estimated at 1%. *Woodland and its edge; **field edges essentially.

The species list associated to land use types varies both in terms of quantity and quality (Table 2). From the species present from 1998 to 2008 in Eyne's low altitude zone, only eight are present in all types, and thus represent a typical assemblage of the bumblebee community found at Eyne's low altitude zone (*B. hortorum*, *B. lucorum*, *B. mesomelas*, *B. pascuorum*, *B. ruderarius*, *B. soroeensis*, *B. subterraneus*, *B. terrestris*; Table 2). The 21 other bumblebee species found at this low altitude zone are rarer or may show more specific ecological preferences, which restricts their habitat to one or two land use types. The proportion of species represented by only one specimen varies considerably according to the land use type. It is very high for the "woodland", "hedges and banks", "crops" and "hayfields" types, with respectively 42.1, 35.7, 31.2 and 28% of the total number of species, compared to other types, i.e. "dwellings", "pastures" and "pseudo-alpine" (respectively 0, 10 and 10%).

The diversity and originality of each land use type based on its bumblebee fauna are presented in Table 3. The diversity and originality indices are very different. The land use type that scores particularly high both in terms of richness and originality is "hayfields", whereas the "dwellings" type is the one showing the lowest scores. The "pastures", "hayfields" and "hedges and banks" types have the highest species richness (Hurlbert expectancy). The "hayfields", "crops" and "woodland" types have a high cumulative rarity index comparable to that of the whole study area. Conversely, the other types show much lower cumulative rarity indices, indicating a more ordinary bumblebee fauna.

Except for "hedges and banks", land use types differ markedly in their specific composition (Table 4) even for the most abundant species of the study area.

The diversity (Hurlbert expectancy) and originality indices (Table 3) on the one hand, and the correlations between land use types (Table 4) on the other hand, emphasise the strong contrast between land use types. They show a marked difference between the urbanised environment ("dwelling" type), which show a weak diversity, and the treeless or shrubless agrarian environments ("hayfields" type), showing a very high density.

The faunistic differences between the various land use types (Table 4) result from the large proportion of singletons, the low number of generalist species in the study area, and hypothetically the biotopographic preferences of some species.

Bumblebee assemblages associated with the different land use types

The biotopographic preferences of many species are difficult to identify. Stations where *B. humilis* was observed (Figure 4b) seem to indicate that this species prefers open

Table 2. Relative proportion of the number of specimens found for each species, for the studied area before and during the 1998–2008 sampling period, and for each land use type during the 1998–2008 period.

Taxa	Studied area (before 1998)	Studied area (1998–2008)	Woodland*	Crops	Dwellings	Hedges and banks	Pastures	Hayfields	Pseudo-alpine
Bombus barbutellus (Kirby) ** [(a)]	0.1 (1)								
Bombus campestris (Panzer) ** [(a)]	0.4 (4)								
Bombus flavidus Eversmann ** [(a)]	2.1 (24)								
Bombus gerstaeckeri Morawitz	1.2 (14)	- (1)				2.0 (1)			
Bombus norvegicus (Sparre Schneider)[(a)]		- (1)	0.7(1)						
Bombus quadricolor (Lepeletier) [(a)]	3.4 (39)	- (1)						0.1 (1)	
Bombus cullumanus (Kirby)	0.3 (3)	0.1 (2)		0.2 (1)				0.1 (1)	
Bombus magnus Vogt		0.1 (3)	0.7(1)					0.1 (1)	0.2 (1)
Bombus ruderatus (Fabricius)	0.2 (2)	0.1 (4)		0.2 (1)			0.5 (2)	0.1 (1)	
Bombus sylvestris (Lepeletier) [(a)]	9.2 (104)	0.1 (4)	0.7 (1)						0.6 (3)
Bombus mucidus Gerstaecker	1.6 (18)	0.2 (6)					0.5 (2)	0.1 (1)	0.6 (3)
Bombus rupestris (Fabricius) [(a)]	2.1 (24)	0.2 (6)		0.2 (1)				0.4 (5)	
Bombus sichelii Radoszkowski	3.0 (34)	0.2 (6)	0.7 (1)				0.3 (1)		0.8 (4)
Bombus mendax Gerstaecker	1.8 (20)	0.2 (7)					1.4 (5)	0.2 (2)	
Bombus bohemicus Seild [(a)]	6.5 (74)	0.3 (10)	0.7 (1)			2.0 (1)		0.3 (3)	0.9 (5)
Bombus hypnorum (L.)	0.3 (3)	0.3 (9)	1.4 (2)			4.1 (2)		0.1 (1)	0.8 (4)
Bombus wurflenii Radoszkowski	3.2 (36)	0.5 (14)					0.5 (2)	0.1 (1)	2.1 (11)
Bombus pratorum (L.)	2.5 (28)	1.1 (31)	5.4 (8)				0.3 (1)	0.9 (10)	2.3 (12)
Bombus confusus Schenk	0.4 (5)	1.3 (36)		0.5 (2)	1.8 (4)		2.7 (10)	1.8 (20)	
Bombus lapidarius (L.)	3.2 (36)	1.8 (51)	0.7 (1)	3.4 (15)			1.6 (6)	2.3 (26)	0.6 (3)
Bombus monticola Smith	0.2 (2)	2.0 (56)	2.0 (3)			2.0 (1)	2.2 (8)	1.6 (18)	4.9 (26)
Bombus pyrenaeus Pérez	6.5 (74)	2.0 (56)	1.4 (2)	0.2 (1)			4.1 (15)	1.2 (13)	4.7 (25)
Bombus terrestris (L.)	0.2 (2)	3.0 (87)	0.7 (1)	3.9 (17)	3.2 (7)	6.1 (3)	9.9 (36)	2.0 (22)	0.2 (1)
Bombus subterraneus (L.)	2.5 (28)	3.9 (112)	0.7 (1)	4.8 (21)	0.9 (2)	2.0 (1)	1.6 (6)	6.1 (68)	2.5 (13)
Bombus pascuorum (Scopoli)	2.2 (25)	4.1 (116)	17.7 (26)	1.1 (5)	13.3 (29)	10.2 (5)	2.1 (8)	1.0 (11)	6.1 (32)
Bombus hortorum (L.)	4.3 (49)	5.1 (145)	5.4 (8)	0.2 (1)	5.5 (12)	6.1 (3)	4.1 (15)	3.9 (43)	12.0 (63)
Bombus mesomelas Gerstaecker	6.9 (78)	5.7 (162)	1.4 (2)	11.9 (52)	5.5 (12)	2.0 (1)	5.2 (19)	5.4 (60)	3.0 (16)
Bombus humilis Illiger	1.1 (13)	8.7 (247)	4.1 (6)	16.1 (70)	14.7 (32)	6.1 (3)	4.1 (15)	10.9 (121)	
Bombus soroeensis (Fabricius)	7.5 (85)	8.9 (254)	8.8 (13)	17.7 (77)	1.4 (3)	6.1 (3)	8.5 (31)	8.4 (93)	6.5 (34)
Bombus sylvarum (L.)	1.0 (11)	11.6 (332)		15.8 (69)	27.5 (60)	12.2 (6)	26.0 (95)	9.2 (102)	
Bombus ruderarius (Müller)	20.4 (231)	19.1 (544)	15.0 (2)	17.7 (77)	22.9 (50)	14.3 (7)	7.4 (27)	19.4 (215)	27.8 (146)
Bombus lucorum (L.)	6.0 (68)	19.2 (546)	32.0 (47)	6.0 (26)	3.2 (7)	24.5 (12)	16.7 (61)	24.3 (269)	23.6 (124)
Total number of specimens	1135	2849	147	436	218	49	365	1108	526
Total number of species	30	29	19	16	11	14	20	25	19

Notes: *Woodland and its edge; **species absent during the 1998–2008 observation period and not taken into account in this study. (a): Exclusively inquiline species. -: species that are present but with low relative proportions. Between brackets, the number of specimens for each species.

Table 3. Estimation of the bumblebee diversity and originality for the studied area (before 1998 and during the 1998–2008 period) and each land use type (during the 1998–2008 period).

	Number of specimens	Number of species	Hurlbert (Number of species expected in a 40 specimens sample)	Cumulative rarity (Specimens^{-1})
Studied area (before 1998)	1135	30	16.2	0.044
Studied area (1998–2008)	2849	29	12.4	0.041
Woodland*	147	19	10.3	0.018
Crops	436	16	9.3	0.018
Dwellings	218	11	8.5	0.005
Hedges and banks	49	14	11.3	0.008
Pastures	365	20	12.3	0.011
Hayfields	1108	25	11.3	0.029
Pseudo-alpine	526	19	10.7	0.011

Note: *Woodland and its edge.

Table 4. Specific composition comparison for the different land use types by a two-tailed Kolmogorov test.

	Woodland	Crops	Dwellings	Hedges and banks	Pastures	Hayfields	Pseudo–alpine
Woodland							
Crops	0.34 (***)						
Dwellings	0.30 (***)	0.11 (NS)					
Hedges and banks	0.18 (NS)	0.19 (NS)	0.20 (NS)				
Pastures	0.36 (***)	0.17 (***)	0.14 (**)	0.21 (*)			
Hayfields	0.19 (***)	0.17 (***)	0.20 (***)	0.07 (NS)	0.25 (***)		
Pseudo-alpine	0.17 (**)	0.30 (***)	0.28 (***)	0.15 (NS)	0.35 (***)	0.14 (***)	

Note: ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$; NS: non-significant.

habitats and particularly the "hayfields" and "crop" types. Stations where *B. pratorum* was observed seem to indicate that this species prefers closed habitats and particularly the "woodland" and "pseudo-alpine" types. Some species seem to have similar affinities for certain land use types. This is the case, for instance, for *B. monticola* (Figure 4e), *B. pyrenaeus* (Figure 6c), *B. sichelii* (Figure 6h) and *B. wurflenii* (Figure 7e), which seem to show affinity for the "pseudo-alpine" type. Stations where, for instance, *B. confusus* (Figure 4a; 11 stations), *B. lucorum* (Figure 4c; 83 stations) or *B. magnus* (Figure 4d; three stations) were present do not allow for a reliable identification of these species' preferences. Superficial examination of the specific distribution maps does not allow determination of the species' biotopographic preferences (Figures 4–7).

Canonical correspondence analysis enables us to calculate the common variance of both matrices [station × species] and [station × land use] (Figure 8a and b respectively). This common variance represents the variation in bumblebee distribution that is determined by land use types. Approximately 11% of the variance in the bumblebee distribution is explained by the seven types. The factors with the highest contribution to the definition of axis 1 are positively related to the degree of closure characterising the land use type analysed (mainly "woodland" and "pseudo-alpine") and negatively related to its degree of openness (mainly "hayfields", "crops" and "pastures"). Axis 2 (Figure 8b) shows an opposition between the treeless and shrubless agrarian environments ("hayfields" and "crops" types) and the other land use types. Projecting the species in the plane defined by the first two canonical axes (69.1% of the variance explained) results in distinct groups of species. These divide (axis 1, Figure 8a) into open habitat species and closed habitat species ("woodland", "hedges and banks" and "pseudo-alpine" types) and split the open habitats (axis 2, Figure 8a) into the treeless and shrubless agrarian types ("crops" and "hayfields") and the other more or less wooded types (Figure 8a). On the basis of these features the species divide into four groups. Group I encompasses many species: *B. bohemicus*, *B. hortorum*, *B. hypnorum*, *B. monticola*, *B. mucidus*, *B. pascuorum*, *B. pratorum*, *B. pyrenaeus*, *B. sichelii* and *B. wurflenii*

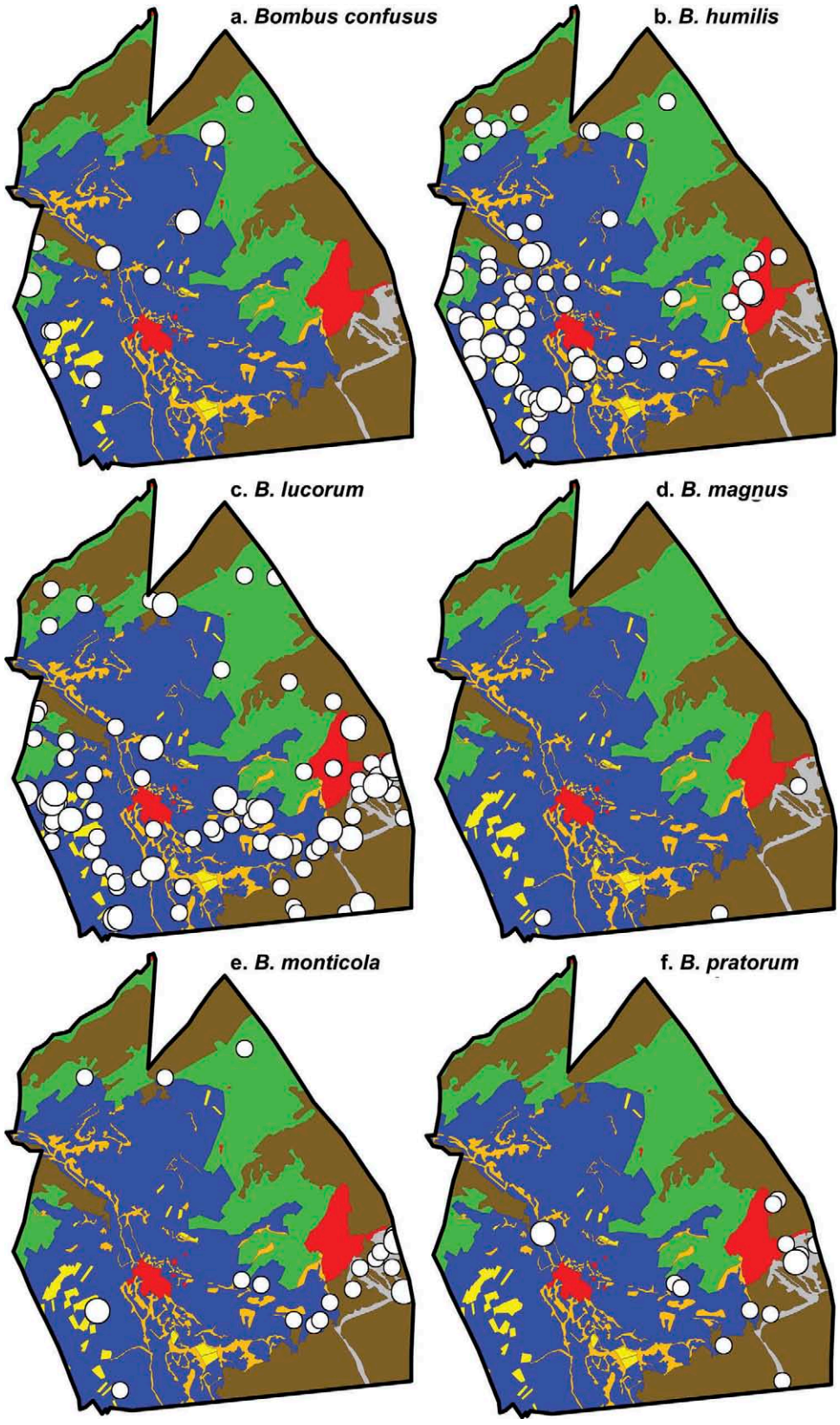

Figure 4. Distribution map of bumblebee species. (a) *Bombus confusus* Schenck (36 specimens, 11 stations). (b) *B. humilis* Illiger (247 specimens, 71 stations). (c) *B. lucorum* (L.) (546 specimens, 83 stations). (d) *B. magnus* Vogt (three specimens, three stations). (e) *B. monticola* Smith (56 specimens, 21 stations). (f) *B. pratorum* (L.) (31 specimens, 14 stations). The data are indicated by dots corresponding to a diameter of either 150 m, when the number of specimens in the station is greater than or equal to five, or a diameter of 75 m, when the number of specimens is less than five.

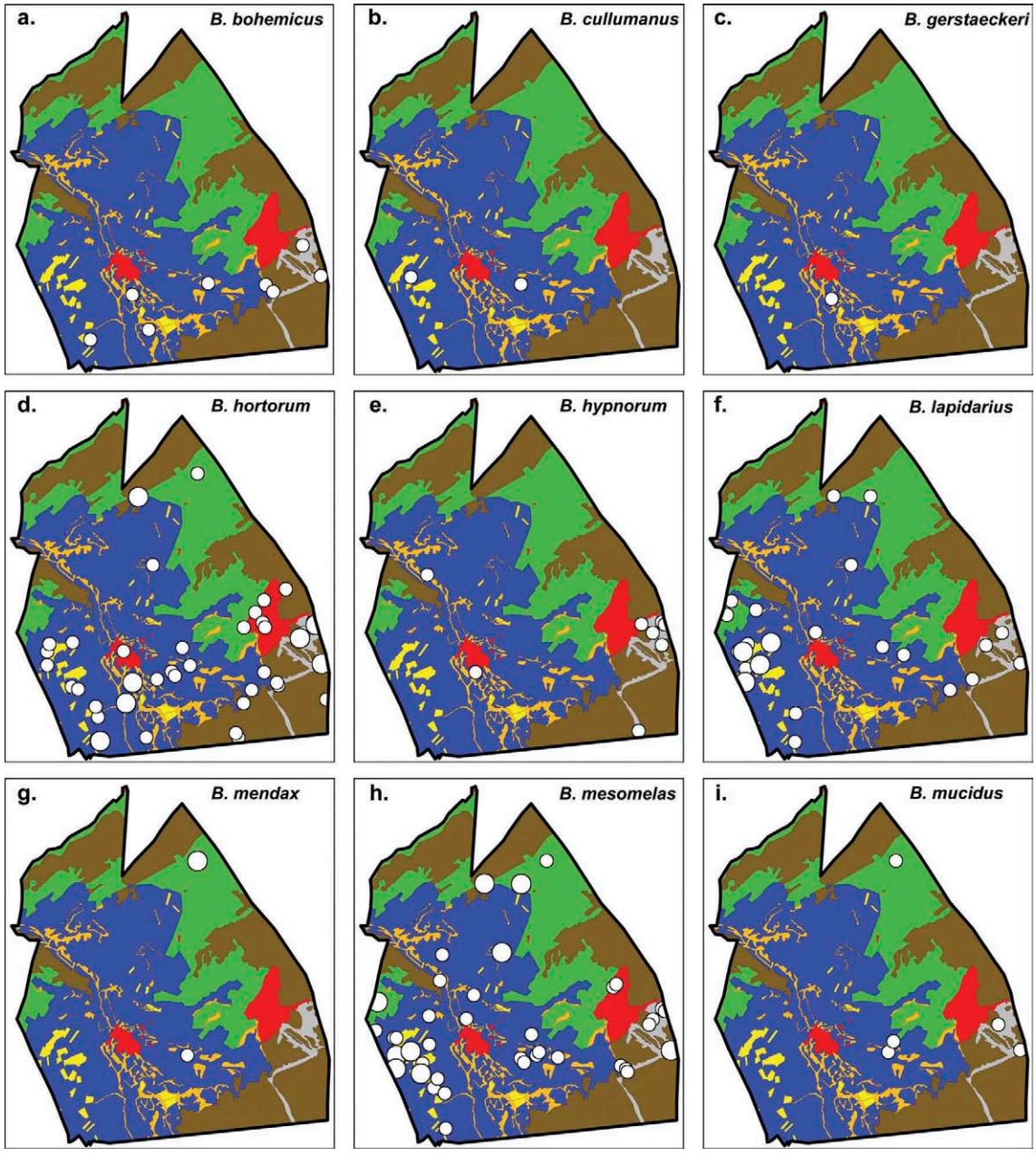

Figure 5. Distribution map of bumblebee species. (a) *Bombus bohemicus* Seidl (10 specimens, eight stations); (b) *B. cullumanus* (Kirby) (two specimens, two stations); (c) *B. gerstaeckeri* Morawitz (one specimen, one station); (d) *B. hortorum* (L.) (145 specimens, 44 stations); (e) *B. hypnorum* (L.) (nine specimens, eight stations); (f) *B. lapidarius* (L.) (51 specimens, 24 stations); (g) *B. mendax* Gerstaecker (seven specimens, two stations); (h) *B. mesomelas* Gerstaecker (162 specimens, 41 stations); (i) *B. mucidus* Gerstaecker (six specimens, five stations). The data are indicated by dots corresponding to a diameter of either 150 m, when the number of specimens in the station is greater than or equal to five, or a diameter of 75 m, when the number of specimens is less than five.

