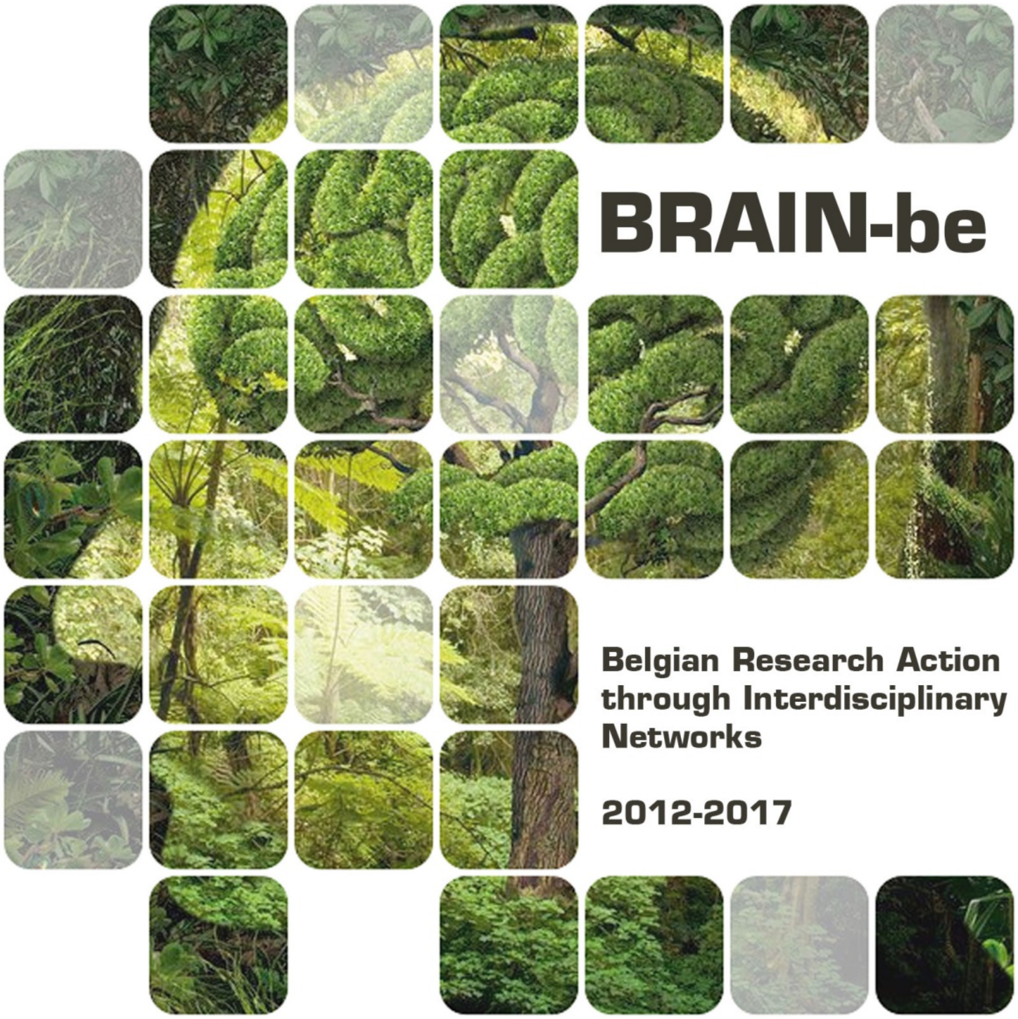

BRAIN-be

Belgian Research Action
through Interdisciplinary
Networks

2012-2017

BELBEES Project

Multidisciplinary assessment of BELgian wild BEE decline to adapt mitigation management policy

Pierre Rasmont (UMONS) – Jean-Luc Boevé (RBINS) – Dirk de Graaf (UGent) – Nicolas Dendoncker (UNamur) – Marc Dufrêne (ULg, Gembloux Agro Bio-Tech) – Guy Smagghe (UGent)

Axis 1: Ecosystems, biodiversity and evolution

NETWORK PROJECT

BELBEES Project

Multidisciplinary assessment of BELgian wild BEE decline to adapt mitigation management policy

Contract - BR/132/A1/BELBEES

FINAL REPORT

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ABSTRACT

The BELBEES project allowed to carefully assess the past and present situation of the wild bee populations in Belgium. The Red List of Belgian wild bees prepared by the BELBEES project shows that on the 399 wild bees recorded in the country: 45 species (12%) are now extinct, 139 species (35%) are threatened or declining, 162 species (41%) are stable or expanding while 53 species (13,3%) are not assessable due to deficient data. Compared to the 1993 assessment, the situation clearly got worse: while already 12% of species appeared declining in 1993, there are now nearly half of the species (48%) that are declining or even extinct. For bumblebees, our analysis revealed that thistles constitute a very important food resource. Their most threatened species are highly dependent on these plants. Floral resource availability decreased significantly, thus threatening most of the specialist bee species and forcing the generalist ones to shift their foraging to other plant species. This resource shift stresses their nutritional fitness. The main agriculture crops in Belgium do not require insect pollination. However, some large areas in Vlaams Brabant and Limburg are devoted to fruit production that could highly suffer from pollinator loss. Climate change appeared as a main threat against bumblebees because most of them are very sensitive to heat stress (occurring during heat waves). Models show that most species may disappear in the next decades. While urbanization has been clearly involved in change in bumblebee fauna, land use and climate change appear as linked factors. No population structuring was found at country scale (Belgium) and only low levels of structuring in an international sampling for seven bumblebee species at continental scale (Europe). This indicates that there is no limitation in gene flow, not even for the restricted bumblebee species. A careful assessment on microbial pathogens in selected wild bee species showed that they include numerous previously unknown taxa, with few or no connection with honey bee diseases. A pilot study with honeybees has been conducted to test the feasibility of adopting a biomarker-driven approach for studying insecticide-induced detoxification mechanisms in bees. Two gene expression biomarkers can be used as an indicator for imidacloprid-induced stress in natural field condition. Meta-analyses to address the multiple effects of drivers showed that there is a significant effect of environmental variables (land use, agriculture intensification, floral resources, altitude) on bee community changes at the regional scale in Belgium, with differences between bee groups. The models reveal that more factors drive shifts in bee communities, and probably local environmental conditions could be underestimated to predict bee species occurrence at large spatial scale. As recommendations and mitigation for Belgian wild bees, we advocate to the inclusion of a significant number of wild bees in habitat protection regulation (threatened ones mentioned in the Red List of Belgian wild bees). Agri-environment Measures (MAE) should maximise the inclusion of relevant foraging flowers like leguminous plants. We recommend the abrogation of the present regulation that constrain the destruction of thistles of agricultural sowed areas. We recommend to study wild bees friendly practices that could specially fit for cities. We underlined the importance to prevent all importation of uncontrolled domesticated bees.

Keywords: Pollination, Conservation Biology, Belgian Wild Bee Decline, Multiple Factors

1. INTRODUCTION

"Seigneur, préservez ceux que j'aime
Frères, parents, amis, et mes ennemis même,
Dans le mal triomphants,
De jamais voir, Seigneur, l'été sans fleurs vermeilles
La cage sans oiseaux, la ruche sans abeilles,
La maison sans enfants."
[Victor Hugo, Les feuilles d'automne, 1832]

In an autobiographic interview, the famous Vietnamese General Giap, who severely fought against the French occupancy, was quoting Victor Hugo, in perfect French. In the poetry that Giap quoted, Victor Hugo explained that nothing can be worse than a life without flowers, birds, bees or children.

We can guess that this could be seen as a simple but very universal way to understand what could be the biodiversity loss. We all hope to live surrounded by flowers, a lot of flowers, birds, many of them, bees, of course more than one bee, and kids. Any failure in these points would be a terrible restriction in our life pleasure.

The publishing of the book "Silent Spring" by Rachel Carsin in 1962 has been the first major alarm about the biodiversity loss. If we continue to use pesticides without care explained Carson, most birds will vanish. This terrible warning deeply upset the occidental civilization. However, the word "biodiversity" itself now so widely mentioned has been invented by E.O. Wilson, as late as 1992, in his "The Diversity of Life". Between these two major steps in ecological awareness, concern about wild bees already emerged.

A serious concern about global biodiversity emerged in the 1960s, leading during the 1990s to an increase focus on Nature Conservation. In this context, important steps were achieved by the Habitat Directive (1992), the Rio Declaration on Environment and Development (1992) and the Aarhus convention (1998). For pollinators, the Sao-Paolo declaration on Pollinators (1999) was the first significant international notification about the key-role of pollinators and their threatened status. The 2016 IPBES report confirmed the importance of pollinator conservation as a symbol of biodiversity conservation.

Belgium is at the centre of this global awareness. The country has one of the most fragmented landscapes among European countries. Most Belgian lands were used over centuries to produce food, timber and fuel and to provide living spaces, leading the whole country to be currently under some form of direct management. Consequently, species occurring in Belgium are to a large extent dependent upon habitats created and maintained by human activity, particularly traditional, non-intensive forms of land management. These habitats are under pressure from agricultural intensification, commercial forestry, urban sprawl, infrastructure development, land abandonment, acidification, and eutrophication. Many species are directly affected by overexploitation as well as alien invasive species, while climate change is set to become an increasingly serious threat in the future.

2. STATE OF THE ART AND OBJECTIVES

Survey of wild bees in Belgium and Western Europe

The 1970s

As far as known, the first warning about the regression of wild bee species came from Peters (1972) in Germany, then from Gaspar *et al.* (1975) in Belgium. These authors underlined that several wild bee species formerly common were disappearing. For several species, as *Melecta luctuosa* or *Coelioxys* spp., this warning was documented with a detailed monitoring and time-series (Gaspar *et al.* 1975). In the same time (1976), Jean Leclercq was explaining in his Zoological lectures at Gembloux, that several bumblebee species completely disappeared from his natal Pays de Herve, the most noticeable being *Bombus sylvarum.* This species, he explained, was very abundant everywhere in Pays de Herve before the WWII but disappeared completely during the 1960s.

Balls (1914; 1921) and Bols (1939) presented a Belgian bumblebee fauna that included about 30 species. Bols (1939) wrote [*en 1937, j'ai observé*] *" en octobre, une dizaine de B. lapidarius s'y sont encore ajoutées de plus une dizaine de B. ruderatus Fab,, une dizaine de B. terrestris L., quelques B. sylvarum L., "* and the next year *" le 26 juin, entre 5 et 6 h. du soir, 50 Psithyrus rupestris ♀♀ et 30 B. ruderatus ♀♀ se cachant individuellement sous les feuilles sèches"* and further, he observed *"les Bombus hortorum Linn., B. pratorum Linn., B. soroeensis Fab., B. hypnorum Linn., B. latreillellus Kirby, B. muscorum Fab. [...] liées localement à l'une ou l'autre flore"*. In the 1970s, it was already impossible to make such records. All these species already disappeared not only from the surrounding of Leuven, where Bols made these observations, but in many parts of Belgium.

The 1980s

The 1980s were marked in adjacent countries of Belgium by very few people involved in wild bee studies and monitoring. One of the worst issues was the collapsing of the Monkswood station that was a founding partner of the EIS-CIE-EEW.

Williams (1982) was the first to clearly quantify the threat in South-England, hypothesizing that landscape fragmentation was the main cause.