(Figure 8a). This group can be described as showing preference for deforested areas, for skiing facilities ("pseudo-alpine" type) or for areas rich in trees and/or shrubs ("hedges and banks" and "woodland") (Figure 8b). Two groups are characterised by biotopographic preferences for open environment. Group II (*B. confusus*, *B. mendax*, *B. terrestris* and *B. sylvarum*) is particularly linked to urbanised and pasture areas whereas group III (*B. humilis*, *B.*

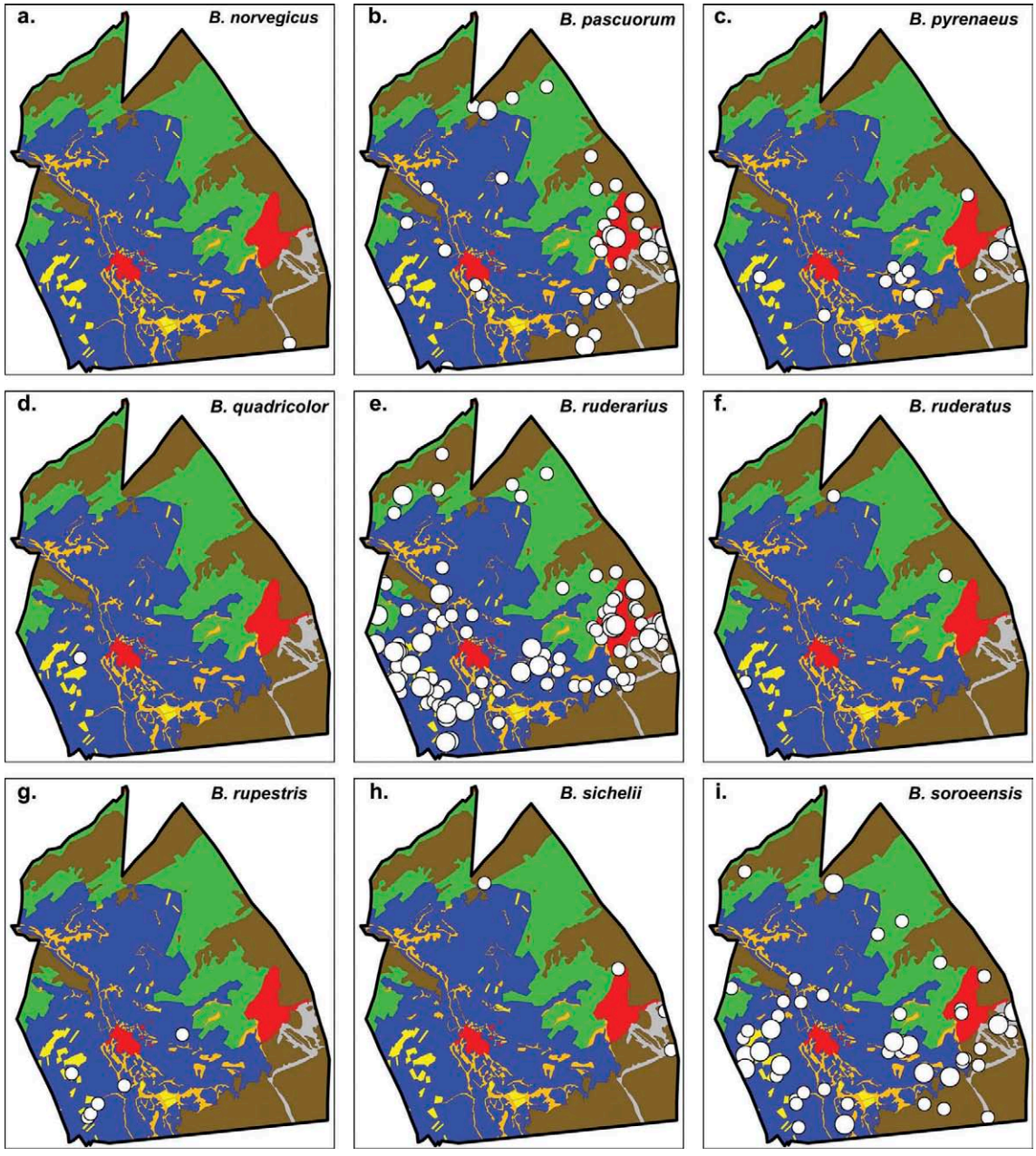

Figure 6. Distribution map of bumblebee species. (a) *Bombus norvegicus* (Sparre Schneider) (one specimen, one station); (b) *B. pascuorum* (Scopoli) (116 specimens. 45 stations); (c) *B. pyrenaeus* Pérez (56 specimens. 17 stations); (d) *B. quadricolor* (Lepeletier) (one specimen, one station); (e) *B. ruderarius* (Müller) (554 specimens. 104 stations); (f) *B. ruderatus* (Scopoli) (four specimens, three stations); (g) *B. rupestris* (Fabricius) (six specimens, six stations); (h) *B. sichelii* Radoszkowski (six specimens, four stations); (i) *B. soroeensis* (Fabricius) (254 specimens. 51 stations). The data are indicated by dots corresponding to a diameter of either 150 m, when the number of specimens in the station is greater than or equal to five, or a diameter of 75 m, when the number of specimens is less than five.

lapidarius, *B. mesomelas*, *B. rupestris*, *B. soroeensis* and *B. subterraneus*) is mainly associated to agrarian areas ("hay-fields" and "crop" types). The most abundant species (*B. lucorum* and *B. ruderarius*, group IV) tend to be generalist and cannot be characterised according to a particular land use type.

Discussion

Land use dynamics

The agricultural decline phenomenon in the Pyrenees has already been demonstrated in the Quérigut district (France, Ariège; Fabre 1977) and in Eyne's valley (Davasse & Galop 1997). As in our study, they observed

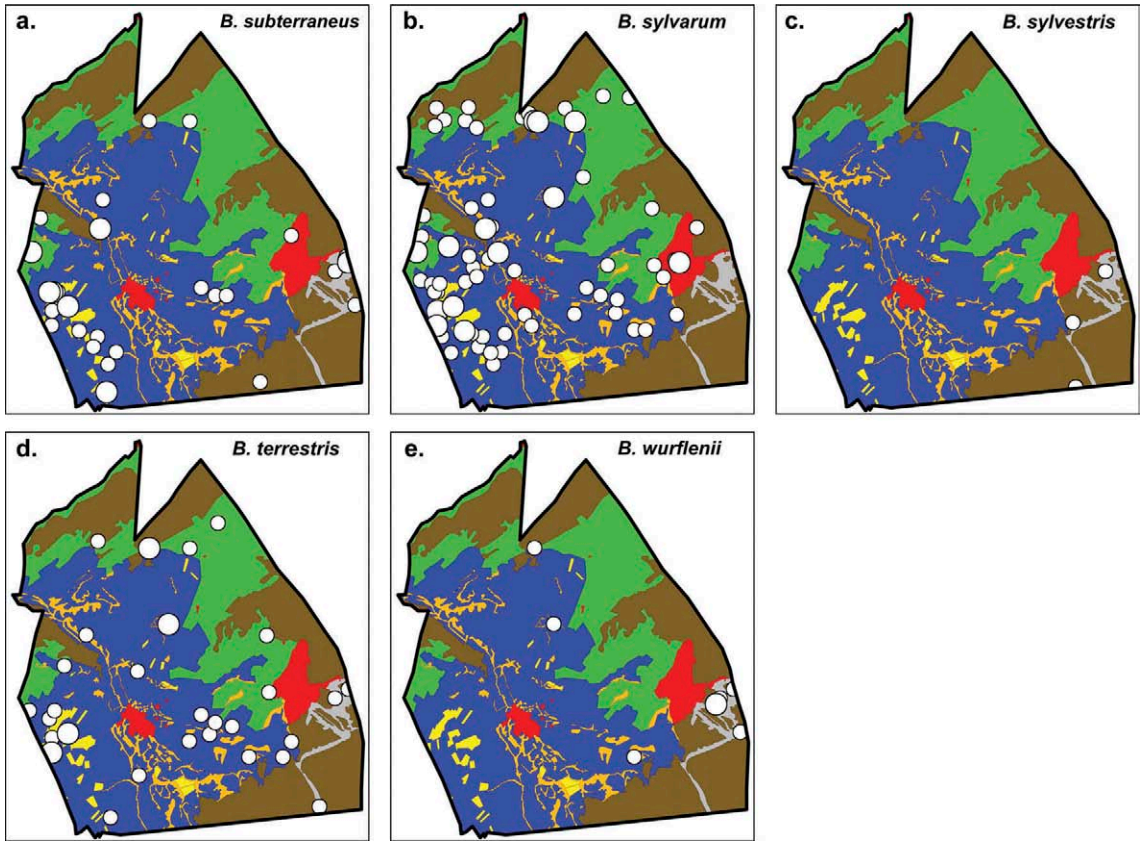

Figure 7. Distribution map of bumblebee species (continuation). (a) *Bombus subterraneus* (L.) (112 specimens, 28 stations); (b) *B. sylvarum* (L.) (332 specimens, 68 stations); (c) *B. sylvestris* (Lepeletier) (four specimens, three stations); (d) *B. terrestris* (L.) (87 specimens, 29 stations); (e) *B. wurflenii* Radoszkowski (14 specimens, seven stations). (See Figure 1d and e for the legend). The data are indicated by dots corresponding to a diameter of either 150 m, when the number of specimens in the station is greater than or equal to five, or a diameter of 75 m, when the number of specimens is less than five.

a continuous trend towards land abandonment and a more extensive use of the remaining agricultural land. Contrary to what takes place in areas undergoing strong agricultural intensification, uncropped plots such as forests, pastures and hedges tend to coalesce. The present agro-pastoral landscape found in Eyne is characterised, among other things, by a dense network of hedges and banks that increases the connectivity between the wooded areas upslope and downslope of the village. Converting the "crops" and "pastures" types into mainly "hayfields" and "woodland" types resulted in a tremendous change in the landscape. The rise of human pressure by urbanisation is clearly noticeable (Figure 2c and d). The dwelling area increased fivefold due to the development of the ski resort and its hamlet "Eyne 2600" (Figure 1). At the same time, ski slopes have been laid out. It is difficult to predict how far this land use type will expand as this is strongly dependent upon economic and touristic contingencies. The current landscape found in Eyne results directly from the overall evolution in land use induced by agricultural decline and the development of the ski resort and its facilities.

Bumblebee assemblages associated with the different land use types

The apparently low value (11%) of the variance in bumblebee distribution explained by the seven land use types could mainly be explained by the inter-annual species abundance variation. We accumulated the species' abundance over 10 years without taking into account the large inter-annual variations that occurred (Iserbyt & Rasmont 2012). Moreover, floral resources, microclimates and altitude are also factors that influence the distribution of bumblebees in this region (Iserbyt et al. 2008; Iserbyt & Rasmont 2012).

The specific richness observed in the "woodland" type may result from the merging of the data pertaining to actual forest with the data concerning its edge. Edges often have a higher index of biodiversity, and so the fact that they are included in "woodland" may overestimate the observed

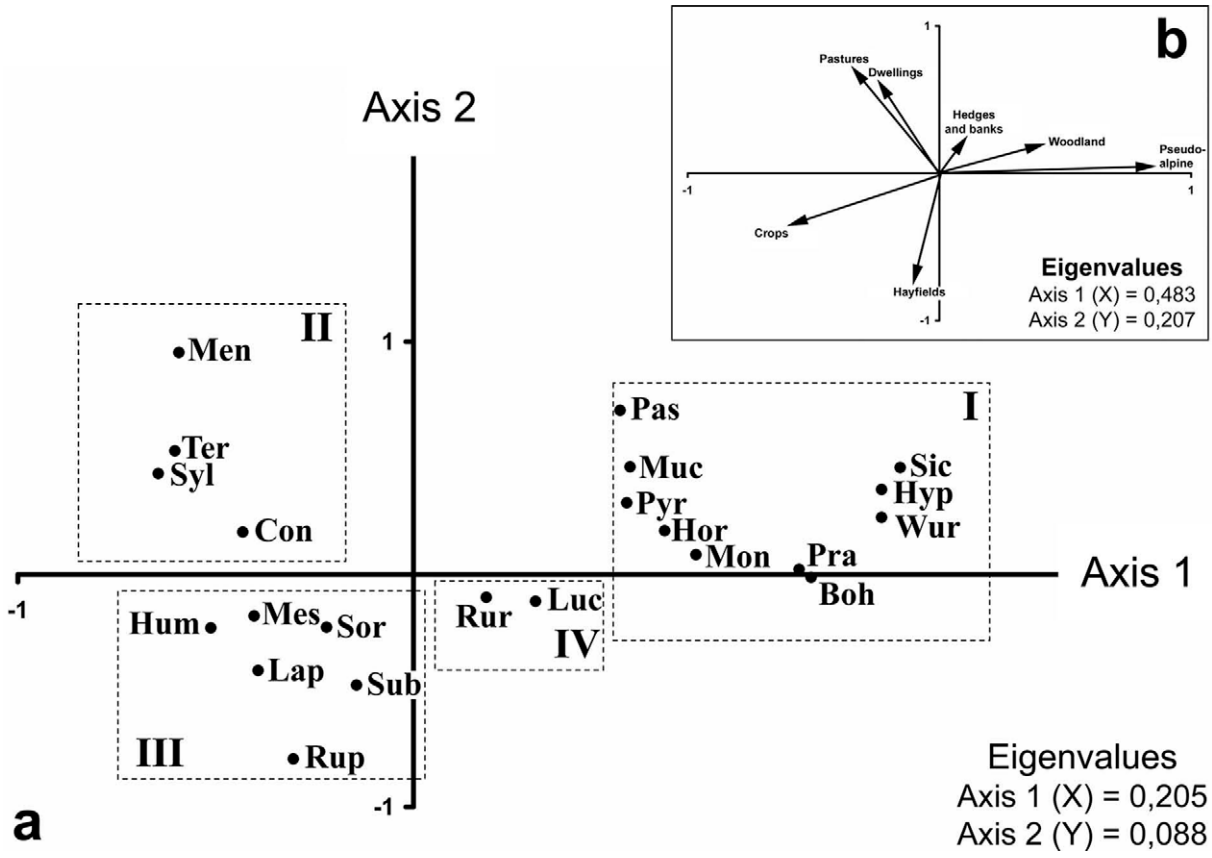

Figure 8. Projection of the species (a) and land use types (b) on the first two axes of the canonical correspondence analysis. The coordinates are indicated in accordance to the scale of eigenvectors. The Roman numerals refer to the biotopographic groups. Abbreviations for the bumblebee species: Boh: *Bombus bohemicus**, Con: *B. confusus*, Hor: *B. hortorum*, Hum: *B. humilis*, Hyp: *B. hypnorum*, Lap: *B. lapidarius*, Luc: *B. lucorum*, Men: *B. mendax*, Mes: *B. mesomelas*, Mon: *B. monticola*, Muc: *B. mucidus*, Pas: *B. pascuorum*, Pra: *B. pratorum*, Pyr: *B. pyrenaeus*, Rup: *B. rupestris**, Rur: *B. ruderarius*, Sic: *B. sichelii*, Sor: *B. soroeensis*, Sub: *B. subterraneus*, Syl: *B. sylvarum*, Ter: *B. terrestris*, Wur: *B. wurflenii*. *Obligatory inquiline species.

diversity of this type. The proportion of singletons is relatively high (42.1%) for this land use type. The species represented by a single specimen include *B. sichelii*, *B. subterraneus*, and the obligatory inquiline species *B. bohemicus*, *B. norvegicus* and *B. sylvestris*. As emphasised by Oertli et al. (2005), a high percentage of singletons results either from a weak sampling or from rarity or sometimes a specific vagrancy (i.e. an individual accidentally found outside the range of its species). Few bumblebee species are linked to the forest environment (*B. hypnorum*, *B. pascuorum* and *B. pratorum*; Reinig 1970; Rasmont 1988; Iserbyt et al. 2008).