Rasmont (1988) and Rasmont & Mersch (1988) made a first quantitative assessment of the Belgian bumblebee fauna. Their monitoring led to the hypothesis that the restructuration of agriculture was the main trigger. An obvious proxy was the total number of horses that passed from 253400 in 1908 to only 26000 in 1985. Another factor that emerged from the agricultural statistics was the past importance of leguminous crops in the landscape. The total crop area of clover, lucern and sainfoin decreased from 163700 ha in 1908 to less than 2500 ha in 1985. As most of the species that vanished from Belgium were Fabaceae visitors, these authors made the relation between this loss of resources and species regression.

The 1990s

The first comprehensive monitoring of the whole Belgian wild bee fauna by Rasmont *et al.* (1993) was, probably also worldwide, a first quantitative assessment at a national scale. On the basis of a statistical method that allows to compare the fauna before and since the pivotal-year of 1950 (Stroot & Depiereux 1989), the conclusions were as follows:

- The survey included museum data for 181894 specimens;
- The Belgian wild bee fauna included 360 species for this time interval;
- From 360 apoid species known in Belgium during the studied period, 91 were decreasing (25%), 145 were more or less stable (40%), 39 were expanding (11%), and 85 were in an undetermined situation (rare species: 23,5%);
- The long-tongued species suffered from a considerable regression compared to the short-tongued ones, suggesting that a crisis of resources in zygomorphic flowers was involved, and thereby validating the Rasmont & Mersch (1988) hypothesis about the role of the drastic regression of Fabaceae crops;
- The cleptoparasitic species also showed a significant regression, including the species that parasite the short tongued bees; the authors made the assumption that it would be the indication of a decrease in population number, even if the area occupied by each short-tongued species did not reduce.

The 2000s

Important initiatives appeared at the end of 1990s and in the early 2000s: the first comprehensive survey of wild bees in the Netherlands (Peeters *et al.* 2000), a first checklist (Rasmont *et al.* 1995) and then a working group "Apoidea Gallica" founded in France. The “Hymenoptera Deutschland” (Aculeata.de) founded in Germany; and in the 2000s the launching of “Bees, Wasps & Ants Recording Society” in UK.

In 2010 was launched the STEP project (*Status and Trends of European Pollinators*), a first international initiative to evaluate wild bee populations at the continental scale. This project targeted a comprehensive assessment of the pollinators of the EU, with a special focus on bees (Potts *et al.* 2015). The project delivered a Red List of European Bees to help direct conservation efforts at the national and continental level (Nieto *et al.* 2014). The project provided a multi-scale and multi-species assessment of the shifts in pollinators across Europe (Bommarco *et al.* 2012), including Belgium (Carvalheiro *et al.* 2013), and it identified simple factors such as climate change (Rasmont *et al.* 2015) and pesticides (Sandrock *et al.* 2014), as well as the key combinations of drivers of change (Gonzalez-Varo *et al.* 2013). STEP also determined which pollinators actually pollinate crops (Riedinger *et al.* 2015), thereby contributing to targeted mitigation measures for those taxa of highest economic importance.

Many recent studies that focus on regional fauna, particular drivers and ecological traits, confirm the early hypotheses proposed in the 1980s on bee decline. As a snapshot of the declining species, most of them are:

- Species foraging on declining resources like Fabaceae or Lamiaceae (e.g. Scheper *et al.* 2014);
- Species sharing parasites with domesticated species (e.g. Murray *et al.* 2013);
- Cold adapted species (e.g. Kerr *et al.* 2015; Rasmont *et al.* 2015) ;
- Species foraging on intensive crops (e.g. Woodcock *et al.* 2016).

Factors of bee decline in Belgium

Changes in agricultural practices (i.e. agricultural motorisation and mechanisation, regression of leguminous crops, introduction of nitrogenous fertilisers and herbicide practices) are considered as the main drivers of bee decline in Belgium and Western Europe (Rasmont 1988; Rasmont & Mersch 1988; Goulson *et al.* 2005, 2008; Rasmont *et al.* 2005; Vray 2018).

Among the many threats linked to modern agriculture is the widespread use of agro-chemicals. The pesticide story is complex. The high toxicity of pyrethrenoids for bees is already known since the 1980s (see Sanchez-Bayo & Goka, 2014), as pure compound or in synergy with fungicides (Vandam & Belzunces, 1998). Recent studies have shown that exposure to neonicotinoid pesticides can lead directly to the loss of honey bees (e.g., Tapparo *et al.* 2012), and commercial *Bombus* in the US (e.g., Gradish *et al.* 2010). Exposure to sub-lethal doses of neonicotinoids has been linked to increased levels of the gut pathogen *Nosema* in honey bees (Pettis *et al.* 2012) and causing colony loss by impairing overwinter survival also in honey bees (Lu *et al.* 2014). Elston *et al.* (2013) report that sub-lethal effects of the neonicotinoid thiamethoxam in conjunction with the DMI fungicide propiconazole, affect colony initiation in bumblebee (*Bombus terrestris*) colonies (see also Godfray *et al.* 2014). Although there is a growing number of laboratory studies (e.g., Goulson 2013, Sandrock *et al.* 2014) describing the sub-lethal effects of neonicotinoid pesticides on some species of bees, wide-ranging field studies are still lacking and represent a gap in our knowledge. However it seems clear that traits related to honey bees make them more robust than many wild bees to resist pesticide effects.

Herbicide application can also negatively impact bee diversity, as it can reduce the availability of flowers on which bees depend, and it can delay the flowering, thus disrupting the timing between food needs for pollinators and food delivery (Boutin *et al.* 2014). Herbicide application can have a significant local effect on bees, especially those species that are specialised pollen foragers (Nabhan & Buchmann 1995). Increasing application of nitrogen-based fertilizers is typical of the widespread intensification of agriculture over much of the continent. Fertilizer use, in addition to encouraging the growth of the target crops, also promotes rank grassland, low in flowering plants (especially Fabaceae) (Wilson *et al.* 1999) and poor for many bees, especially some *Bombus* species and Fabaceae specialists (Rasmont, 2005).

Moreover, climate change is also considered to be an important driver of increased extinction risk, and 159 bee species appear to be threatened by climate change, following the European Red List (Nieto *et al.* 2014). Studies by Maracchi *et al.* (2005) and Olesen & Bindi (2002) show that climate change in Europe will lead to more widespread and prolonged heat waves and summer droughts. Increase in temperature across the Boreal, Arctic and Alpine regions will severely impact the vegetation composition. This is already having an effect on the species associated with these habitats, the bumblebee species of these biomes facing an increased threat of extinction (Callaghan *et al.* 2004; Ødegaard *et al.* 2009, Rasmont *et al.* 2015).

3. METHODOLOGY

The methodology is included in the results section.

4. SCIENTIFIC RESULTS AND RECOMMENDATIONS

PART I. SCIENTIFIC RESULTS

4.1. WP1. Project coordination

4.1.1. WP1. Task 1. Coordination of wild bee database networking

One of the first steps of the project was to compile old and recent data of wild bees into a database, the BDFGM ("*Banque de données fauniques de Gembloux et Mons*"). Most of the old data present in institutions were digitalized and encoded. Recent data were accumulated thanks to the work of the scientific partners but also by our partner Natuurpunt.

4.1.2. WP1. Task 2. Project web site and dissemination of information on Internet

A website was developed as part of the BELBEES project (http://www.belbees.be/). Some information related to the BELBEES project was disseminated to the large public via social networks (Facebook https://fr-fr.facebook.com/BelbeesProject/ and Twitter https://twitter.com/belbeesproject). Wild bees' data accumulated during the BELBEES project allowed an update of the data (ecological information, maps and photos) included in the website Atlas Hymenoptera (http://www.atlashymenoptera.net/liste_them.asp?them=Belgium). These data were also included in the GBIF portal.

4.1.3. WP1. Task 3. Follow up committee

Several BELBEES meetings were organised with the Consultative Committee. The first meeting was organised during the first year of the project (12/06/2014) and allowed to identify the stakeholders involved in the project as well as their opinions and needs in terms of wild bee conservation. During the following year a second meeting entitled "BELBEES Halfway Stakeholders meeting" was organised (21/10/2015) followed by a BELBEES day event

(12/12/2015). The last meeting entitled "Transposing sciences results to wild bee conservation" took place during the last year of the project (30/05/2018) and allowed us to review the project with stakeholders and to develop a draft of recommendations.

4.1.4. WP1. Task 4. Periodic and final reports

The effective collaboration between scientific partners of BELBEES allowed us to produce annual reports, including all the activities carried out and those in progress. Regular meetings were held between the partners throughout the project, to discuss needs, problems and future steps. Collaboration between partners also resulted in the production of several publications (see under heading Publications). All the scientific partners were actively involved in the redaction of reports. Finally, a final meeting between the scientific partners (25/05/2018) was organised in order to make a final assessment of the BELBEES project and to organise the final report.

4.2. WP2. Wild bee data collecting, digitization and distribution analyses

4.2.1. WP2. Task 1. Identification and digitization of museum collections

A substantial effort has been provided for the digitalization and identification of old and recent specimens of wild bees. Identification of all the specimens used in this project has been confirmed by a validation team that gathers Belgian taxonomist experts (e.g. A. Pauly, P. Rasmont, D. Michez, N. Vereecken). The database includes data coming from institutions (RBINS, UMONS, Gembloux Agro-Bio Tech), Natagora and Natuurpunt, citizen entomologists and from specimens collected during the BELBEES project. Two foreign museums were also visited in order to study and collect data about given bee species which are rare in Belgian collections: the *Museum für Natürkunde* (Berlin, Germany) and the *Museum National d'Histoire Naturelle* (Paris, France). In parallel, data digitalized in the 1980s have been re-coded by UMONS since the spatial resolution in this period was noticeably too low (10 km) for present standards (0.1 - 1 km). That operation was conducted on nearly 30,000 records being carefully checked by consulting relevant topographic maps. Thanks to all these new and/or upgraded data, the database currently contains a total number of 797,604 encoded specimens (see TABLE I). Moreover, description and pictures of each Belgian wild bee species are available on www.atlashymenoptera.net.

TABLE I. Number of specimens encoded in each wild bee family.

Family	Number of specimens
Andrenidae	244,132
Apidae	352,877
Colletidae	52,110
Halictidae	55,499
Megachilidae	71,393
Melittidae	21,593
	797,604

4.2.2. WP2. Task 2. Definition of taxonomical tools and taxonomical validation for the network

Identification tools were developed for both amateur (citizen entomologists) and scientific purposes. Identification keys were established for Belgian wild bee species belonging to Apidae (*Bombus)*, Andrenidae (*Andrena)* (performed by UMONS), the Halictidae and Megachilidae families (performed by the RBINS). These keys include high-resolution pictures of specific morphological elements leading to an easier identification of specimens than with the older identification keys. Information about the phenology and distribution of each species are also detailed. The purpose is to develop an “Atlas of Belgian wild bees” including identification, geographical and ecological information about all the Belgian species of wild bee families.

In addition, DNA barcoding was performed on specimens of Halictidae. This method allowed the detection and description of five new cryptic species within the species complex *Seladonia smaragdula* (Halictidae) at the European scale. One of them reaches the Belgian frontier, near Maastricht.

Species taxonomy of bumblebees (*Bombus* Latreille, 1802) is well known to be problematic due to a potentially high intra-specific variability of morphological traits while different species can converge locally to the same colour pattern (cryptic species). Assessing species delimitation requires to arbitrarily select variable traits whose accuracy continues to be debated. The integrative taxonomy based on the unified species concept (De Queiroz, 2007) aims to overcome limitations due to unsettled adequacy of selected diagnostic traits and limited sampling. First, the approach considers multiple independent lines of evidence to evaluate inter-population differentiation processes and taxonomic statuses. This reduces the likelihood of false taxonomic conclusions driven by single trait. Second, analysing multiple traits to investigate inter-population differentiation allows to increase the amount of information available despite a limited sample size.