Between 1953 and 2000 most cropped zones were converted into "hayfields" zones. This evolution may have contributed to the exceptional bumblebee species richness in Eyne (Iserbyt et al. 2008). Hayfields have the highest specific richness of all land use types encountered in the studied area, with high diversity and originality indices (Table 3). The high proportion of singletons in this land use type results from the presence of rare species (*B. cullumanus*, Figure 5b; *B. magnus*, Figure 4d; *B. rupestris*, Figure 6g) but mostly from the vagrancy – facilitated by the laying out of ski slopes (Figure 1) – of species that are typical of the middle and high altitudes (*B. mucidus*, Figure 5i; *B. wurflenii*, Figure 7e), or the forest (*B. hypnorum*, Figure 5e). The presence in this area, shaped by mountain agro-pastoral activity, of species such as *B. cullumanus, B. confusus*, *B. humilis*, *B. ruderatus*, *B. subterraneus* and *B. sylvarum*, generally considered as regressing in Europe as a whole (Rasmont et al. 2005; Xie et al. 2008; UKBAP 2009; Goulson 2010), shows the importance of careful conservation efforts. Eyne is one of the last two places in the world where the presence of *B. cullumanus* has been documented (Rasmont et al. 2005, email from G. Mahé to P. Rasmont; unreferenced).

Estimation of the potential impact of agricultural decline on the bumblebee fauna in Eyne

Having determined the habitat preferences of the different species, and the greater or lesser role of the different land

use types in the diversity of bumblebees, we can evaluate the potential impact that further agricultural decline could have on the bumblebee communities.

As Michel (2006) suggested for small mammals in the Armorican region (France, Ille-et-Vilaine and Manche), in Eyne the current extensification of agriculture, mostly through reallocation of crops to hayfields, could initially contribute to maintaining the specific diversity that has been observed for half a century (Delmas 1976; Iserbyt et al. 2008). Moreover, as described by Oertli et al. (2005) in Switzerland, the main factors explaining a high bumblebee diversity are the coexistence of the different habitat types and the weak intensity of agricultural practices. This is precisely the present situation in Eyne as a whole but more specifically in the low altitude part of its territory.

However, the agricultural decline process leads to a progressive tree and shrub encroachment due to pastoral abandonment (Davasse & Galop 1997). While crops around the town are converted into hayfields, the older and more distant hayfields are used as extensive pastures for a short period in spring and autumn, allowing shrubs and trees to grow. As a result, we are witnessing the progressive emergence of wooded habitats. Subsequent loss of open land habitats is a threat to plant (Dirnböck et al. 2003) and animal diversity (Labaune & Magnin 2002; Laiolo et al. 2004). If these land dynamics are to last, urbanisation, shrub encroachment and reforestation are likely to result in a reduction in bumblebee specific diversity. Indeed, urbanisation has a negative impact on bumblebee gene flows and dispersal (Jha & Kremen 2013). Furthermore, several works showed that bumblebee abundance and specific richness increase proportionally to the extent of pastures and meadows (Hatfield & LeBuhn 2007) and decrease proportionally to the extent of forest (Diaz-Forero et al. 2011 2012). The reforestation process that took place between 1953 and 2000, mostly at the expense of pastures, could induce a reduction of specific richness in the future.

Sooner or later agricultural decline will have a negative impact on grassland bumblebee species (*B. confusus*, *B. cullumanus, B. humilis*, *B. ruderatus*, *B. subterraneus* and *B. sylvarum*; according to Rasmont 1988; Benton 2006; Iserbyt et al. 2008) to the benefit of edge-loving species (e.g. *B. hortorum*, *B. pascuorum* and *B. ruderarius*; according to Pittioni & Schmidt 1942; Rasmont 1988; Benton 2006) and forest-loving species (e.g. *B. pratorum* and *B. hypnorum*; according to Pittioni & Schmidt 1942; Reinig 1970; Rasmont 1988; Benton 2006; Iserbyt et al. 2008; Crowther et al. 2014). More specifically, it is likely that the rare species found in Eyne, such as *B. confusus*, *B. cullumanus* and *B. ruderatus*, which are stringently dependent on legume-rich dry grasslands (Rasmont 1988; Benton 2006; Iserbyt et al. 2008), will regress if the current land use dynamics persists (i.e. reforestation and urbanisation). Indeed, the decline of these bumblebee species as observed among other places in Britain (Goulson et al. 2005), Belgium and France (Rasmont et al. 2005), seems to result from changes in plant communities induced by changes in agricultural practices.

As to species strictly dependent on woodland such as *B. pratorum* and *B. hypnorum* (Pittioni & Schmidt 1942; Reinig 1970; Rasmont 1988; Benton 2006; Iserbyt et al. 2008; Crowther et al. 2014), the current reforestation process should sooner or later enable the dispersal and expansion of these ordinary and already abundant species, which are expanding in the Western Palaearctic (Rasmont 1989; Goulson & Williams 2001; Williams et al. 2009; Crowther et al. 2014).

The linear uncultivated landscape features such as "hedges and banks" or field edges have an important ecological function for bumblebees, as habitats for species with a liking for transitional environments (e.g. *B. hortorum*, *B. pascuorum* and *B. ruderarius* according to Pittioni & Schmidt 1942; Reinig 1970; Rasmont 1988; Benton 2006), as shelters from predation, as nesting sites owing to the numerous possibilities afforded (Svensson et al. 2000), or as corridors for population flows (Steffan-Dewenter et al. 2002; Cranmer et al. 2012). In the UK, some studies showed that hedges do not represent a barrier to the movement of bumblebees (Krewenka et al. 2011), and even that they could facilitate their spatial orientation during travel (Cranmer et al. 2012). However hedges tend to get thicker and bigger during land abandonment process and therefore could act as barriers to the movement of bumblebees. This, coupled with the expansion of woodland areas, could therefore increase the fragmentation of open land habitats. As observed generally in the Western Palaearctic (Williams 1988; Osborne & Corbet 1994; Goverde et al. 2002; Fahrig 2003), habitat loss and fragmentation could in the long run lead to a decrease of Eyne's bumblebee diversity, at least among species preferring open environments.

Kreyer et al. (2004) did not observe any restriction in the foraging activity of *B. pascuorum* and *B. terrestris* in Hesse (Germany) due to the presence of a forest barrier in a plain. The situation is different in Eyne (Figure 1), where the pine forest makes up a homogenous ecological barrier at an altitude ranging from 1800 to 2100 m. This barrier splits the Eyne valley, with its alpine and subalpine grasslands from the tableland surrounding the village, with its mountain "pastures", "crops" and "hayfields". The laying out of ski slopes east of the village has involved the clearing of long strips of forests between subalpine heathlands and grasslands, and the cultivated tableland (Figure 1). It is precisely along these corridors or at their lower end that several bumblebee species normally linked exclusively to alpine and subalpine heathlands and grasslands come to forage (*B. mendax*, Figure 5g; *B. monticola*, Figure 4e; *B. mucidus*, Figure 5i; *B. pyrenaeus*, Figure 6c; *B. sichelii*, Figure 6h; subalpine species according to Pittioni &

Schmidt 1942; Rasmont 1988; Iserbyt et al. 2008). The opening of these ski slopes thus decompartmentalised the vegetation altitudinal zonation and brought high altitude species to the vicinity of the village.

Preserving the spatiotemporal heterogeneity of the landscape through agro-pastoral practices (Marage 2004) is essential to maintain specific diversity; but this is largely contingent on natural and human disruptive patterns (Burel 1999; Lambin et al. 2001).

Nevertheless, the impact of climate change can also significantly affect the population dynamics of bumblebees (Rasmont et al. 2015), especially in mountain environments. Indeed, our previous studies showed that climate is an important driver of changes in bumblebees abundance and diversity in the Eyne valley (Iserbyt & Rasmont 2012), and that bumblebees are highly vulnerable to extreme temperatures, such as heat waves (Rasmont & Iserbyt 2012). The increase of forest cover at the entrance of the Eyne valley could become a barrier and stop low altitude bumblebee communities from moving to higher altitude zones, in response to global warming. This trend is offset by the laying out of ski slopes, which could act as corridors and enable species to disperse to higher altitudes. Moreover, changes in land use may affect local climate because of the modification of solar partitioning. For example, conversion of woodland in open land induces a decrease in humidity and an increase in temperature (Vitousek 1994). Micro-climatic conditions of a landscape depend not only on regional climate, but also on landscape structure and configuration.

Conclusion

In summary, it should be underlined that the land use types favourable for specific diversity and originality in Eyne's bumblebee population are hayfields, pastures, hedges and banks, and cleared corridors (ski slopes). Conversely, the least favourable are forestland and urbanised zones. Urbanisation and reforestation due to agricultural decline cause an extension of this unfavourable area. It is therefore crucial for bumblebee conservation that agricultural policies allow for the maintenance of open land habitats and habitat diversity.

The main limitations of this study are the availability of data, and especially their accuracy. Indeed, data prior to 1998 have not been used because of their lack of precision, which failed to allocate bumblebee species to the different land use types for the period of 1950. Therefore, we have not been able to demonstrate a dynamic of bumblebee species in relation to the land use.

Next steps in this research could involve the development of prospective scenarios of land use change in these mountain regions marked by agricultural decline. In order to assess the impact of these scenarios on bumblebee populations, they should take into account the socio-economic evolutions related to agriculture (intensification or abandonment) and tourism (e.g. skiing), coupled to climate change.

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Annexe III

Maebe K., Meeus I., Vray S., Claeys T., Dekoninck W., Boevé J.-L., Rasmont P., Smagghe G. 2016.

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A century of temporal stability of genetic diversity in wild bumblebees

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Since the 1950s, bumblebee (*Bombus*) species are showing a clear decline worldwide. Although many plausible drivers have been hypothesized, the cause(s) of this phenomenon remain debated. Here, genetic diversity in recent *versus* historical populations of bumblebee species was investigated by selecting four currently restricted and four currently widespread species. Specimens from five locations in Belgium were genotyped at 16 microsatellite loci, comparing historical specimens (1913–1915) with recent ones (2013–2015). Surprisingly, our results showed temporal stability of genetic diversity in the restricted species. Furthermore, both historical and recent populations of restricted species showed a significantly lower genetic diversity than found in populations of co-occurring widespread species. The difference in genetic diversity between species was thus already present before the alleged recent drivers of bumblebee decline could have acted (from the 1950's). These results suggest that the alleged drivers are not directly linked with the genetic variation of currently declining bumblebee populations. A future sampling in the entire distribution range of these species will infer if the observed link between low genetic diversity and population distribution on the Belgium scale correlates with species decline on a global scale.

One of the important variables in evolutionary biology and population genetics is the effective population size (N_e)[1]. N_e is the size of an ideal population which has the same genetic diversity as the actual population of interest[2]. Due to violations of the ideal scenario (e.g. all specimens should have equal reproduction and survival probabilities), wild populations usually depart from N_e which is generally smaller than the corresponding census population size (N_c)[2]. Although many factors are linked with population viability, by estimating N_e one can determine the viability of a population (e.g. ref. 3) and in turn the conservation status of this population or species[1].

The most important conservation consequence of a decrease in N_e is that it will result in a loss of genetic diversity[2,4–6]. The presence of sufficient genetic variation is crucial for the persistence of populations, as the loss of genetic variation will lead to a lower adaptive ability in response to current and future changes in the environment, such as new pathogens, climate change and habitat loss, and can ultimately lead to extinction[4–6]. On a short term, populations with a low N_e, are more vulnerable to random processes, such as genetic drift (e.g. refs 4–6). The higher effects of drift within small populations cause a (further) decrease in genetic variation. In turn, the chance of inbreeding will increase, and can lead to a decreased fitness due to inbreeding depression (e.g. refs 4–6). In social insects such as bumblebees, N_e will be very low in relation to the observed number of specimens, as most bumblebee species are monoandrous and their colonies consist mostly out of only one founder queen (e.g. refs 7–9).

Currently, several methods are described to estimate N_e in natural populations. In general, temporal studies, which estimate N_e by examining allele frequency changes over time, showed the best results (reviewed by refs 6,10–12). Although favourable, these approaches will be limited to only a few organisms as it requires time series, i.e. the availability of multiple specimens from populations sampled at two or more points in time (as reviewed by Habel *et al.*[6]). Although challenging, temporal sampling methods were already successfully applied to estimate N_e in natural populations (reviewed in ref. 6). In this manner several studies demonstrated a decrease of genetic

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diversity or an increase in genetic differentiation (e.g. refs 6,13,14). For instance, Athrey *et al.*[15] compared historical and recent populations of the endangered golden-cheeked warbler (*Dendroica chrysoparia*) to assess the impact of demographic changes. Over a period of 100 years, genetic diversity decreased, while genetic differentiation increased. In contrast, other studies clearly showed temporal stability of population structures[16–18]. The absence of significant shifts over time could be attributed to past population dynamics which has a direct impact on genetic drift and gene flow.

In bumblebee species, which are all essential pollinators in natural and managed ecosystems (e.g. refs 9,19), new data has recently raised the debate whether the population structure remained temporally stable or whether shifts in genetic diversity occurred during recent times[20,21]. Many bumblebee species show major population declines in different parts of the world (e.g. refs 22–24). Specifically in Europe, 24% of the 68 European bumblebee species are threatened with extinction. More generally, bumblebee populations tend to decline (46%), remain stable (29%) or increase (13%)[25,26]. In populations of the restricted and currently declining bumblebee species, a lower genetic diversity than contemporary widespread and stable bumblebee species was observed[20,21,27]. So it remained unclear whether this difference is caused by the decrease in population size or whether it is an intrinsic characteristic of the currently declining species[20,21,27]. The generally accepted hypothesis says that the lower genetic diversity in the declining species is due to a reduction of genetic diversity caused by the drivers of bee decline (e.g. refs 27,28). This decline is possibly due to, or at least influenced, by (a combination of) the following drivers: pathogen infection and/or spill-over from domesticated pollinators, use of pesticides, food depletion, climate change, and landscape modifications (e.g. refs 29–31). Although land cover changed substantially during the last three millennia due to anthropogenic activity[32], the increasing loss of habitats and forage resources due to the agricultural intensification, is thought to be the key driver of the observed bumblebee decline in Europe which started around the 1950s[24,30,33].

Due to the impact of these drivers, the effective population size of restricted bumblebee species may decrease. Genetic drift (e.g. for changes of allele frequency in a population due to random sampling of organisms) may lead to a decrease of genetic variation within a population[4,5]. This stochastic loss of alleles will be more pronounced in small populations[4,5], and if the population is isolated this effect may be less buffered by dispersal. This difference in vulnerability towards the impact of genetic drift will be reflected in the observed level of genetic variation, with lower and decreased levels within the populations of the restricted bumblebee species. However, this hypothesis is solely based on recent contemporary samples[22,27,28]. In contrast, studies using only historical specimens revealed similar differences in genetic diversities between declining and stable bumblebee species in North-America[20] and Europe[21], which suggests that no recent major reduction in genetic diversity occurred[20,21]. In an alternative hypothesis, populations of restricted bumblebee species have already a lower genetic diversity due to past population dynamics. These species may maintain the lower level of genetic diversity in their populations by (i) receiving enough dispersing specimens from neighboring subpopulations to counter the effects of drift, and/or (ii) as their populations may always been small, most of their genetic variation may already be removed due to selection and drift. The latter suggests that a further decrease of genetic variation will become difficult to notice over short time periods. Testing which hypothesis is causing the observed lower genetic variation in the restricted and declining species is necessary to improve conservation strategies and secure the pollination services of wild bumblebees[5,27]. Indeed, lower genetic diversity will predispose these populations to have a more limited ability to adapt to the changing environment[4,5]. For instance, genetically pauperized bumblebees are more susceptible to diseases. Research performed by Whitehorn *et al.*[34] in the UK showed that populations of the large carder bee or moss carder bee (*B. muscorum*) with a lower level of heterozygosity showed a higher prevalence of the gut parasite *Crithidia bombi.* The same negatively correlated interaction between parasite prevalence and genetic diversity was found for the parasitic mite *Locustacarus buchneri* in *B. muscorum* but not in *B. jonellus*[35]. Furthermore, in North-America, researchers found also a link between the level of genetic diversity and an increased vulnerability to the pathogen *Nosema bombi*[22].

In this study, we compared two groups of bumblebee species, those currently widespread and found in different parts of Belgium *versus* those currently restricted and limited to specific localities, to investigate whether a reduction in genetic diversity has occurred over time. More specifically, we compared the genetic diversity of eight bumblebee species before and after the general bee decline that started in Europe around the 1950s[35,36]. Pin-mounted museum specimens from 100 years ago (1913–1915) were compared to specimens collected recently (2013–2015), both sets originating from the same five locations in Belgium. As pollinator regression is intensively described in Belgium and concerns bumblebees[36] but also other wild bees[37,38], hoverflies and butterflies[36], this country was chosen as our main study area. Among the eight chosen bumblebee species, four are currently restricted (*B. ruderarius*, *B. sylvarum*, *B. humilis* and *B. soroeensis*) and four are currently widespread (*B. pascuorum*, *B. hortorum*, *B. pratorum* and *B. lapidarius*) in Belgium. For each location and species, samples were genotyped with microsatellite DNA markers. Here, we hypothesize that (i) widespread bumblebee species have larger effective population sizes than sympatric restricted bumblebee species; (ii) genetic diversity in the potentially smaller populations of restricted species will decrease over time by the influence of genetic drift.

Results

Data analysis. Each of the 16 microsatellites amplified successfully in each *Bombus* species. Genotype replications for all loci were consistent, with a correct repetition of 99.71%. Based on our exclusion step of maximum 6 loci of missing values allowed within the genotype profile of a single specimen, 85 specimens were excluded from all further analyses for the historical data, and 11 for the recent ones (Table 1). Furthermore, an extra 114 and 86 specimens were removed as Colony 2.0 and Kinalyzer analyses identified them as being full-sibs within a population. Indeed, when these analyses detected a full-sib pair within one population (specimens from the same location and time period), only one random selected specimen per sibship was kept for further analysis (Table 1). After these two exclusions steps, 357 out of 566 historical and 436 out of 533 recent specimens remained in our

Species	Subgenera	Historical					Recent				
		Pop	n	NA	FS	*N*	Pop	n	NA	FS	*N*
B. hortorum	*Megabombus*	4	97	3	6	88	4	94	1	8	85
B. humilis	*Thoracobombus*	2	33	2	12	19	1	15	1	6	8
B. lapidarius	*Melanobombus*	5	100	18	13	69	5	122	3	8	111
B. pascuorum	*Thoracobombus*	4	101	14	20	67	5	140	0	31	109
B. pratorum	*Pyrobombus*	3	69	9	22	38	4	97	3	12	82
B. ruderarius	*Thoracobombus*	3	75	16	23	36	2	20	2	1	17
B. soroeensis	*Kallobombus*	1	25	12	2	11	1	21	0	12	9
B. sylvarum	*Thoracobombus*	3	66	11	16	39	1	24	1	8	15
Total		25	566	85	114	367	23	533	11	86	436

Table 1. Number of specimens genotyped in the analysis categorized per *Bombus* species. With indication of their division in *Bombus* subgenera, and with pop = the number of populations sampled, *n* = the total number of specimens genotyped, *NA* = the number of specimens that were not amplifiable, *FS* = the number of detected and removed full sibs, and N = the final number of workers used in all further analyses.

dataset, which we used to estimate the different genetic parameters of all populations of each species (Table 1). In addition, our analyses detected no significant linkage disequilibrium between microsatellites, but found significant deviations of HW for some loci in the populations of each species. Although this difference may be due to the presence of null alleles, our analysis performed with MICROCHECKER 2.2.3 revealed only very low frequencies of null alleles (<5%) in these involved microsatellite loci.