In this context, Lecocq *et al.* (2015) proposed an approach derived from the method established by Schlick-Steiner *et al.* (2010) applied to bumblebees. This approach based on consensus between several independent alternative traits (i.e. DNA, eco-chemical traits) provides a solution to better understand bumblebee systematics. Lecocq *et al.* explain the benefit of this strict approach for species delimitation. Although a differentiation in one character can be enough to highlight speciation process, if this particular character is actually doubtful for species delimitation (since it is always difficult to determine the suitability of a character for species delimitation) the resulting taxonomic conclusion could be wrong. Moreover, as science advances some widely used characters today can become considered as unsuitable in the future. Therefore we advocate for a strict approach (i.e. a taxon deserving a species status is a taxon with differentiation in all studied traits).

Among operational criteria to assess specific status, the reproductive traits involved in the pre-mating recognition (i.e. the male cephalic labial gland secretions, CLGS) have been premium information. Since these secretions are supposed to be species-specific, these chemical traits can bring essential information where species delimitation is debated. However, the chemical analysis of CLGS is very technical and time consuming (i.e. integration of each peak and alignment of all compounds for all specimens). Moreover, the comparison of each peak is usually done *via* the retention time (RT). However, when specimens are injected at different times, the RT for a same compound could be different due to the degradation of the column of the gas chromatograph. According to the method described by Dellicour & Lecocq (2013a), before each sample injection, a standard (Kovats) was injected containing a mix of hydrocarbons (alkanes) from C10 (decane) to C40 (tetracontane) to facilitate the alignment of compounds and their identification. Kovats indices were calculated with GCKovats 1.0 Dellicour & Lecocq (2013a). To standardize and make the alignment of each relative proportion of compound as objective as possible, Dellicour & Lecocq, 2013b developed a JAVA application to perform alignment (GCAligner 1.0).

4.2.3. WP2. Task 3. Selection of species and locations to have a representative dataset

Thanks to this identification and digitization works it was possible to select species and locations with a representative dataset to be used in other tasks of the BELBEES project. The target localities were chosen based on the availability of extensive historical bumblebee datasets from the 20th century (Figure 1).

The target species chosen for genetic analyses were: *Bombus confusus, B. distinguendus, B. humilis, B. jonellus, B. magnus, B. muscorum, B. pomorum, B. ruderatus, B. soroeensis, B. subterranus, B. sylvarum* and *B. veteranus*, whereas all other collected bee species were used in other analyses.

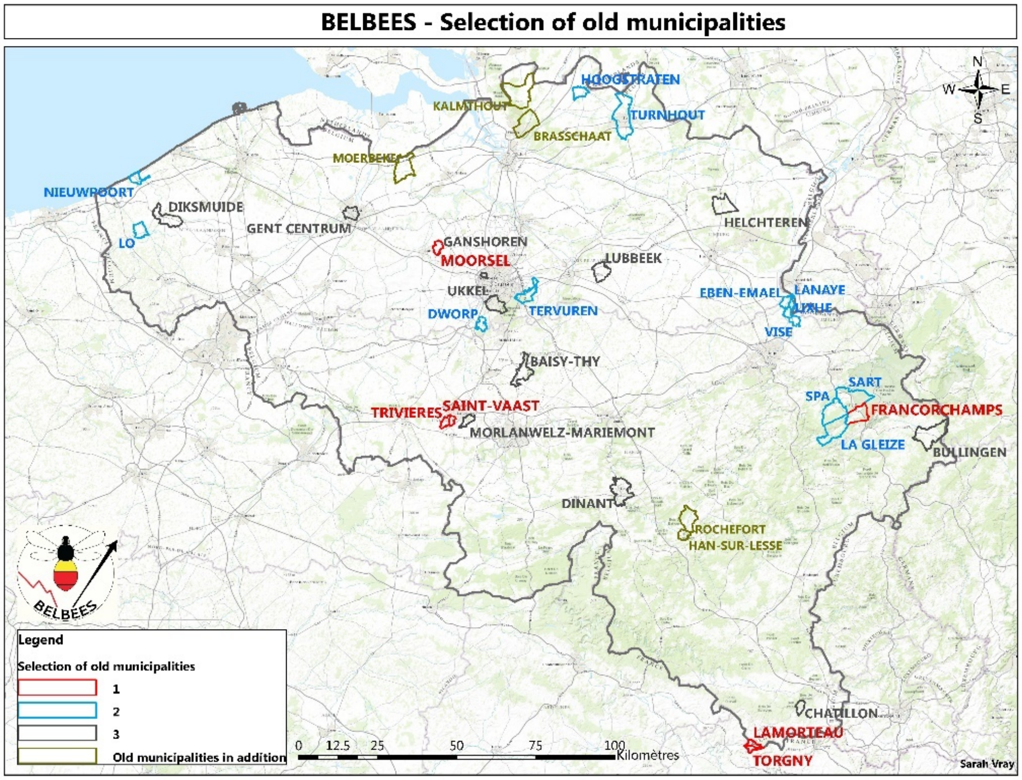


Figure 1. Map including the target localities in Belgium sampled during the BELBEES project (Map made by S. Vray). In red: locations with the most intensive monitoring both present and past, blue: location with past monitoring with few recent sampling, green: location with few past data and numerous past ones, grey: location with past data but few or no recent ones.

4.2.4. WP2. Task 4. Collection of old samples (specimens, pollens)

A very important historical material was available in the collections of the RBINS. The taxonomic identifications have been checked and encoded in the database BDFGM. This digitization of old specimen allowed to increase our database from 100, 326 to 797, 604 specimens.