Estimation of genetic diversity. For all *Bombus* species, the genetic diversity of all populations was estimated in the two time periods (1913–1915 and 2013–2015). Within the recent populations of the widespread *Bombus* species, the genetic diversity parameters (A_R and H_E) were high, ranging from 3.820 to 6.590 and from 0.409 to 0.755, respectively (Table 2). The observed level of genetic diversity within the populations of the restricted bumblebee species was lower than within the populations of the widespread species ranging from 2.560 to 3.810 and from 0.307 to 0.434 (A_R and H_E, respectively; Fig. 1).

Within the historical bumblebee populations, we found a similar result, with A_R and H_E for the widespread *Bombus* species ranging from 3.430 to 9.040 and from 0.420 to 0.728, and a lower genetic diversity within the populations of the restricted species ranging from 2.240 to 3.870 and from 0.313 to 0.509 (A_R and H_E respectively; Table 2 and Fig. 1).

Genetic diversity in restricted versus widespread species. After running the LMM, we found no decrease of H_E over time. Indeed, the factor "'period" was not present in the best models (delta > 2; Table 3). Therefore, H_E remained stable over time for the restricted species with 0.385 *versus* 0.351 (mean H_E in 1913–1915 and 2013–2015, respectively) and for the widespread species with 0.589 *versus* 0.594 (mean H_E in 1913–1915 and 2013–2015, respectively; Table 2). Although, mean A_R remained fairly stable for the restricted species from 3.127 to 3.198 and for the widespread species with 5.519 *versus* 5.443 (mean A_R and H_E in 1913–1915 and 2013–2015, respectively; Table 2), time period was included in the best LMM models for A_R (delta = 0.000, Table 3). However, the effect of time period was not significant (LMM, $P = 0.758$, Table 4), neither with the interaction of "distribution" and the different "subgenera" (LMM, $P = 0.910$, and $P = 0.088$–0.802, respectively; Table 4). In general, these results show that the historical and recent genetic diversity within the populations of the restricted and widespread species did not decrease over 100 years; at least not in a consistent manner.

The models (delta < 2) fitting the observed pattern of the genetic diversity variable H_E best were the model with species "distribution" and "subgenera" as fixed factors and with or without the interaction between "distribution" and "subgenera". The model (M12) was the best fitting model (delta = 0.000) and had the highest weight (0.632; Table 3). For the variable A_R, the models with species "distribution", "period" and "subgenera" as separate main fixed factors and with or without the interaction between "distribution" and "subgenera" and between "subgenera" and "period" had the lowest delta AIC score (Table 3). Although model (M12) showed a similar significant result for A_R (delta = 0.648, weight = 0.181; Table 3), the models including "period", with or without the interaction of "period" with "subgenera", were always better fitting the data (for both M40 and M48, delta = 0.000 and weight = 0.250, respectively; Table 3). Therefore, model M12 and model M48 were selected and performed as best fitting models for H_E and A_R, respectively (Tables 3 and 4).

Species distribution was significantly explaining the observed pattern of H_E (LMM, *t*-test, $t = -5.803$, $p < 0.001$) and A_R (LMM, *t*-test, $t = -3.520$, $p < 0.001$) (Table 4), which means that the widespread bumblebee species had a higher genetic diversity than the restricted species, within and between both time periods. For both parameters, species subgenera had also a significantly effect on the observed pattern of genetic diversity. Indeed, compared with the other subgenera, the species of the subgenera *Melanobombus* had a higher A_R and H_E (LMM, *t*-test, $t = 4.889$, $p < 0.001$; $t = 1.965$, $p < 0.049$; respectively; Table 4) and in *Thoracobombus* a lower H_E (LMM, *t*-test, $t = -3.348$, $p < 0.001$; Table 4).

Effective population size estimation. The effective size of each population, with data from two time points available, were measured with MLN_e (Table 5). Within one species our estimations of N_e varied remarkably depending on the population. Although this complexes the comparison of N_e between species, we can clearly

	Species	Location	Historical time period (1913–1915)					Recent time period (2013–2015)				
			N	H_E	SE	A_R*	SE	*N*	H_E	SE	A_R*	SE
Restricted species	*B. soroeensis*	Torgny	11	0.479	0.079	3.530	0.514	9	0.434	0.095	3.810	0.680
	B. humilis	Trivières	13	0.358	0.079	3.220	0.527		—	—	—	—
	B. humilis	Torgny	6	0.313	0.077	2.310	0.339	8	0.349	0.080	3.250	0.556
	B. ruderarius	Moorsel	10	0.333	0.085	2.750	0.504		—	—	—	—
	B. ruderarius	Nieuwpoort		—	—	—	—	6	0.307	0.095	2.560	0.500
	B. ruderarius	Trivières	15	0.354	0.092	3.400	0.658		—	—	—	—
	B. ruderarius	Torgny	11	0.440	0.075	3.490	0.501	11	0.318	0.101	3.100	0.691
	B. sylvarum	Moorsel	7	0.316	0.089	2.440	0.484		—	—	—	—
	B. sylvarum	Trivières	14	0.366	0.078	3.130	0.566		—	—	—	—
	B. sylvarum	Torgny	18	0.509	0.074	3.870	0.498	15	0.345	0.089	3.270	0.720
		Mean	**11.7**	**0.385**	**0.068**	**3.127**	**0.496**	**9.8**	**0.351**	**0.045**	**3.198**	**0.400**
Widespread species	*B. pascuorum*	Francorchamps	17	0.458	0.093	4.120	0.673	26	0.478	0.085	4.340	0.727
	B. pascuorum	Moorsel	21	0.493	0.083	4.410	0.639	21	0.459	0.086	4.170	0.738
	B. pascuorum	Nieuwpoort		—	—	—	—	19	0.454	0.090	4.420	0.833
	B. pascuorum	Trivières	17	0.491	0.087	4.530	0.684	23	0.442	0.083	4.000	0.690
	B. pascuorum	Torgny	12	0.420	0.072	3.430	0.478	20	0.409	0.087	3.820	0.686
	B. hortorum	Francorchamps	18	0.596	0.087	6.320	0.952	25	0.584	0.092	6.080	0.981
	B. hortorum	Moorsel	24	0.557	0.094	5.800	0.911	20	0.580	0.095	5.950	0.929
	B. hortorum	Nieuwpoort	25	0.576	0.082	5.720	0.849		—	—	—	—
	B. hortorum	Trivières	21	0.568	0.088	5.460	0.883	17	0.572	0.084	5.720	0.845
	B. hortorum	Torgny		—	—	—	—	23	0.563	0.089	5.710	0.907
	B. pratorum	Francorchamps	18	0.554	0.081	9.040	0.686	22	0.557	0.078	5.070	0.751
	B. pratorum	Moorsel	12	0.606	0.061	4.380	0.568	19	0.630	0.071	5.560	0.764
	B. pratorum	Trivières	8	0.601	0.076	8.130	0.539	21	0.626	0.076	5.500	0.726
	B. pratorum	Torgny		—	—	—	—	20	0.639	0.075	5.480	0.652
	B. lapidarius	Francorchamps	16	0.728	0.040	5.600	0.413	23	0.722	0.056	6.250	0.546
	B. lapidarius	Moorsel	14	0.690	0.040	5.350	0.506	20	0.728	0.054	6.310	0.519
	B. lapidarius	Nieuwpoort	15	0.716	0.039	5.640	0.534	24	0.755	0.055	6.590	0.584
	B. lapidarius	Trivières	8	0.701	0.059	4.810	0.567	22	0.746	0.047	6.520	0.534
	B. lapidarius	Torgny	16	0.671	0.052	5.560	0.564	22	0.745	0.052	6.480	0.643
		Mean	**16.4**	**0.589**	**0.091**	**5.519**	**1.377**	**21.5**	**0.594**	**0.111**	**5.443**	**0.898**

Table 2. Comparison of the genetic diversity within historical and recent populations of *Bombus* species. For each population the mean values (and SE) of the expected heterozygosity (H_E) and the allelic richness (A_R) over all microsatellite loci are given. Furthermore, species are grouped based on their distribution in Belgium. With N = the number of populations of each species. *Allelic richness calculated based on 10 diploid specimens.

distinguish the lower N_e in the populations of *B. sylvarum* and *B. soroeensis* ($N_e = 160.6$ and $N_e = 239.0$, respectively) in comparison with the N_e within the populations of all stable bumblebee species (Table 5). Furthermore, the estimates also showed large population sizes for *B. hortorum* (Table 5). *B. pascuorum*, *B. pratorum* and *B. lapidarius* showed similar values in N_e as those observed in *B. ruderarius* and *B. humilis* populations (Table 5), although the *B. pascuorum* population of Torgny showed a very low N_e ($N_e = 219.0$) comparable with the N_e observed in the populations of *B. sylvarum, B. soroeensis,* and *B. ruderarius* in Torgny.

Discussion

Lower genetic diversity in restricted species. Hundred years ago, and still today, restricted bumblebee species compared to widespread species had, and have, a significantly lower genetic diversity (Fig. 1). This result confirms those on the difference in level of genetic diversity between declined and more stable bumblebee species in The Netherlands by using only historical bumblebee specimens[21]. Hence, the here unique experimental setup of comparing recent with historical genetic diversity of different bumblebee species coming from the same locations in Belgium enabled us to show, moreover, that the levels of genetic diversity remained fairly stable over time in the studied populations. Indeed, in general, no major reduction in genetic diversity is observed over time. These results do not support the hypothesis based on solely recent specimens, which explains the difference in genetic variation between stable and declining species by a reduction of genetic diversity due to population declines in response to environmental drivers which acted around 1950 (e.g. refs 27,28). The present results rather corroborate the hypothesis that for some species (here, the restricted species) the levels of genetic diversity were already low at the beginning of the 20th century, thus well before the 1950s when the agricultural revolution started with a massive use of pesticides and fertilizers. Although our results clearly support the latter hypothesis, we do not rule out the possibility that a small reduction in genetic diversity has occurred during the last 100 years. A possible and small reduction in genetic diversity may be undetectable due to the lack of statistical power and the rather small sampling sizes within the experimental setup of our study. Although we admit that an increase in

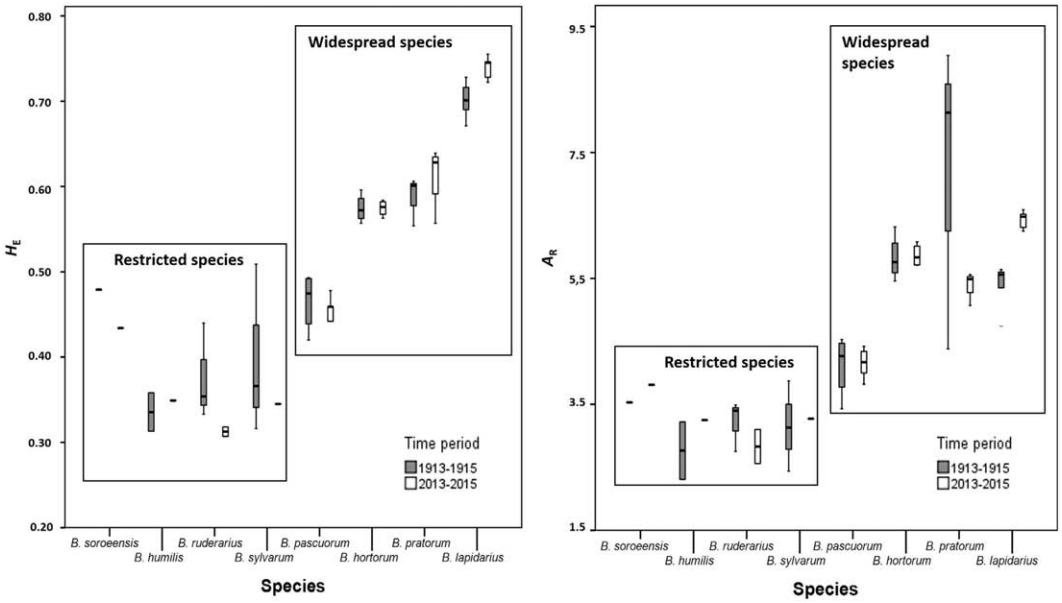

Figure 1. Comparison of the historical and recent genetic diversity within each *Bombus* species. Box-plots of H_E and A_R for each species and for both time periods 1913–1915 and 2013–2015.

sample size would be preferable to strengthen the power of the results in our study, the potential reduction would be much smaller than the here observed difference between restricted and widespread species. Furthermore, we provide here the best possible sampling and setup considering the possible difficulties when using and genotyping historical old museum and collection material. Hence, a tendency towards a small decrease in genetic diversity over time can be seen for the restricted bumblebee species, *B. ruderarius* and *B. soroeensis* (Fig. 1). However, this reduction is too small to explain the observed difference between the restricted and widespread species.

Change of genetic diversity over time. Our results are indicative for a temporal stability of genetic diversity within the studied populations of the restricted bumblebee species. A comparable example of temporal stability of genetic diversity is shown in Danish populations of the large blue butterfly (*Maculinea arion*)[39]. Although declined the population showed no shift in genetic variation over 77 years. The authors mentioned several possible hypotheses to explain this result: i) the population decline was not strong enough to cause a reduction of genetic diversity; ii) the butterfly might decline as it relies on a host ant species, *Myrmica sabuleti*, which experiences a decline, while the butterfly's genetic diversity is maintained by gene flow or dispersal; iii) too low statistical power in the analysis of genetic diversity due to insufficient historical sampling, or iv) the historical populations were already genetically impoverished before the start of their study. The latter explanation turned out to be the most likely one with a lower genetic variation due to long-term isolation from nearby populations[39]. Here, we can formulate some similar explanations. Although we cannot totally exclude the possibility that the population decline was not severe enough to cause a reduction of genetic diversity in bumblebee species, this seems less possible, considering the major population declines observed for most bumblebee species[24,30,33]. As described above, the low statistical power due to insufficient historical sampling may be another possibility here. Especially, if one takes into account that all comparisons between historical and recent genetic diversity levels in the restricted species were assessed from one location (Torgny; Table 2). Finally, the absence of recent specimens at the other locations for these species is already indicative of their population decline, and may also represent a non-measurable loss of genetic diversity at these locations.

Although no significant reduction of genetic diversity is detected, a significant increase of genetic diversity over time was observed for the widespread species *B. lapidarius* (subgenera *Melanobombus;* Table 5 and Fig. 1). A possible explanation of this observed effect is an increase in effective population sizes and/or a higher level of gene flow by dispersal between populations in this species.

Other possible causes of the lower genetic diversity in the restricted bumblebee species. The question about genetic reduction now somewhat shifts. If it did not occur over the last 100 years, did it occur earlier? The low genetic profile within the historical populations of the restricted species was perhaps already altered due to genetic bottlenecks occurring even before the beginning of the 20th century. In Europe, land cover use changed substantially due to anthropogenic activity, such as a large scale deforestation during the Industrial Revolution (ca. 1790–1900)[32] which may have caused earlier bumblebee declines. In turn, most of the genetic variation could be already removed from their populations, with only the necessary adaptive genetic variation remaining[40]. The detection of an additional reduction, within the 100 years of our study, would thus become even more difficult to detect. Although having low levels of genetic variation, without a strong (new) external pressure on the environment, these populations would not be heavily affected[40]. However, once the possible drivers of bee decline acted in the 1950's, they may have caused the trigger that started the decline and even the extinction of

A	H_E	(Intercept)	Distribution	Subgenera	Period	Distribution: Subgenera	Distribution: Period	Subgenera: Period	df	logLik	AIC	delta	weight
	M4	0.554	+	+	*NA*	*NA*	*NA*	*NA*	9	71.762	−120.79	0.000	0.496
	M12	0.554	+	+	*NA*	+	*NA*	*NA*	9	71.762	−120.79	0.000	0.496
	M8	0.584	+	+	+	*NA*	*NA*	*NA*	10	68.195	−110.44	10.343	0.003
B	A_R	**(Intercept)**	**Distribution**	**Subgenera**	**Period**	**Distribution: Subgenera**	**Distribution: Period**	**Subgenera: Period**	**df**	**logLik**	**AIC**	**delta**	**weight**
	M40	4.878	+	+	+	*NA*	*NA*	+	14	−45.151	131.030	0.000	0.250
	M48	4.878	+	+	+	+	*NA*	+	14	−45.151	131.030	0.000	0.250
	M4	4.742	+	+	*NA*	*NA*	*NA*	*NA*	9	−54.470	131.678	0.648	0.181
	M12	4.742	+	+	*NA*	+	*NA*	*NA*	9	−54.470	131.678	0.648	0.181
	M56	4.915	+	+	+	*NA*	+	+	15	−44.719	134.438	3.408	0.046

Table 3. Selection of best fitting model explaining the genetic diversity in *Bombus*. Of all possible models run under MUMIn[55] using species distribution, species subgenera, location and both time periods as fixed effects and species as a random effect, the best fitting linear mixed-effect models (with a delta < 4) are given. The final selected models for A. H_E and B. A_R were indicated in bold following their high (negative or positive) Akaike's Information Criterion (AIC) and weight of fitting the pattern. With + = parameters included in the model, and NA = not included parameters.

A.	H_E	Estimate	SE	*t*-value	*p*
	Distribution	−0.098	0.017	−5.803	**<0.001**
	Megabombus	0.013	0.035	0.371	0.710
	Melanobombus	0.166	0.034	4.889	**<0.001**
	Pyrobombus	0.048	0.035	1.370	0.171
	Thoracobombus	−0.098	0.029	−3.348	**<0.001**
B.	A_R	**Estimate**	**SE**	***t*-value**	***p***
	Distribution	−1.067	0.303	−3.520	**<0.001**
	Period	−0.280	0.940	−0.298	0.766
	Megabombus	0.988	0.803	1.229	0.219
	Melanobombus	1.552	0.790	1.965	**0.049**
	Pyrobombus	0.525	0.803	0.654	0.513
	Thoracobombus	−0.745	0.729	−1.031	0.302
	*Period * Megabombus*	0.239	1.052	0.228	0.820
	*Period * Melanobombus*	−0.758	1.030	−0.736	0.462
	*Period * Pyrobombus*	2.061	1.069	1.927	0.054
	*Period * Thoracobombus*	0.291	0.988	0.294	0.768

Table 4. Output of the selected linear mixed - effect models (LMM). Impact of the different factors in the models on A. H_E and B. A_R. With the estimate, standard error (SE) and *p*-value of each factor or interaction in the model obtained by *t*-tests. Significant factors are indicated in bold.

these species. Although a very possible explanation, but in the absence of robust collection material from the 19th century, it is impossible to test this possibility, let alone the general technical difficulties in using haplodiploid species. In our study we present species belonging to different subgenera which could represent a different genetic diversity and could bias the difference observed between restricted and widespread species. Indeed, the over representing of different species of the subgenus *Thoracobombus* could bias the observed lower genetic diversity within the group of restricted bumblebee species. However, we speculate that this is not the case. Indeed, even within the subgenera *Thoracobombus*, the widespread species (*B. pascuorum*) had a significantly higher genetic diversity than the restricted species (*B. ruderarius*, *B. sylvarum* and *B. humilis*; Table 2 and Fig. 1). The lower levels of genetic diversity in the restricted bumblebee species may be a specific character of these species, and thus do not necessarily mean that there was a population bottleneck or decline. Many species have vast differences in their effective population sizes and genetic diversity[2]. For instance, Romiguier *et al.*[41] revealed a strong influence of life-history traits (such as body mass, longevity, and reproductive strategy) on genetic diversity by a comparative analysis of patterns of diversity across several animals. A bumblebee species in which the mother queen produces much reproductives (daughter queens and/or males) may have a higher level of genetic diversity than a species which produces less reproductives. Whether this is effectively the case is not known. More research concerning the role of species specific characteristics on the observed difference in genetic diversity between these bumblebee species might bring more clarity. The relatively low genetic diversity of the restricted species may be explained by the smaller population sizes of these species. Indeed, restricted bumblebee populations may have smaller population sizes and thus can have a reduced genetic diversity as a result of higher genetic drift and if isolated also

Species	Location	LMNe	95% CI
B. soroeensis	Torgny	**239.0**	167.6–354.0
B. humilis	Torgny	**1343.7**	467.7–∞
B. ruderarius	Torgny	**455.8**	266.8–918.1
B. sylvarum	Torgny	**160.6**	121.1–214.0
B. pascuorum	Francorchamps	**1971.0**	1096.5–5041.6
B. pascuorum	Moorsel	**984.6**	627.23–1730.0
B. pascuorum	Trivières	**1615.0**	875.2–4315.0
B. pascuorum	Torgny	**219.0**	167.9–288.3
B. hortorum	Francorchamps	**∞**	11493.0–∞
B. hortorum	Moorsel	**∞**	∞–∞
B. hortorum	Trivières	**4896.4**	17313.0–∞
B. pratorum	Francorchamps	**449.9**	339.6–607.1
B. pratorum	Moorsel	**396.1**	299.2–538.5
B. pratorum	Trivières	**503.1**	364.5–726.4
B. lapidarius	Francorchamps	**939.8**	656.9–1446.5
B. lapidarius	Moorsel	**658.1**	477.7–955.2
B. lapidarius	Nieuwpoort	**840.4**	610.5–1216.5
B. lapidarius	Trivières	**2851.4**	1310.2–∞
B. lapidarius	Torgny	**599.9**	453.5–816.6

Table 5. Estimation of the effective population sizes (N_e) with different temporal methods. For each method, the 95% confidence interval (CI) of N_e is given. The harmonic mean is calculated over all temporal methods for each population and over all populations of each species.

Species	European Population Trend[1]	Belgian collection data[2]		Francorchamps		Moorsel		Nieuwpoort		Torgny		Trivières		N	
		<1950	>1950	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2
B. soroeensis	Decreasing	526	49	+	+	−	−	−	−	+	+	+	−	3	2
B. humilis	Decreasing	857	27	+	−	+	−	−	−	+	+	+	−	4	1
B. ruderarius	Decreasing	1599	185	+	−	+	−	+	+	+	+	+	−	5	2
B. sylvarum	Decreasing	622	35	+	−	+	−	−	−	+	+	−	−	3	1
B. pascuorum	Increasing	20176	3995	+	+	+	+	+	+	+	+	+	+	5	5
B. hortorum	Stable	5529	865	+	+	+	+	+	+	+	+	+	+	5	5
B. pratorum	Increasing	3603	3597	+	+	+	+	+	+	+	+	−	+	4	5
B. lapidarius	Increasing	10714	971	+	+	+	+	+	+	+	+	+	+	5	5

Table 6. Distribution and abundance data of the different bumblebee species. The division of the selected *Bombus* species in restricted and widespread species is based on three sets of available data: (i) the species population trend in Europe[25]; (ii) the number of bumblebee specimens within the RBINS collection before 1950 and between 1955–1993[36,37]; and (iii) the presence or absence of the species at each location during the historical and recent bumblebee foraging season 1913–1915 (=T1) and 2013–2015 (=T2), respectively. With N the total number of locations where a species was found, and with V = the presence and – = the absence of the species at that specific location. [1]Data from[25]. [2]Data from[36,37].

a reduced gene flow[4,6]. Although it seems that genetic drift did not result in an extra reduction over the last 100 generations, our results showed that restricted bumblebee species have lower effective population sizes. Species' smaller distribution is thus a valid explanation of the low genetic diversity observed in the restricted species which are now more heavily affected by the drivers of the decline. Hence, the question can perhaps be rephrased: is a low H_E and A_R intrinsically linked with the biology of a certain species, or with being locally restricted? Here we defined the distribution range based on data from a small ecological scale (see Table 6 and Fig. 2). A sampling on a larger ecological scale, within the whole distribution range of these species, will allow for comparison of the same species being locally widespread and locally restricted. This will determine the link between species and an intrinsic level of genetic diversity. In turn, this will have great influence on the implementation of future conservation strategies.

Methods

Sampling of historical and recent specimens. Historical bumblebee specimens were collected from the Hymenoptera collection of the Royal Belgian Institute of Natural Sciences (RBINS) in Brussels. Five locations in Belgium were selected due to the presence of sufficient available historical bumblebee specimens of multiple species for genetic analysis, and since these five locations represent the main bio-geographical units of the country.

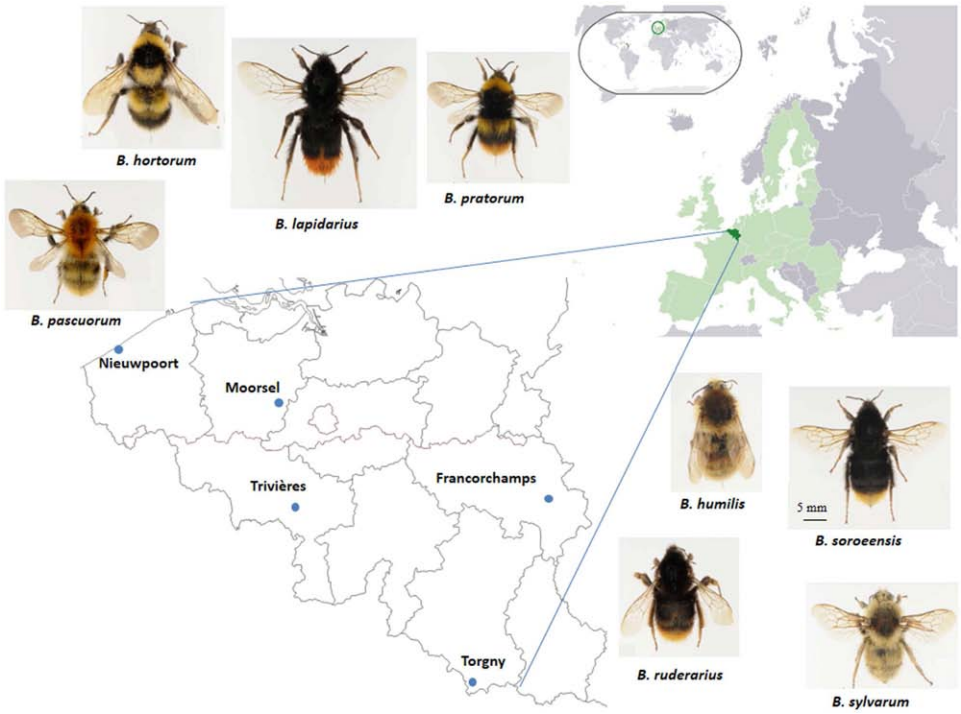

Figure 2. Overview of the bumblebee species sampled at five locations in Belgium. Specimens for each species were collected at the same five locations in Belgium in 1913–1915 and 2013–2015. Pictures of species are from Rasmont & Pauly[60], and an adapted map of Belgium from http://www.d-maps.com/conditions.php?lang=en.

In these five locations (Francorchamps, Moorsel, Nieuwpoort, Trivières, and Torgny; Fig. 2), bumblebees were collected within a $5 \times 5\,km^2$ frame. Two neighboring localities were merged together for Trivières (Trivières and St-Vaast) and Torgny (Torgny and Lamorteau) to allow a comparable sampling area as within the other localities. Historical specimens from the RBINS collection were collected in the bumblebee foraging season 1913, 1914, and 1915 (Table 1), while recent specimens were sampled in 2013, 2014 and 2015 at the same locations. For both historical and recent time periods, 20 to 25 specimens were selected when possible from each location for genetic analyses resulting in the selection of 566 historical and 533 recent specimens (Table 1).

All bumblebee species belong to only one genus, *Bombus*, but are divided in different subgenera (for a full division of species in subgenera, see ref. 42). The eight bumblebee species selected here belong to five different subgenera: *Kallobombus*, *Megabombus*, *Melanobombus*, *Pyrobombus*, and *Thoracobombus* (see Table 1)[42].

Here, the further division of the eight bumblebee species in two groups of four currently restricted and four currently widespread species is based on three layers of available distribution or abundance data: (i) the available abundance data of these species within the Belgian collection[36,37], with a clear significant difference between both groups within the two time periods by using a linear mixed model (LMM, lmer(log(abundance) ~ distribution + (1|species), data = Data) in R studio[43] with R package lme4 version 1.1–10[44] (LMM, *t*-test, $t = 6.721$, $p < 0.001$; Table 6); (ii) the presence and/or absence of the species at each location during the historical and recent bumblebee foraging season (Table 6): and (iii) the population trend of these species within Europe, with the group of restricted species having a "decreasing" population trend and the widespread species a "stable" or "increasing" population trend (ref. 25; Table 6).

Bumblebee DNA extraction and microsatellite protocol. One middle leg of each individual bumblebee specimen was used for DNA extraction. DNA extraction, PCR amplification with 16 microsatellite markers (four multiplexes of four loci), and visualization with capillary electrophoreses on an ABI-3730xl sequencer (Applied Biosystems), were performed with the method as described in Maebe *et al.*[21]. The four microsatellite markers (B11, B100, B126, B132) developed by Estoup *et al.*[45]; and the four loci (BL02, BT04, BT08, and BT10) developed by Reber-Funk *et al.*[46] were chosen based on Maebe *et al.*[21]. Of the other remaining eight markers, five loci (BL13, BT02, BT05, BT23, BT24) were developed by Reber-Funk *et al.*[46] and three loci (0294, 0304 and 0810) by Stolle *et al.*[47]. From the 1099 genotyped specimens, 128 random selected specimens (11.65%) were re-genotyped to examine the genotypic error rate.

Data analysis. Some of the genotyped specimens were excluded prior to data analyses, after applying several validation steps following Maebe *et al.*[21]. In short, specimens were removed when they could not be scored in a reliable manner for a minimum of 10 microsatellite loci, and only one random specimen per sibship was kept after sister identification with the programs Colony 2.0[48] and Kinalyzer[49]. Furthermore, genotypic linkage

disequilibrium, deviations from Hardy-Weinberg equilibrium (HW), and evidence of null alleles were tested using the programs FSTAT 2.9.3[50], GENALEX 6.5[51] and MICROCHECKER[52], respectively.

Estimation of genetic diversity. For each population we determined the genetic diversity based on two parameters: the allelic richness (A_R) estimated as the sample size-corrected private allelic richness with the program HP-Rare 1.1[53] calculated and normalized on 10 diploid specimens for all populations, and Nei's unbiased expected heterozygosity (H_E;[54]) calculated with the program GENALEX 6.5[51].

Comparison of genetic diversity between species groups. To examine whether genetic diversity differed between species, and/or whether other factors such as species distribution, species subgenera, locations and/or time period had an effect on genetic diversity, we conducted LMM's in RStudio[43]. Species and sample location were chosen as random factors: (i) species, since the genetic diversity of a specific species is correlated over time and location; and (ii) location, as specimens were resampled at each location. Fixed factors were: time period (1913–1915 or 2013–2015), species subgenera (belonging to which bumblebee subgenera), and species distribution (widespread or restricted, see explanation above and in Table 6). The model that best fitted the pattern in genetic diversity was selected by using the Akaike's Information Criterion (AIC). The MUMIn package with the dredge command allowed us to calculate all possible combinations and thus model selection[55]. As described in Maebe *et al.*[21], problems linked with the interpretation of inter-specific differences could arise, for instance, in microsatellite mutation rates and levels of polymorphisms. Therefore, we added species as a random factor in the model. Furthermore, species belonging to the same subgenera could have comparable levels of genetic diversity due to similar mutation rates and dispersal abilities. Thus, over-representing of species belonging to one subgenera in the two groups (widespread *versus* restricted bumblebee species) could cause bias in our analyses. For instance, bumblebee species of the subgenera *Pyrobombus* may have higher dispersal abilities than *Thoracobombus* species[56,57]. As species with more limited dispersal rates will have less chance of a successful recolonization, they will be more vulnerable to genetic drift and thus may have less genetic variation. Thus, as division in subgenera could influence the level of genetic diversity, subgenera was included in the LMM. The best LMMs were run in R studio with R package lme4 version 1.1–10[44].

Effective population size. The estimation of N_e of each population was performed using one multiple temporal method: a maximum-likelihood approach implemented in the program MLN_e[58]. This method recently became recommended for the estimation of N_e in natural populations under both ideal and migration scenarios (see ref. 59). In short, and contradicting other methods, this method successfully takes migration into account when estimating N_e which otherwise could bias N_e estimation. A generation time of 1 year was used, as most bumblebee species have one life-cycle per year.

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Author Contributions

Conceived and designed the experiments: K.M., I.M., G.S. Collection of old specimens: K.M., W.D., J.L.B. Sampling of recent specimens: K.M., S.V. Performed the experiments: K.M., I.M., T.C. Analyzed the data: K.M., I.M., T.C. Wrote the paper: K.M., I.M., S.V., W.D., J.L.B. P.R., G.S.

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Annexe IV

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The interplay of climate and land use change affects the distribution of EU bumblebees

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Abstract

Bumblebees in Europe have been in steady decline since the 1900s. This decline is expected to continue with climate change as the main driver. However, at the local scale, land use and land cover (LULC) change strongly affects the occurrence of bumblebees. At present, LULC change is rarely included in models of future distributions of species. This study's objective is to compare the roles of dynamic LULC change and climate change on the projected distribution patterns of 48 European bumblebee species for three change scenarios until 2100 at the scales of Europe, and Belgium, Netherlands and Luxembourg (BENELUX). We compared three types of models: (1) only climate covariates, (2) climate and static LULC covariates and (3) climate and dynamic LULC covariates. The climate and LULC change scenarios used in the models include, extreme growth applied strategy (GRAS), business as might be usual and sustainable European development goals. We analysed model

performance, range gain/loss and the shift in range limits for all bumblebees. Overall, model performance improved with the introduction of LULC covariates. Dynamic models projected less range loss and gain than climate-only projections, and greater range loss and gain than static models. Overall, there is considerable variation in species responses and effects were most pronounced at the BENELUX scale. The majority of species were predicted to lose considerable range, particularly under the extreme growth scenario (GRAS; overall mean: 64% ± 34). Model simulations project a number of local extinctions and considerable range loss at the BENELUX scale (overall mean: 56% ± 39). Therefore, we recommend species-specific modelling to understand how LULC and climate interact in future modelling. The efficacy of dynamic LULC change should improve with higher thematic and spatial resolution. Nevertheless, current broad scale representations of change in major land use classes impact modelled future distribution patterns.

KEYWORDS

biodiversity loss, dynamic, future, land use change scenarios, pollinators, projections, species distribution models (SDMs), wild bees

1 | INTRODUCTION

Recent scientific consensus suggests that we are facing a sixth mass extinction event, correlated strongly to anthropogenic factors (Ceballos et al., 2015). To avoid the dramatic loss of biodiversity and associated ecosystem services, immediate and thorough conservation efforts are required (Barnosky et al., 2011). An important role of biodiversity conservation research is to understand and estimate potential changes in biodiversity alongside changing abiotic and biotic conditions (Elith, Kearney, & Phillips, 2010; Porfirio et al., 2014).

In an effort to understand these effects experts have produced scenarios of climate, and land use and land cover (LULC) change. Land use and land cover change scenarios use potential climate change, policy decisions and strategies to represent socioeconomic developments which will inevitably shift land use and management (Rounsevell, Ewert, Reginster, Leemans & Carter, 2005; Van Vuuren et al., 2011; Verburg, Rounsevell & Veldkamp, 2006). Scientists have developed scenarios with the goal to evaluate the impact of environmental changes on biodiversity (Spangenberg et al., 2012). Their role in biodiversity analyses is to allow the production of dynamic land use variables which better reflect future habitat suitability for a species and may be useful to explain additional drivers of distributional changes alongside climate change. There is strong consensus that both climate and LULC change are important in driving the observed patterns of biodiversity declines (Luoto, Virkkala & Heikkinen, 2007; Ostberg, Schaphoff, Lucht & Gerten, 2015). Historically, LULC change has been the dominant cause of observed biodiversity changes and researchers expect that it will remain an ongoing threat to worldwide biodiversity (Millennium Ecosystem Assessment, 2005, Ostberg et al., 2015). Climate and land use change underlie a multitude of environmental pressures that may have a greater joint impact on biodiversity than when operating in isolation (Clavero, Villero & Brotons, 2011; Mantyka-Pringle, Martin & Rhodes, 2012). Therefore, models which exclude LULC change from modelling biodiversity in the future neglect a significant factor in potential drivers of species distribution change, even if these projections are coarse and at broad spatial scales.