Concerning old *Bombus* data, a huge work was performed to encode the bumblebee specimens from F.J. Ball's collection (Ball 1914, 1920), stored at the RBINS, and covering mainly the period from 1910 to 1930. A part (about 21,300 specimens) of the collection was encoded by Pierre Rasmont during his thesis (Rasmont 1988). The remaining (about 41,000 specimens) was encoded by Morgane Folschweiller, Pierre Rasmont and Sarah Vray (Vray 2018). Even if all specimens were identified by Ball, the identification of each had to be checked and sometimes corrected. Indeed, in addition to the errors of identification, Ball did not know the diversity of species of the subgenus *Bombus*, discovered by Rasmont (1984), as well as the presence of *Bombus cullumanus* (Rasmont 1982). All the specimens have been encoded with the exception of the 16,000 workers of the four species belonging to the subgenus *Bombus* (*B. cryptarum, B. lucorum, B. magnus,* and *B. terrestris*), because it is still impossible to differentiate them on the basis of morphology with current methods. For each specimen, locality, date and collector's name as mentioned on the labels, were encoded.

4.2.5. WP2. Task 5. Collection of new samples (specimens, pollens)

In order to compare the bumblebee historical fauna (from RBINS collections) to the current one, six well-sampled municipalities during 1910-1930 were re-sampled by Sarah Vray in 2013, 2014, and 2015. New samplings were performed in each target locality in three periods: (i) March-May, (ii) June-July, and (iii) August-September. The sampling protocol involved: to use nets, to collect at least along roads, in meadows and among hedges, to take photographs, to note the plant species on which the specimen was foraging, and finally to pin and label all samples. In total, more than 3,900 specimens were collected and encoded. These specimens were used in Sarah Vray's thesis as well as in other publications from other BELBEES partners (e.g. Maebe *et al.* 2016). The number of specimens sampled during 2013 and the period '2013-2015' are detailed in TABLE II.

TABLE II. Number of bumblebee specimens sampled during 2013 and the period 2013-2015 in selected Belgian localities (Moorsel, St-Vaast/Trivières, Francorchamps, Torgny/Lamorteau, Nieuwpoort, Hoogstraten, Dworp, Lo and Montagne-St-Pierre) for the BELBEES project.

Localities	Sampling 2013	Sampling 2013-2015
Moorsel	-	796
St-Vaast/Trivières	-	1044
Francorchamps	-	1093
Torgny/Lamorteau	-	1016
Nieuwpoort	188	-
Hoogstraten	7	-
Dworp	9	-
Lo	20	-
Montagne-St-Pierre	30	-

Other wild bee species were collected (by Alain Pauly) in Han-sur-Lesse, Angleur (Streupas), Auderghem (Jardin Jean Massart), Brugge and Kalmthout. Main results from these collections are described below, most of them having been published.

Finally, in addition to the old data and data collected in the field, Pierre Rasmont completed the BDFGM with the naturalist database of Observations.be/Waarnemingen.be belonging to Natagora / Natuurpunt, who are partners of the BELBEES project. This database contains opportunistic data from hundreds of observers across the country (mostly in Flanders).

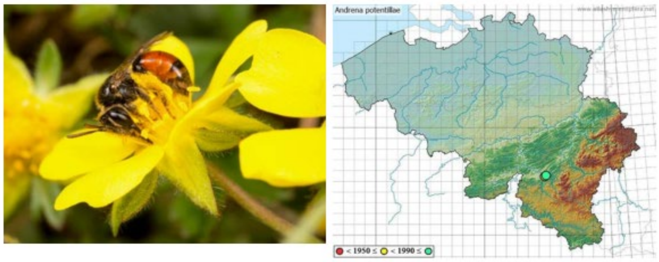
Heathlands of Streupas (Angleur) (Pauly, 2018)

Sampling of wild bees were performed in the heathlands of Streupas localized in Angleur (Liège, Belgium). The management of this natural reserve has been handled by the University of Liège (ULg) and involved special practices like deforestation, mowing and the removal of the upper layer of the soil since 1989. Wild bee observation showed that some species are specifically linked to plant species: *Andrena fuscipes* and *Nomada rufipes* foraged on *Calluna vulgaris,* whereas *Dufourea vulgaris* and *Melitta haemorrhoidalis* on *Campanula rotundifolia*, and *Panurgus calcaratus* and *P. banksianus* on Asteraceae species. Heathlands also provide favourable nesting sites for wild bee genera such as *Andrena*, *Halictus* and *Colletes*. Warm and dry micro-climate, short vegetation as well as bare soil in places and numerous slopes are several factors that are beneficial to the nesting of those wild bees. Afterwards, the current fauna in wild bees was compared to past observations during the 1970-1980s. This comparison revealed that 54 bee species were found in the past in this heathland while 47 bee species are currently present. We have noticed that two common bee species (*Andrena helvola* and *Lasioglossum fulvicorne*) and one rare bee species of the 1970-80s (*Dufourea dentiventris*) have not been observed since the early 2000's.