Species distribution models (SDMs) represent a powerful tool for understanding patterns in biodiversity. They combine species occurrence data with environmental conditions to estimate the distribution of species in space and time (Elith & Leathwick, 2009). Often used to project species distributions into unsampled areas, or areas of possible invasion, they also project species distributions into the future (Franklin, 2010). The majority of future distribution models include only climate change variables and do not include LULC variables or use only LULC variables based on current conditions (static; Bellard, Bertelsmeier, Leadley, Thuiller & Courchamp, 2012; Titeux et al., 2016). At broad spatial scales, climate is expected to be the main constraint to species distributions, but at finer resolutions, the effect of LULC covariates increase; landscape-specific features that provide nesting and feeding resources occur at this finer scale (Luoto et al., 2007; Rahbek et al., 2007; Thuiller, Araújo & Lavorel, 2004). Therefore, improved estimations of biodiversity change require detailed land use change scenarios (Titeux et al., 2016).

Even though studies recommend the inclusion of LULC variables to avoid producing unrealistic projections, few studies have used dynamic LULC covariates to model biodiversity patterns in the future. Reasons for this is that projections of LULC change are rarely available or only at coarse resolution and with few land use classes (Titeux et al., 2016). However, climate predictions offer similar

limitations with resolution and parameters often not directly relevant to the habitat suitability of species. Interestingly, the studies that explicitly include dynamic LULC variables in the SDM process show considerable variation in the effect this has on species distribution patterns, specifically range change (Barbet-Massin, Thuiller & Jiguet, 2012; Chytrý et al., 2012; Ficetola et al., 2010; Martin, Van Dyck, Dendoncker & Titeux, 2013; Riordan & Rundel, 2014; Sohl, 2014; Wisz et al., 2008). The variation is most likely due to differences in species, spatial scale and explanatory variables included in these studies. Likewise, the performance of SDMs usually depends strongly on the modelling framework used, the species modelled, the distribution, quality and quantity of collection data, and the resolution of the species occurrence data and covariates (Aguirre-Gutierrez et al., 2013; Bellard et al., 2012; Harris et al., 2013; Warren & Seifert, 2011). Testing the effect of dynamic LULC covariates with multiple species, different resolutions and covariates is essential to understand their role in SDMs (Martin et al., 2013).

In this study, we evaluate the effects of LULC change scenarios available for Europe, on the distributional changes projected by SDMs for 48 European bumblebee species projected onto Belgium, the Netherlands, and Luxembourg (BENELUX), and at the European scale. We use three land use change scenarios (business as might be usual [BAMBU], growth applied strategy [GRAS], sustainable European development goals [SEDG]) representing alternative socioeconomic futures, which have been specifically developed to evaluate the impacts of environmental changes on biodiversity (Assessing LArge-scale environmental Risks with tested Methods (ALARM) Scenarios; Spangenberg et al., 2012). We expect to observe differences in the projected distributions produced by climate-only models vs. models which include LULC. We expect that the differences between static and dynamic LULC models will be less pronounced and species-specific, and will likely depend on the spatial scale and resolution at which the LULC covariates are projected (Luoto et al., 2007; Martin et al., 2013). Overall, we aim to illustrate the bias associated with using climate change-only scenarios when modelling bumblebees that land use change will undoubtedly affect. We also aim to show how presently available dynamic LULC projections affect the modelled distributions for multiple species. Following this important step, we discuss the extent to which our results provide improvements to land use change scenarios in development and the conservation implications of using such SDMs.

2 | MATERIALS AND METHODS

2.1 | Target species

Our study group is the genus *Bombus*, for which we have detailed, long-term, biogeographical records for most of Europe, and which has shown significant decline in the last one hundred years (Biesmeijer et al., 2006; Carvalheiro et al., 2013; Kerr et al., 2015; Rasmont et al., 2005). Forty-eight European bumblebee species were included in the analysis (see Table S1). The species modelled share similar life histories, but exhibit vastly different ranges and distributions in Europe (Rasmont et al., 2015). According to the IUCN Red List of threatened species, *Bombus* in Europe includes species of all threat levels (Nieto et al., 2014). Climate change impacts have been modelled for the genus *Bombus* at the European scale, projecting severe declines and northerly shifts for the majority of the species (Rasmont et al., 2015). However, loss of habitat for feeding and nesting resources has been cited as a major driver of past *Bombus* decline (Biesmeijer et al., 2006; Carvalheiro et al., 2013; Goulson et al., 2010; Williams & Osborne, 2009). Therefore, climate might not necessarily be the only significant driver of change for this group over the next one hundred years. Furthermore, the distribution patterns of wild bee species are reported to be affected by change in major land use classes, particularly the presence of arable land (Aguirre-Gutiérrez et al., 2015; Senapathi et al., 2015).

2.2 | Species presence data

This study includes bumblebee collection records from 22 European countries and multiple sources including professional and amateur scientists (see Fig. S1). The data were collated as part of the EU FP7 project STEP (Potts et al., 2011), and is aggregated and available to view on the Atlas Hymenoptera webpage (Rasmont & Iserbyt, 2013). We used records from 1970 until 2000, as these represent the 'current' period of climate data, which we used to train the species distribution models. We had 462,636 records available to use.

2.3 | Spatial extent and resolution

The spatial extent was limited to the extent of the ALARM projections of European land use, which in turn limited the species collection records available to use (see Fig. S1). Europe in the context of this study is defined as the European Union without Ireland, Romania, Bulgaria, Canary Islands and Cyprus, and including Norway and Switzerland. We created 5 × 5 km, 10 × 10 km and 20 × 20 km European grids for training the SDMs to project onto the BENELUX (Belgium, Netherlands and Luxembourg) region. We also created a 50 × 50 km European grid for training the SDMs to project onto the original spatial extent of Europe. All map projections use the European terrestrial references system 1989 (ETRS89).

2.4 | Climate and Land Use Data

Variables of current climatic conditions were produced from monthly interpolated rainfall and temperature data from 1971 to 2000, at a 10′ resolution (Fronzek, Carter & Jylhä, 2012; Mitchell, Carter, Jones, Hulme & New, 2004). We considered 14 climate variables for the modelling process (see Table S2). However, because climate variables are often strongly correlated. Including all climate variables in the models would have added redundant information. Therefore, to avoid collinearities, we conducted a selection according to Pearson correlation coefficients (<0.7; Dormann et al., 2013). When two variables were highly correlated, we selected the variable that we estimated to have the greatest ecological relevance to *Bombus* species.

We selected total annual growing degree-days (>5°c), which was correlated with other temperature variables, because it is linked to the presence of wildflowers and flowering crops, both important food sources for bumblebees. Furthermore, we chose water balance, which was correlated with the majority of other precipitation variables because it is representative not only of total precipitation, but has a direct link with temperature, making it an important influence for terrestrial vegetation (Gerten, Schaphoff, Haberlandt, Lucht & Sitch, 2004). Five climate variables were used as explanatory covariates in the model: average precipitation of the wettest month; total annual number of growing degree-days above 5°C; mean diurnal range (mean of monthly difference between daily maximum and minimum temperatures); annual temperature range (maximum temperature of warmest month–minimum temperature of coldest month); and annual water balance (mean monthly precipitation minus the monthly potential evapotranspiration; Gerten et al., 2004).

Each of the five climate variables was aggregated to the 50 × 50 km and 20 × 20 km grids, and downscaled to the 10 × 10 km and 5 × 5 km grids using bilinear interpolation (Randin et al., 2009). All spatial analyses were conducted using Rstatistics 3.3.2 (R Core Team, 2016), the Raster package (version 2.5-2; Hijmans, 2015) and ARCGIS 10.2 (ESRI, 2016).

The future land use projections were built in congruence with a set of global change scenarios and associated climate change as part of the European ALARM project (Spangenberg et al., 2012). These climate scenarios were derived from a coupled Atmosphere-Ocean General Circulation Model (HadCM3; New, Hulme & Jones, 1999) and include the scenarios as outlined in the IPCC Special Report on Emission Scenarios (IPCC, 2001). We produced the same five climate variables in the current period for each of three scenarios of climate change (BAMBU, GRAS, SEDG) in 2050 and 2100 for the four grid resolutions.

The three scenarios are:

- 'Business as Might Be Usual' (BAMBU)—IPCC A2 scenario (see Spangenberg et al., 2012, for more information); mean projected temperature rise in Europe at 2100 is 4.7°C; an intermediate change scenario based on extrapolated current socioeconomic and policy decisions.
- 'Growth Applied Strategy' (GRAS)—IPCC A1FI; mean projected temperature rise in Europe at 2100 is 5.6°C; a maximum change scenario driven by policies of deregulation and economic growth.
- 'Sustainable European Development Goal' (SEDG)—IPCC B1 scenario; mean projected temperature rise in Europe at 2100 is 3.0°C; a moderate change scenario driven by economic, social and environmental policies, related to stabilizing atmospheric greenhouse gases emissions and stopping the loss of biodiversity.

Current land use was obtained from the Coordination of Information on the Environment (CORINE) Land Cover at 250 × 250 m resolution (Bossard, Feranec & Otahel, 2000). The CORINE classes were reclassified as six classes to match the future projections. We removed the class 'others' from our analysis because it represents diverse land use types and was inexplicable in an ecologically relevant context for bumblebee species. Future land use was obtained from the ALARM EU project downscaled to 250 × 250 m for each of the three scenarios for 2050 and 2100 (Dendoncker, Bogaert & Rounsevell, 2006; Spangenberg et al., 2012). At each grid resolution, we determined the percentage cover for each land use covariate. The final five land use layers were: percentage cover arable land; percentage cover forest; percentage cover grassland; percentage cover permanent crops; and percentage cover urban.

The role of the covariates will be tested in three ways using three variable sets in the models: (1) Dynamic climate-only models, suggesting that only climate variables matter in the future distribution of bumblebee species. (2) Static land use and dynamic climate, suggesting that land use variables are important in delimiting species habitat suitability, but that their future change will be driven only by climate change and changes in land use are redundant. (3) Dynamic climate and dynamic land use, suggesting that future distribution patterns will be dependent on the interaction between changing climate and changing land use.

2.5 | Species distribution modelling

We used a SDM approach to compare the role of dynamic land use data in the future distribution patterns of bumblebees. We modelled the distribution of 48 species using R (R Core Team, 2012) with the *biomod2* package (version 3.3-3; Thuiller, Georges, & Engler, 2015). We chose an ensemble modelling approach, which creates a consensus of the predictions of multiple algorithms and is an established method to account for projection variability (Thuiller, 2014). Even small differences between algorithms can lead to different projections of future distribution change. Ensemble modelling aims to limit the many uncertainties of forecast modelling and has become increasingly used in studies of biodiversity change (Thuiller, 2014).

We chose three algorithms to include in the ensemble model based on their previous performances with analogous collection data for a similar insect species group (Aguirre-Gutierrez et al., 2013). The three algorithms chosen were a generalized linear model, GLM (Nelder & Wedderburn, 1972), with linear and quadratic effects, and stepwise selection based on the Akaike Information Criteria (AIC); a generalized boosted model, GBM (Friedman, 2001), with 3,000 trees and five cross-validation folds; and Maximum Entropy Modelling (MAXENT; Phillips & Dudík, 2008), with linear and quadratic features. We decided to choose simplicity and ecological clarity over model complexity by dropping detailed features, such as product, threshold, hinge and polynomial.

Models for each species were trained at multiple resolutions at the European scale; 5 × 5 km, 10 × 10 km, 20 × 20 km and 50 × 50 km. We had 462,636 records available to use; these were aggregated as unique species occurrences for each grid cell resolution. The number of occurrences per resolution is as follows: 67030 at 5 × 5 km, 49146 at 10 × 10 km, 30104 at 20 × 20 km and 21,162 at 50 × 50 km. We modelled 48 species (see Table S1) with at least 50 unique records, and for which there are no ongoing

taxonomic debates surrounding their species definition (see Rasmont et al., 2015). A number of occurrences in the database were not point level GPS coordinates, but were recorded as UTM grids of varying sizes. To be confident in the spatial accuracy of collection records we removed occurrences that were recorded as UTM grids larger than 1 × 1 km. As the sampling methods were diverse and nonsystematic, there are likely spatial biases amongst the records. To deal with this potential spatial autocorrelation between closely sampled locations we selected a subset of points per species. A random starting observation was selected and all points in adjacent grid cells removed; this was then repeated for all remaining points. This produced a more even spread of observations and minimized the effects of heavy sampling at particular locations.

As true absences were not available (it is not possible to accurately say that a bee species is not present during sampling) we generated randomly distributed pseudo-absences for GBM and GLM and selected a background sample for MAXENT (Elith et al., 2011; Phillips et al., 2009). We used target-group sampling to select our background points (Mateo, Croat, Felicísimo & Muñoz, 2010; Phillips et al., 2009). We specified that the background samples and pseudo-absences could only be selected from areas where other bumblebees have been recorded since 1970. This approach is more objective than taking the background and pseudo-absence samples from sites that have not been sampled, accounting for potential sampling bias (Elith et al., 2011; Phillips et al., 2009) and providing more accurate results (Mateo et al., 2010). To account for within algorithm variation we trained the models 10 times for each of the 48 species, the three algorithms, the three model hypotheses, and the four grid resolutions. This resulted in 360 models per species. We used a bootstrap approach where random subsets of 80% of the data were used for model training and the remaining 20% to produce Area Under the Curve (AUC) values to test model performance (Bahn & Mcgill, 2013; Jiménez-Valverde & Lobo, 2007). For each covariate included in the model, we calculated variable contribution as the change in correlation between the covariates and the response with and without the selected variable (Thuiller et al., 2015). We then produced an ensemble model for each of the three model hypotheses, creating a median representation of the predictions of the 10 runs and three algorithms together. We chose the median value as it is less sensitive to extreme values than the mean.

We projected the models trained at 5 × 5 km, 10 × 10 km and 20 × 20 km, onto BENELUX. BENELUX comprises no novel conditions under the scenarios (i.e., there are no conditions in BENELUX in 2100 that do not already occur within Europe). Therefore, no forecasting into unknown ecological space occurred (Fig. S2). We also projected the data trained at 50 × 50 km onto the entire European study area. For each species we produced habitat suitability maps of the median ensemble predicted distribution. One map was produced for each of the three model types at 2050, and 2100 under the three change scenarios at the 4 grid resolutions. Habitat suitability maps were converted to binary presence absence maps using the values under which specificity and sensitivity is optimized (Thuiller et al., 2015).

2.6 | Statistical analysis

Analyses were conducted on the ensemble model map projections of binary presence/absence. To compare the projected distributions of the three model hypotheses we measured the change in three distribution metrics. We calculated range change by looking at changes per species in areas of occupancy between the current and future periods. Specifically, we analysed the percentage of grid cells lost (present in the current period and absent in the future) by each species under the different scenarios and the percentage of grid cells gained (percentage of absent cells in the current period occupied in the future). To examine spatial shifts we took the centroid of the species range from the present (2000) and the future (2050 and 2100). A positive value indicates northerly shift and negative, a southerly shift.

To determine the role of the different models, (i.e. climate-only model [COM], dynamic LULC model [DLM] and static LULC model [SLM]), we created separate mixed effects models for each of the three metrics for both Europe and BENELUX projections. We included species as a random effect, as we were interested in how changes in distribution of the species vary across the different model types, periods and scenarios, and not in the inherent variation between species. Furthermore, to determine if our results are related to the structure of the data we also included the current range of the species as a covariate. Due to large numbers of zeros both range loss and range gain at the BENELUX scale were analysed with two separate mixed models: Bernouli distributed models of the probability of gain or loss and a linear mixed effects model of values given range loss/gain were projected.

Finally, in addition to presenting results for bumblebees as a group, we chose two species, *Bombus argillaceus* (Scopoli, 1763; increasing in range) and *B. veteranus* (Fabricius, 1793; decreasing in range), to look more closely at the difference between model projections with and without LULC covariates. We chose these two species as they are at opposite end of the spectrum of climate risk, both had high model performance values, both have a large number of collection records within Europe and we believe them to be representative of two futures, i.e. considerable range gain and considerable range loss, respectively (Rasmont et al., 2015). The current distribution of *B. argillaceus* is in Southern and South Eastern Europe as well as Western Asia (Rasmont & Iserbyt, 2013). In previous climate-only models of future conditions *B. argillaceus* was projected to increase its range considerably in Western Europe (Rasmont et al., 2015). *Bombus veteranus* exhibits an already patchy distribution in the plains of Northern Europe and has already declined in Belgium, shifting from an abundant species to one which is barely present (Rasmont & Iserbyt, 2013). Under future climate-only projections *B. veteranus* is expected to decrease in range considerably (Rasmont et al., 2015).

3 | RESULTS

3.1 | Model training fit and variable contribution

For models trained on the current period, we assessed model fit using AUC scores. An AUC value below 0.5 indicates a model fit that

is not better than random, values above indicate enhanced model fit. We used AUC values to compare the change in model fit per species with LULC vs. a COM (Figure 1). The mean AUC values for all species are above 0.7, indicating better than random model fit. For all 48 species, model fit improves by the addition of LULC covariates. A paired Wilcoxon rank sum test indicates that the mean difference between the AUC values of the models with LULC and the COMs is 0.013 ± 0.004 (p value <.001).

We also compared the variable contributions of the different explanatory covariates included in the models (Figure 2). Climatic variables are the most important in explaining the current distribution of all species. The total annual number of growing degree-days was included amongst the four most important variables for 44 of the species modelled. The most important LULC covariate is the percentage cover of arable land but the percentage cover of forest is also important for a number of species (Figure 2). Overall LULC variables contribute 15% of total variable importance.