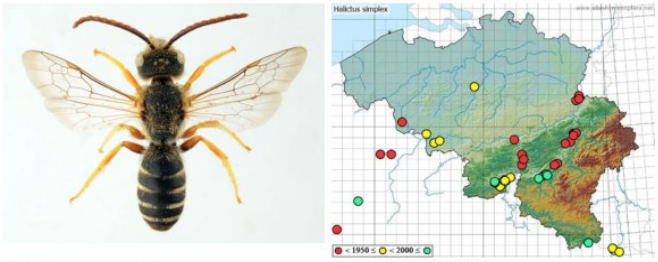
The disappearance of the two commons species may be explained by the repeated removal of the upper layer of the soil since 1989 which has increased the area of bare soil. Regarding *D. dentiventris* the regression seems to occur at the Belgium scale. At the same time, eight new bee species were observed in the heathland (*Andrena nitida*, *A. vaga*, *A. ventralis*, *Colletes cunicularius*, *Nomada alboguttata*, *N. flava*, *N. lathburiana* and *Sphecodes albilabris*). It is also interesting to note that the main floral resources in Streupas are *Campanula rotundifolia*, *Lotus corniculatus* and Asteraceae species. The data obtained from the heathlands of Streupas were then compared with the available data for the calaminarian halde of Plombières (Liège, Belgium), known for being a wild bee diversity hotspot. Whereas 79 species of wild bees were identified between 1978 and 2015 at Streupas, 130 species were found at Plombières. A great amount of *Andrena* and *Nomada* species were observed in both sites. Megachilidae family was mainly collected in the calaminarian embankment of Plombières, probably due to the presence of a more diversified flora including *Echium* and *Lotus*. As for the wild bees related to heathers (*Calluna vulgaris*) they were only found at Streupas. Among all bee species found is Streupas, seven of them are strictly protected in Wallonia: *Andrena fuscipes, Anthidium punctatum, Anthophora retusa, Colletes cunicularius, Macropis fulvipes, Panurgus banksianus* and *P. calcaratus* (Pauly, 2018).

Han-sur-Lesse (Pauly & Vereecken, 2018)

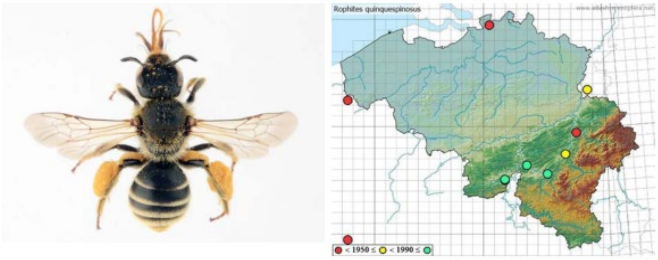
Since the fifties 131 species have been listed in the municipality of Hans-sur-Lesse (which represents one third of the Belgian bee species). The most abundant bee family are the Megachilidae due to the significant presence of Fabaceae and Lamiaceae in Han-sur-Lesse. The plants genera *Anthyllis*, *Hippocrepis*, *Lotus* and *Stachys* are very attractive for the *Osmia*, anthidies and trachuses. Halictidae were also abundant. We noticed that *Lasioglossum pauxillum* was more observed during our recent sampling than in the 1950s. A rare species, *Halictus simplex*, was also recorded and foraged on a specific plant species of the site, *Geranium sanguineum* (Figure 2).



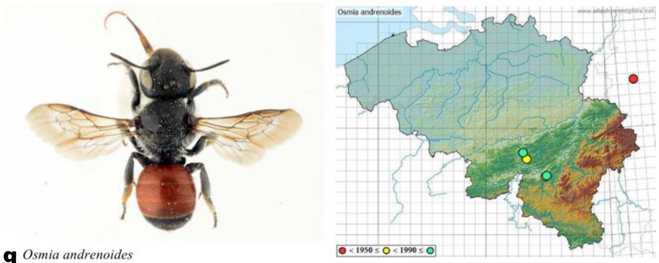
a *Andrena potentillae* (© NV). **b** *Andrena potentillae* (© AP).



c *Halictus simplex* (© AP). **d** *Halictus simplex* (© AP).



e *Rophites quinquespinosus* (© AP). **f** *Rophites quinquespinosus* (© AP).



g *Osmia andrenoides* **h** *Osmia andrenoides* (© AP).

Figure 2. Specific species of the calcareous grasslands in Han-sur-Lesse and their distribution in Belgium (Pauly & Vereecken, 2018)

In contrast, the family Andrenidae was less represented with the exception of *Andrena potentillae* that was abundant and that foraged on *Potentilla verna*. Regarding the family Apidae, honey bees are almost completely absent. In the genus *Bombus*, *B. lapidarius*, *B. terrestris*, *B. pascuorum*, *B. hortorum* and *B. pratorum* are the most abundant, in opposition to *B. ruderarius*, *B. soroeensis* and *B. veteranus*. We also observed the presence of six wild bee species specific to calcareous grasslands: *Osmia aurulenta*, *O. bicolor*, *O. rufohirta*, *O. spinulosa*, *O. andrenoides* and *Trachusa byssina*. The presence of cracks into the rocks allowed the nesting of *Lasioglossum nitidulum*, *L. morio* and *L. laticeps*. Other environmental elements can be exploited by some species for nesting. *Osmia andrenoides*, *O. aurulenta*, *O. bicolor*, *O. rufohirta* and *O. spinulosa* can be found in empty snail shells whereas *Osmia leucomelana* and *Ceratina cyanea* nest in stems of wild roses growing among rocks.

Floral preference of wild bees has also been analysed. The plant species most foraged were *Aster lynosiris*, *Helianthemum nummularium*, *Geranium sanguineum*, *Hippocrepis comosa*, *Potentilla verna*, *Prunus spinosa* and *Seseli libanotis*. The oligolectic bee species are linked to the Fabaceae, Lamiaceae and to the two plant species *Potentilla verna* and *Allium sphaerocephalum*. The following step in our Working Package was to compare current and past data (which were performed between 1951 and 1955 by Paul Maréchal, and between 1950 and 1987 by Jacques Petit). From the 89 wild bee species recorded in Han-sur-Lesse, 12 species have still not been observed since the 1950's (i.e. *Andrena combinata, A. curvungula, A. falsifica, A. labialis, A. schencki, Megachile centuncularis, M. maritima, M. pilidens, Osmia pilicornis, O. ravouxi, O. spinulosa* and *Bombus humilis*). Two rare species (*Rophites quinquespinosus* and *Halictus simplex*) have been observed in 2005 but have not been collected between 2014 and 2017. The presence of localized bee species (*Andrena potentillae* and *Osmia andrenoides*), species of calcareous grasslands and helicicole species (*Osmia aurulenta*, *O. bicolor* and *O. rufohirta*) already observed in the 1950s, supports the conclusion that the fauna of Han-sur-Lesse is relatively stable. The location of Han-sur-Lesse also represents a unique refuge in Belgium for *Osmia andrenoides, Andrena potentillae* and *Rophites quinquespinosus*. Two new species have been recorded in 2017, *Andrena vaga* and *Nomada lathburiana*.