3.2 | The future of bumblebees projected at the BENELUX scale

Of the distribution change metrics analysed, the largest discrepancies were found in the projected range loss (Figure 3a,b). There is considerable variability between species and between scenarios but model type has a significant effect on the projections of whether species will lose range and how much range will be lost (Table 1). Overall species are more likely to lose range under DLMs than both COMs and SLMs ($p < .001$ and .002; Table 2). However, given range loss occurs (i.e. excluding species that showed no range loss) then greater loss is projected by COMs than both SLMs and DLMs (1.3%; $p < .001$; Table 2). However, this relationship is highly variable and species specific. Under COMs 11 species show greater mean range loss averaged across scenario and resolution, however, five species show greater range loss under DLMs (Figure 3a). The relationship between projected range loss of SLMs and DLMs, while not significant at the BENELUX scale, (Table 2) also appears to be species specific, with some species below the equal projection line, indicating greater range loss under DLMs (Figure 3b). There are no significant interactions between model type and other explanatory variables, suggesting a consistent effect of model type across scenarios, periods and resolutions (Table 1).

Model type, period, scenario and resolution at which the modelling occurred significantly influence the probability of range gain (Table 1). Only 50% of species were projected to gain any range at all within BENELUX by 2100 (Figure 4a,b). The odds of range gain are significantly higher for DLM projections than for COM and SLM ($p < .0001$; Table 2). When range gain occurs there is no significant difference between COMs and DLMs, however, both projected significantly higher loss than SLMs (1.4 and 1.2%, $p < .0001$ & .03; Table 2). This can be visualized in Figure 4a, where variation

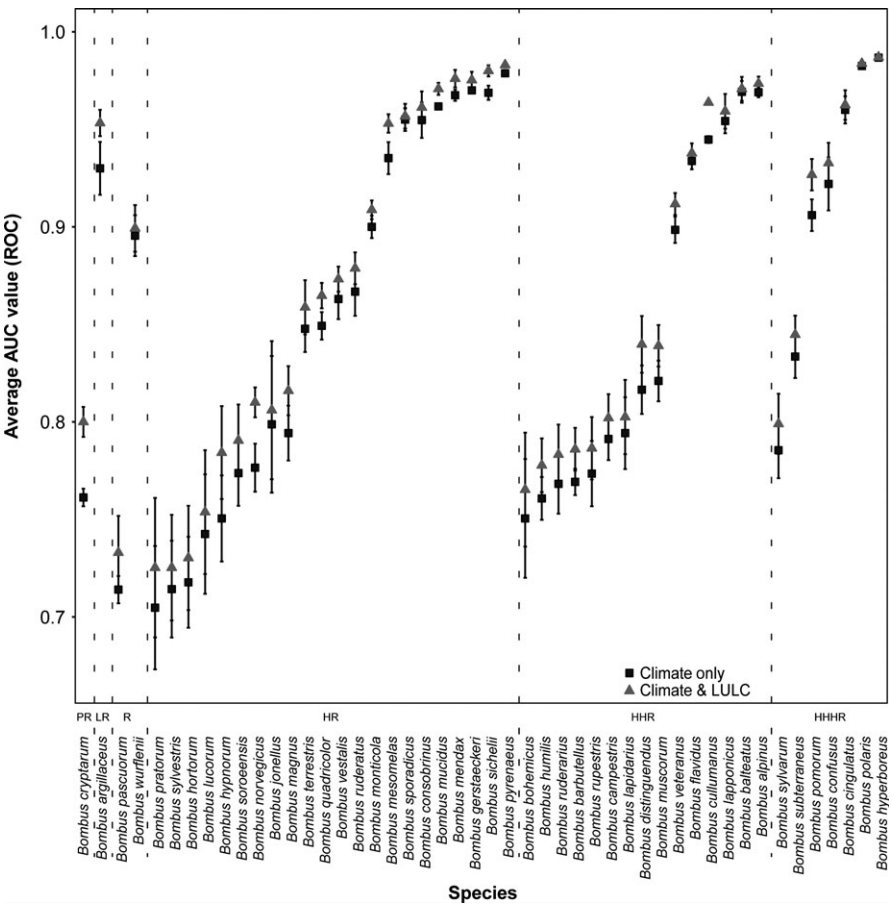

FIGURE 1 Area under the curve (AUC) statistics for median-ensemble-model performance visualized per species. Black squares represent models with only climate covariates and grey triangles models with land use land cover (LULC) covariates and climate covariates. Groupings represent Climatic risk as calculated by the Climate Risk Atlas for Bumblebees (Rasmont et al., 2015). Potential risk (PR), low risk (LR), Risk (R), high risk (HR), very high risk (HHR), extreme risk (HHHR)

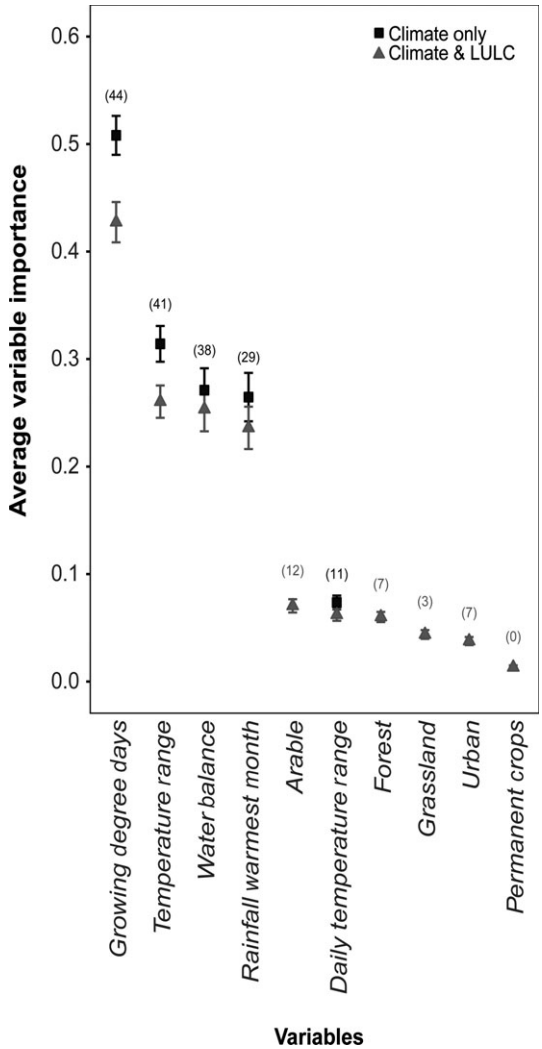

FIGURE 2 Average variable importance values and standard errors of all covariates included in the training models. Black squares represent models with only climate covariates and grey triangles models with land use land cover (LULC) covariates and climate covariates. The numbers in the brackets represent the number of species for which this variable was one of the four most important variables

between species is evenly distributed and clustered at zero and Figure 4b, where seven species have a considerably greater range gain under DLMs.

Period and scenario at which the modelling occurred significantly influence the directional shift of the distribution centroid ($p < .001$; Table 1 and Figure 1). Model type did not significantly affect the projected shift.

3.3 | The future of bumblebees projected at the European scale

At the European scale with lower spatial resolution (50 × 50 km), SLMs project significantly less range loss than both COMs and DLMs (2.9% and 1.7%; $p = <.001$ and .02, Table 2). Overall, all 48 species are projected to lose at least some range and the relationships between the different model types shows a strong linear correlation, but with considerable deviation from the assumption of the projections being equal (Figure 5a,b). Eighteen species are projected to lose greater range under COMs whilst fourteen species are projected to lose greater range under DLMs (Figure 5a). The relationship between DLMs and SLMs is clearer with a higher number of species below the equal protection line than above, which supports the significant effect found in the mixed models (1.21%, $p < .01$; Figure 5b and Table 1).

At the European scale greater range gain is projected by COMs than SLMs and DLMs (2% and 1.6%; $p < .001$; Table 2). DLMs project greater range gain than SLMs (1.2%, p value = .01; Table 2). This relationship is visible in Figure 6a with the majority of species considerably above the equal projection line. The same pattern is observed for SLMs and DLMs, with 12 species below the equal projection line. The majority of species only illustrate modest range gain, and the differences between model types are emphasized when range gain is high (Figure 6a,b).

Centroid distributional shifts are greater under COMs than SLMs and DLMs (48.2 and 51.7 km; $p < .001$). There is no significant difference in centroid distributional shift between SLMs and DLMs (Figure 7).

3.4 | The role of other explanatory variables in the mixed models

Scenario, period, and resolution are included in the majority of best models. The effect of these explanatory variables is consistent across the different distribution change measures and scales. The more extreme change scenario (GRAS) projects greater loss and northern shift of the centroid than business as usual (BAMBU) and sustainable scenarios (SEDG). The probability of range gain is lowest under the GRAS scenario and the largest range gain occurs under SEDG. In the period 2000–2050 lower percentage range loss, and lower centroid shift were projected. The SEDG scenario showed a significant interaction with period with range loss and centroid shift much lower for the period 2050–2100. The effect of resolution at the BENELUX scale did not interact significantly with model type, however, overall lower range loss and greater gain occurs at the finer resolutions. Finally, the current size of the distribution was also included in some best models, at the European scale more widespread species lose less and gain more range (for full details of all models see Table S3 and Figs. S3–S10).

3.5 | Focus on one atypical and one representative species

Bombus argillaceus is atypical compared to the majority of European bumblebees. It is one of only two species projected to increase in range under climate change. At the 5 × 5 km resolution *B. argillaceus* increases in range and latitude under all model types and scenarios. The projected range gain percentage is larger for COMs (BAMBU: 16%, GRAS: 42%, SEDG: 14%; Figure 8a–c) than DLMs

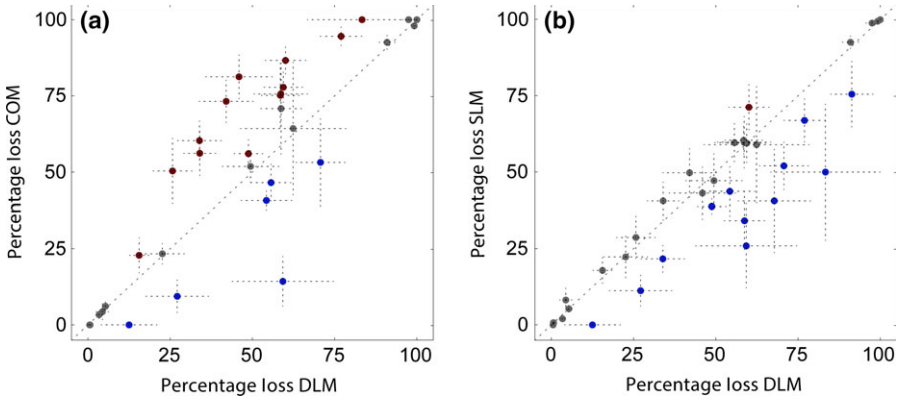

FIGURE 3 Comparison of percentage loss projections between model types for BENELUX 2000–2050. (a) Climate-only Models (COM) and Dynamic Land Use Models (DLM) and (b) Static Land Use Models (SLM) and DLM. (a) $N = 36$, (b) $N = 38$. Results are averaged across resolution (5 × 5, 10 × 10 and 20 × 20 km) and scenario (BAMBU, SEDG, GRAS), represented by standard error bars (dashed lines). The equal projection line (dashed line 0,0 to 100,100) represents the point at which the two model projections are equal. Red = above the equal projection line, Blue = below the equal projection line, Grey = overlapping the equal projection line

TABLE 1 Effects of SDM variability on the distributional change in bumblebees

	BENELUX (20 × 20, 10 × 10, 5 × 5 km)					Europe (50 × 50 km)		
Explanatory variables	Percentage gain	Centroid shift	Probability of gain	Percentage gain	Centroid shift	Percentage loss	Percentage gain	Percentage loss
Single terms								
Range size present Europe	***	***	–	–	–	***	***	***
Model Type (COM, DLM, SLM)	***	***	***	**	–	***	***	***
Period (2000–2050, 2050–2080)	***	***	***	***	***	***	***	***
Scenario (BAMBU, GRAS, SEDG)	–	***	***	***	***	***	–	***
Resolution (20 × 20, 10 × 10, 5 × 5 km)			***	–	–			
Two-way interactions								
Range size present × model type	–	–	–	–	–	–	–	–
Range size present × period	***	***	–	–	–	–	***	–
Range size present × scenario	–	–	–	–	–	–	–	–
Range size present × resolution			–	–	–			
Model Type × period	–	–	***	–	–	–	–	–
Model Type × scenario	–	–	–	–	–	–	–	–
Model Type × resolution			–	–	–			
Period × scenario	–	***	***	–	–	***	–	***
Period × resolution			–	–	–			
Scenario × resolution			–	–	–			
Degrees of freedom	856	847	1617	726	1361	853	856	853

p values: $.01 \leq p \leq .05$ = *, $.001 \leq p \leq .01$ = * * and <.001 = ***.
The most parsimonious models as chosen by Bayesian information criteria (BIC) for the percentage range loss, percentage range gain, and shift in the distributional centroid for 48 bumblebee species at European and BENELUX scales. The significance of each term included in the model is shown. The symbol "–" represents a variable not included in the best model. The random term for all models was '1 | species.' For a detailed version of the table see Table S3.

(9%, 34%, 7%; Figure 8d–f) or SLMs (10%, 36%, 10%; Figure 8g–i). At the BENELUX scale only new areas of habitat suitability are projected. At the European scale we observe that *B. argillaceus* is one of the few species to significantly increase in range. This range gain is much less under SLMs and DLMs than COMs. Under COMs *B. argillaceus* is projected to gain considerable range in the West and East

TABLE 2 Pairwise comparisons between model types

Contrasts	BENELUX (20 × 20, 10 × 10, 5 × 5 km)					Europe (50 × 50 km)		
	Percentage gain	Centroid shift (km)	Probability of gain (Odds Ratio)	Percentage gain	Centroid shift (km)	Percentage loss	Percentage gain	Percentage loss
COM—DLM	1.62***	51.7***	0.30***	1.17	NA	1.17	1.62***	1.17
COM—SLM	1.97***	48.2***	0.58*	1.45**	NA	2.91***	1.97***	2.91***
DLM—SLM	1.21**	−3.5	1.93***	1.24*	NA	1.74*	1.21**	1.74*

p values: .01 ≤ *p* ≤ .05 = *, .001 ≤ *p* ≤ .01 = ** and <.001 = ***.
Showing the fixed effect and the significance of the best models as chosen by Bayesian information criteria BIC. Null hypothesis tested: that the difference between contrasts is equal to 0. Values are averaged over other explanatory variables included in the model (see Table S1.)

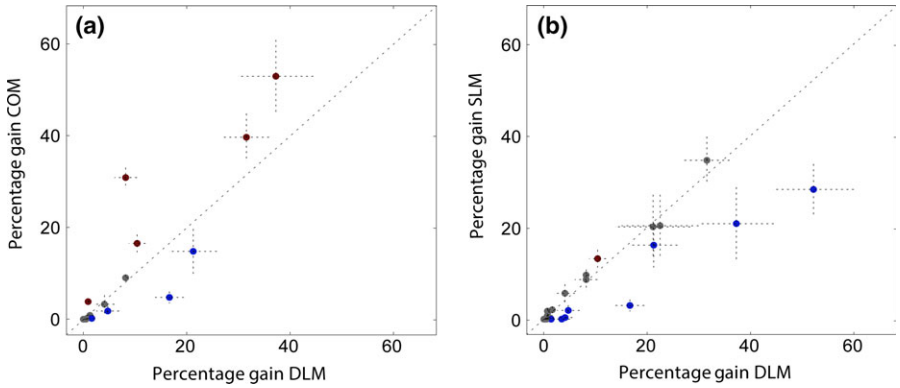

FIGURE 4 Comparison of percentage gain projections between model types for BENELUX 2000–2050. (a) Climate-only Models (COM) and Dynamic Land Use Models (DLM) and (b) Static Land Use Models (SLM) and DLM. (a) *N* = 25, (b) *N* = 35 bumblebee species in BENELUX for 2000–2050. Results are averaged across resolution (5 × 5, 10 × 10 and 20 × 20 km) and scenario (BAMBU, SEDG, GRAS), represented by standard error bars (dashed lines). The equal projection line (dashed line 0,0 to 70,70) represents the point at which the two model projections are equal. Red = above the equal projection line. Blue = below the equal projection line. Grey = overlapping the equal projection line

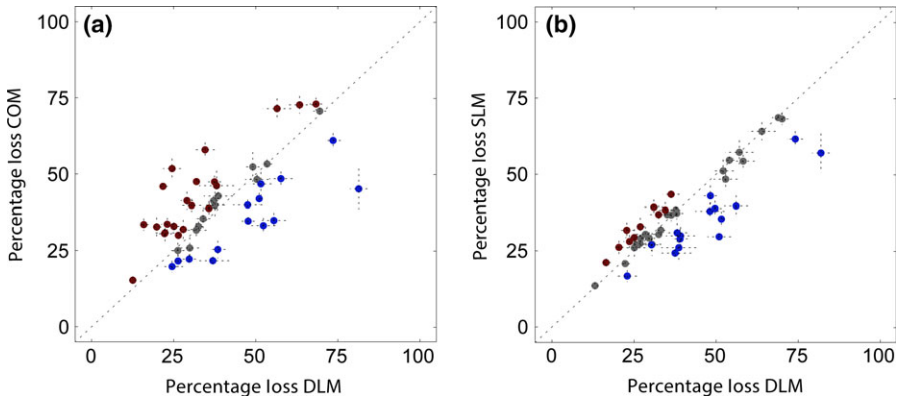

FIGURE 5 Comparison of percentage loss projections between model types for Europe 2000–2050. (a) Climate-only Models (COM) and Dynamic Land Use Models (DLM) and (b) Static Land Use Models (SLM) and DLM. *N* = 48. 50 × 50 km resolution. Results are averaged across scenario (BAMBU, SEDG, GRAS), represented by standard error bars (dashed lines). The equal projection line (dashed line 0,0 to 100,100) represents the point at which the two model projections are equal. Red = above the equal projection line. Blue = below the equal projection line. Grey = overlapping the equal projection line

of Europe (Figure 9). A large amount of the projected range loss is in areas with novel climatic conditions, making the predictions unreliable.

Bombus veteranus is one of the numerous European bumblebee species projected to lose a large part of its suitable habitat under climate change; it is therefore representative of the majority of

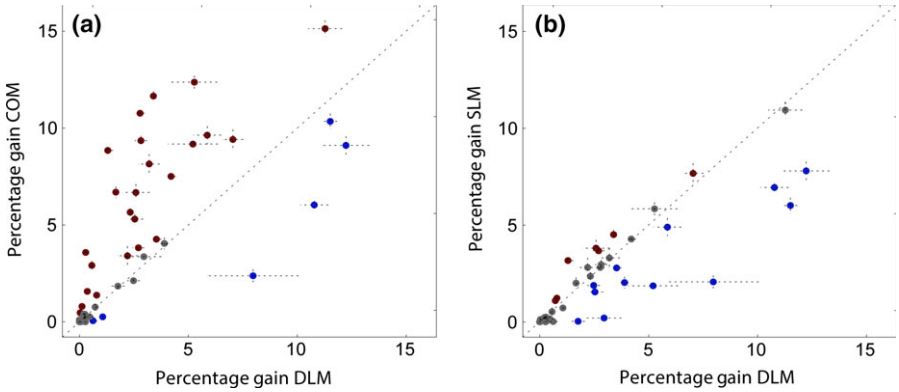

FIGURE 6 Comparison of percentage gain projections between model types for Europe 2000–2050. (a) Climate-only Models (COM) and Dynamic Land Use Models (DLM) and (b) Static Land Use Models (SLM) and DLM. N = 48. 50 × 50 km resolution. Results are averaged across scenario (BAMBU, SEDG, GRAS), represented by standard error bars (dashed lines). The equal projection line (dashed line 0,0 to 15,15) represents the point at which the two model projections are equal. Red = above the equal projection line. Blue = below the equal projection line. Grey = overlapping the equal projection line

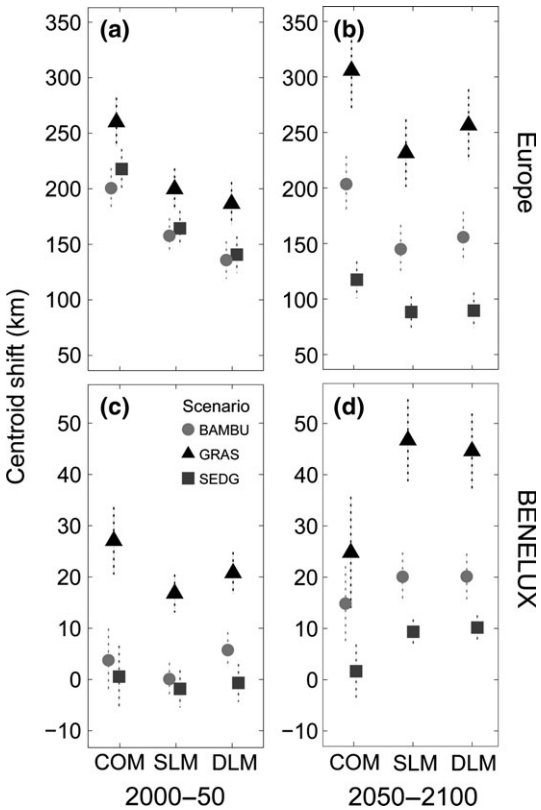

FIGURE 7 Mean and standard error of directional shift of species distribution centroid. For Climate-only Models (COM), Dynamic Land Use Models (DLM) and Static Land Use Models (SLM) at Europe at 2050 (a) and 2100 (b) and BENELUX at 2050 (c) and 2100 (d) for three change scenarios (BAMBU, GRAS, SEDG)

bumblebees in Europe. *Bombus veteranus* under BAMBU and GRAS is expected to lose almost its entire suitable habitat in BENELUX. The species is not projected to go extinct at 5 × 5 km resolution, but projections of the GRAS scenario show only a tiny pocket of remaining suitable habitat in South-east Belgium (Figure 5k,n,q). Significant gain is only projected under SEDG for COMs (25%; Figure 8l). At the European scale *B. veteranus* loses more range under COMs (54%, 67%, 38%; Figure 9j–l) than SLMs (32%, 50%, 19%; Figure 9p–r) and DLMs (40%, 55%, 26%; Figure 9m–o). *Bombus veteranus* is projected to expand into Northern Europe, further under COMs. Overall SLMs project more persistence in the landscape but less Northern shift. Finally, the centroid of the distribution of *B. veteranus* is projected to shift further North overall under DLMs than SLMs (BAMBU: +95 km, GRAS: +68 km SEDG: +98 km, Figure 9m–r).

4 | DISCUSSION

This study shows that incorporating dynamic LULC change scenarios, even those with low precision and few classes, can have significant effects on the projected distributions of bumblebee species. Species can only occur in a location at any time when a series of critical conditions are met, including both suitable climate and land use and land cover types that allow them to feed, grow, survive and reproduce. Therefore, it is surprising that the use of climate change projections is commonplace, whereas LULC change projections are rarely used in species forecasting (Titeux et al., 2016). We tested the effect of dynamic LULC variables on projecting future distribution changes for 48 European *Bombus* species in 2050 and 2100. *Bombus* being a genus for which change in major land use classes has affected historical distribution patterns (Aguirre-Gutiérrez et al., 2015; Senapathi et al., 2015).

4.1 | Models including LULC compared to climate-only models

All models improved in fit (AUC) when adding LULC covariates. However, this refers to goodness-of-fit and does not necessarily mean greater predictive ability (Thuiller et al., 2004). A number of

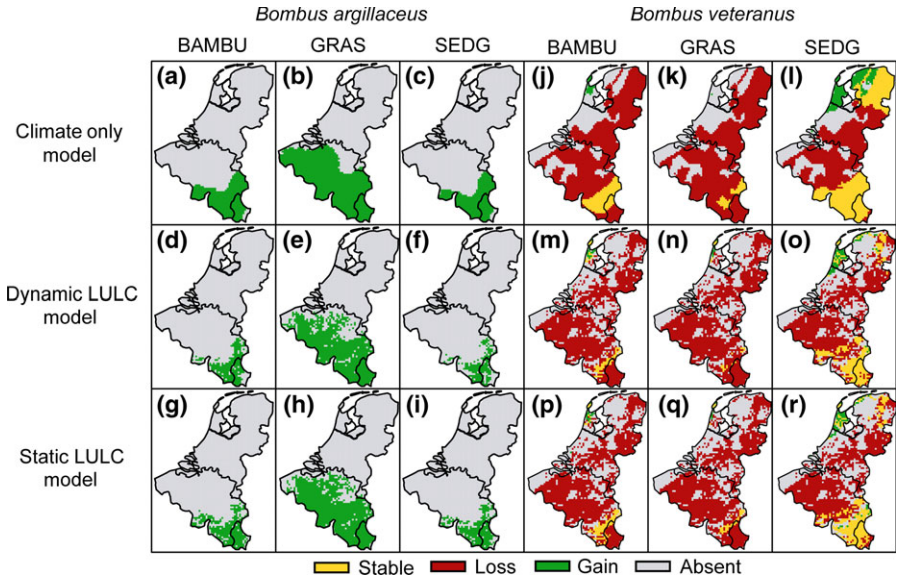

FIGURE 8 BENELUX maps showing 5 × 5 km resolution of change in habitat suitability between 2000 and 2100 for two species, *Bombus argillaceus* (a–i; atypical) and *Bombus veteranus* (j–r; representative of many species). Habitat suitability change is shown for three future change scenarios (BAMBU, GRAS, and SEDG) and for three model types (Climate-only [a–c, j–l], Dynamic LULC [d–f, m–o], and Static LULC [g–i, p–r]). Yellow: cells that have remained as suitable habitat; Red: cells that were suitable in 2000 but unsuitable in 2100; Green: cells that were unsuitable in 2000 but suitable in 2100; Grey: cells that were never projected as suitable habitat

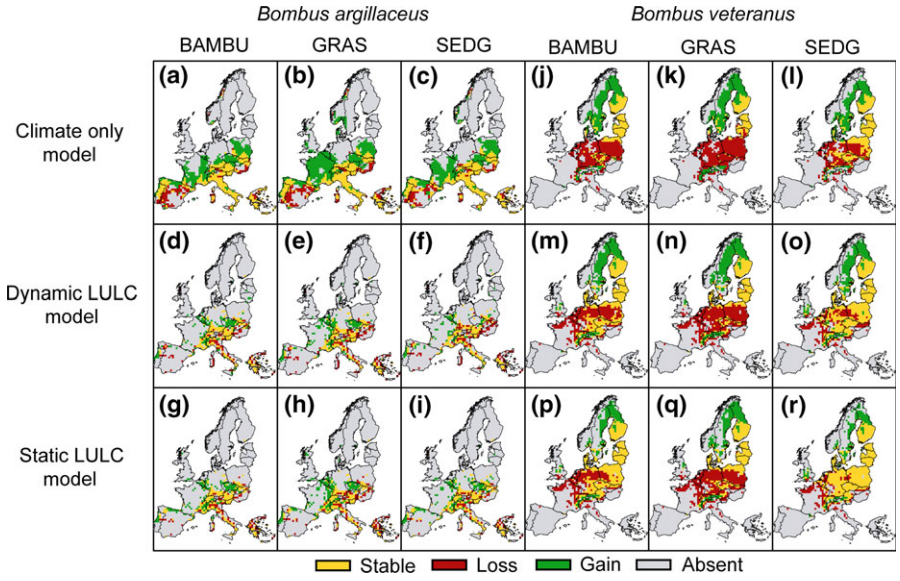

FIGURE 9 European maps showing 50 × 50 km resolution of change in habitat suitability between 2000 and 2100 for two species, *Bombus argillaceus* (a–i; atypical) and *Bombus veteranus* (j–r; representative of many species). Habitat suitability change is shown for three future change scenarios (BAMBU, GRAS, and SEDG) and for three model types (Climate-only [a–c, j–l], Dynamic LULC [d–f, m–o], and Static LULC [g–i, p–r]). Yellow: cells that have remained as suitable habitat; Red: cells that were suitable in 2000 but unsuitable in 2100; Green: cells that were unsuitable in 2000 but suitable in 2100; Grey: cells that were never projected as suitable habitat

species are influenced by LULC covariates, in particular the percentage cover of arable land and forest. The results support research showing that using only climate covariates may over-represent the species range in the present (Luoto et al., 2007; Sohl, 2014; Stanton, Pearson, Horning, Ersts & Reşit Akçakaya, 2012). This is likely to misrepresent species range change as well as the shift of species range limits. The importance of LULC change is dependent on whether habitat requirements, namely nesting and feeding resources (Busch, 2006), can be adequately captured by the relationship between these six land use covariates and the climate change covariates. Therefore, we saw variation for bumblebees as they differ significantly in their landscape requirements (Goulson et al., 2010; Persson, Rundlöf, Clough & Smith, 2015). A result unique to our study is that COMs (at the European scale) projected greater range

loss and lower range gain than when land use covariates were included. This is in part due to greater range size in the present under COMs. However, there were also examples of areas that became suitable for certain bumblebees with the introduction of LULC covariates. These results suggest that for some species including LULC covariates, projects, on average, a wider bioclimatic envelope and is more likely to project persistence in the landscape. In other words LULC covariates, provide a habitat filter over the climate prediction. However, we did not observe the same pattern for all species, and there were species, which showed greater loss and gain with dynamic land use covariates. Overall, the relationship was highly variable (see Figs. S7–S10). This inconsistent relationship indicates that dynamic LULC model predictions are not simply a level up or down from climate-only models. Additionally, the introduction of LULC covariates projected an inability of most bumblebees to completely track Northern climate shifts, particularly into Scandinavia, supporting historical patterns (Kerr et al., 2015).

4.2 | Models including dynamic LULC compared to static LULC models

Including static LULC change in SDMs is based on the incorrect assumption that LULC will not change in the future or that this change is negligible in comparison to climate change (Stanton et al., 2012). In this study, loss and gain of suitable habitat was more likely with dynamic LULC covariates. The distribution patterns of DLMs represent more variable suitable habitat conditions in time than SLMs under equivalent climate change, resulting in greater projected range loss and gain. However, this pattern varied between species and was more discernable for some over others. This variability is supported by other studies; including dynamic LULC covariates previously led to more accurate model predictions for invasive bullfrogs (Ficetola et al., 2010) and central European plants (Chytrý et al., 2012), but not so for a European butterfly species (Martin et al., 2013). Our multispecies study indicates that a number species show projected distribution changes under different model types, however, some do not show any. This, in and of itself, is not surprising as species differ in their dependency on specific characteristics of climate and land use. Therefore, including dynamic LULC covariates, even at coarse thematic resolution, can significantly alter the projected distributional changes of certain species.

4.3 | Inclusion of LULC in models for individual species distribution projections

We focused on the projections of two species, *B. argillaceus* was atypical compared to the majority of species, demonstrating range. The results suggest that dynamic LULC limits the availability of suitable habitat in the North. Overall, this illustrates the necessity of dynamic LULC in prospective SDMs, and that change in major land use classes such as grassland and urban affect observed species range change under climate change. *Bombus veteranus* is representative of the patterns observed for many species. Climate drove the distribution but LULC models projected extra areas of suitable habitat, which were rarely continuous and perhaps indicative of real world patterns. Fragmented suitable habitat increases the probability of losing local populations and decreases the probability of establishing new populations, both of which severely affect a species' tracking of global change.

4.4 | LULC-inclusive models for forecasting and guiding conservation efforts

The importance of including LULC projections depends on the goals and desired outcomes of the modelling process. As a tool, SDMs explore unknown areas and periods where conditions may be suitable for species occurrence, observe the role of environmental covariates and influence conservation management (Franklin, 2010). However, due to limitations in data availability and modelling methods their value to conservation and ability to predict occurrence should not be overestimated (Lobo, 2016), particularly in the case of undersampled and geographically and taxonomically restricted insect data (De Palma et al., 2016). Regarding covariate influence, we observe that for at least some species dynamic LULC covariates significantly affect projected distributions. Regarding conservation management, variation between model types, model performance and projected distributions suggests that using DLMs to inform conservation practices would be suitable at the broad scale. The absence of dynamic LULC covariates could lead to significantly underfitted potential distributions for specific landscapes or species with implications for management. (Franklin, 2013; Porfirio et al., 2014; Wright, Hijmans, Schwartz, Shaffer & Franklin, 2015). Overall, species and purpose-specific approaches to covariate selection should be preferred.

4.5 | The generation of dynamic LULC scenarios deserves more attention

The observed patterns strongly support the case for more detailed LULC change scenarios. This supports the conclusions of similar studies (Barbet-Massin et al., 2012; Martin et al., 2013). The scenarios presented here intend to provide a platform on which to relate species conservation to socioeconomic factors and policy decisions, they also aim to make it possible to assess which improvements at landscape level are needed to improve species persistence (Van Vuuren et al., 2011). However, it is likely that the LULC change maps produced by these scenarios will become superseded by updated, more detailed LULC change scenarios, linked to new climate change models. Finer resolution and more detailed classes would greatly improve LULC projections (Busch, 2006; Verburg, Van De Steeg, Veldkamp & Willemen, 2009). In the case of bumblebees, we know that to model wild bee species adequately we need ecologically relevant LULC covariates that represent local management (Aguirre-Gutiérrez et al., 2015; Marshall et al., 2015; Scheper et al., 2015). New scenarios should emphasize a relevance to biodiversity and land use management, for example, separating between natural-grassland

and agricultural-grassland, and intensive and less intensive farming systems. While the incidence of and change in forest and arable land cover correlates with habitat suitability, this is an indirect effect. The coarseness of these classifications does not provide an adequate foundation to extract causal information or infer on the importance of land use management (Thuiller et al., 2004). Moreover, national and international policies, such as the CAP in Europe, tend not to change land cover per se (grassland remains grassland), but the management level and thus biodiversity value. For example, arable land cover is the most important LULC covariate for the majority of bumblebees as defined by the correlative variable importance values (see Table S3). However, the ecological significance of this importance could relate to agricultural intensification, pesticide use, availability of floral resources, or most likely, a combination of these factors.

4.6 | Differences between the data sources

Among the 48 bumblebees modelled there are examples of polytypic species representing significant intraspecific variation (Rasmont, 1983). For example, SDMs at subspecies level for *B. terrestris* performed differently from aggregated models with all subspecies as a single unit (Lecocq, Rasmont, Harpke & Schweiger, 2016). We did not utilize this variation; we modelled the habitat requirements of each species using all available records. Occurrence points were selected to represent the highest resolution possible to model at 5 × 5 km resolution, and many points were removed. However, due to the nature of the data and the multitude of sources it is still likely that some point records were not accurately recorded, though we expect this number to be minimal (Duputié, Zimmermann & Chuine, 2014).

There were distinctions between the resolution of the climate and land use sources in the past and in the future. Due to the coarse nature of Atmosphere-Ocean General Circulation Models (AOGCMs) used to calculate the climate-change covariates they do not represent accurately fine scale effects (Fronzek et al., 2012). This means at the 10 × 10 and 5 × 5 km resolutions that fine-scale topographic effects of climate may be lost. This may result in a more homogenous representation of climate at these resolutions, which may over-represent range size and connectivity. However, this is representative of climate data often used in studies of this type to model in the future, and in general climate is more homogenous than land use, particularly at the BENELUX scale. To understand in detail the climate effects on biodiversity, fine scale climate change projections are required. The land-use change maps were downscaled to match the availability of current LULC data at European scale. However, the downscaling algorithm is likely to produce some clustering for the future LULC covariates (Dendoncker et al., 2006). Therefore, we focused on percentage cover covariates and it was not possible to include covariates of connectivity and edge effects, as they would not be comparable to present conditions. Furthermore, the land-use change models were created in congruence with climate variables; this means that present and future comparisons are valid at the different modelled resolutions (Rounsevell et al., 2006).

Finally, there are many methods for SDM and changes to the modelling algorithms, covariates and resolutions can affect the results (Aguirre-Gutierrez et al., 2013; Warren & Seifert, 2011). We chose to use simplified algorithms in an ensemble modelling approach to account for this variation (Thuiller, 2014).

5 | CONCLUDING REMARKS

This work represents a detailed analysis of the effect of dynamic LULC scenarios at different scales on the projected distributions of multiple species. We show species dependent responses to the effect of dynamic LULC, which demonstrates that these types of scenarios can play a significant role in projecting species distributions under climate change. Climate variables alone, whilst driving habitat suitability, are unlikely to project accurately the detailed distribution patterns of all species. Therefore, we advocate repeated use and testing of these available scenarios with multiple species. However, new scenarios and projections of LULC change at finer spatial and thematic resolutions that indicate management practices will be necessary to better assess biodiversity change in an uncertain future.

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SUPPORTING INFORMATION

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