The data was finally compared to that obtained on the calcareous grasslands of Treignes and Montagne Saint-Pierre. With 119 species, a greater number of species were identified at Treignes compared to Hans-sur-Lesse. However, the sampling methods at Treignes were different from Hans-sur-Lesse and the station contained a more ruderal fauna and flora. More species were collected at Montagne Saint-Pierre than in Treignes. But the former location involves both sandy soils and chalky soil, whereas Treignes is characterized by a calcareous soil only. Thus, biotopes are variably diversified in these two localities (Pauly & Vereecken, 2018).

Botanical Garden "Jean Massart" (Auderghem)

This site is potentially interesting for wild bees due to numerous melliferous plants growing there (Figure 3). The garden was prospected in 1975, and revisited in 2004, 2013, 2014 and 2015. The bees were collected with a hand net and the foraged flowers systematically noted. Two Malaise traps and 10 yellow pan traps were placed from May 15, 2015 to May 30, 2016, in the "evolution garden" plot, near a hedge and at the edge of a wood, and surveyed weekly. A total of 112 bee species were identified. Among the rarer ones are *Lasioglossum majus, L. pygmaeum, L. sexnotatum, Andrena pilipes, A. rosae, A. tarsata* and *A. viridescens*. An overviewing manuscript is almost ready for publication.

Figure 3. Botanical garden "Jean Massart" (Anderghem) (A. Pauly)

Brugge

Four plots of sandy heathlands around Brugge (Beisbroeck, Chartreuzinnenheide, TerHeyde and Zevenkerken) were surveyed using yellow pan traps in 2015 and 2016 (Figure 4). About 1,000 specimens of Andrenidae, 900 Halictidae, 300 *Nomada* and 200 *Bombus* were identified. An ongoing publication will compare the results with those from other sandy areas studied in Belgium. Most interesting species collected in Brugge were *Andrena argentata, Panurgus banksianus* and *Lasioglossum major*.

Figure 4. One of the collection area in Brugge.

Kalmthout

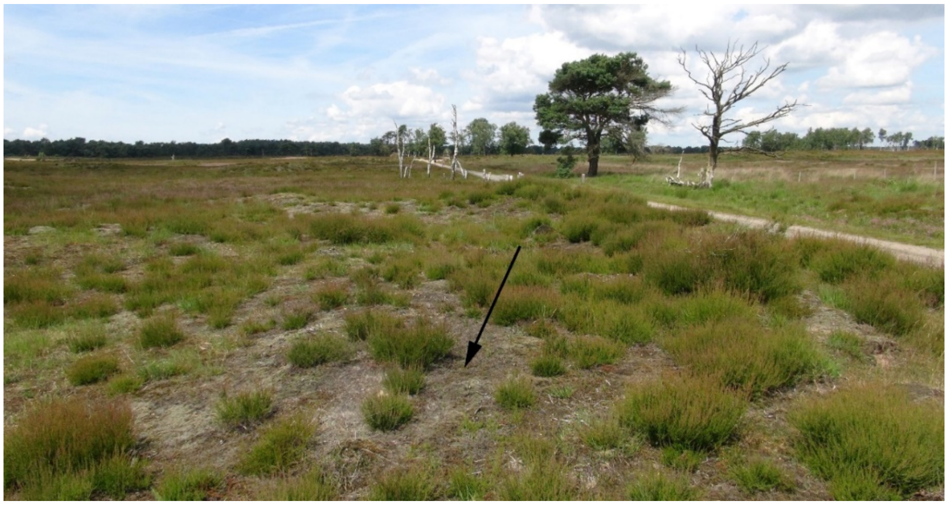


Figure 5. One of the collection area in Kalmthout.

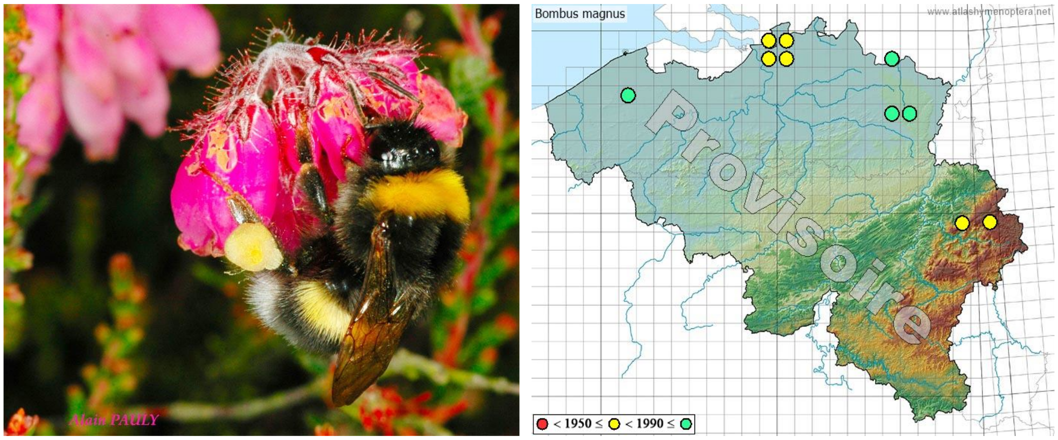


Figure 7. Distribution map of Bombus magnus in Belgium (A. Pauly)

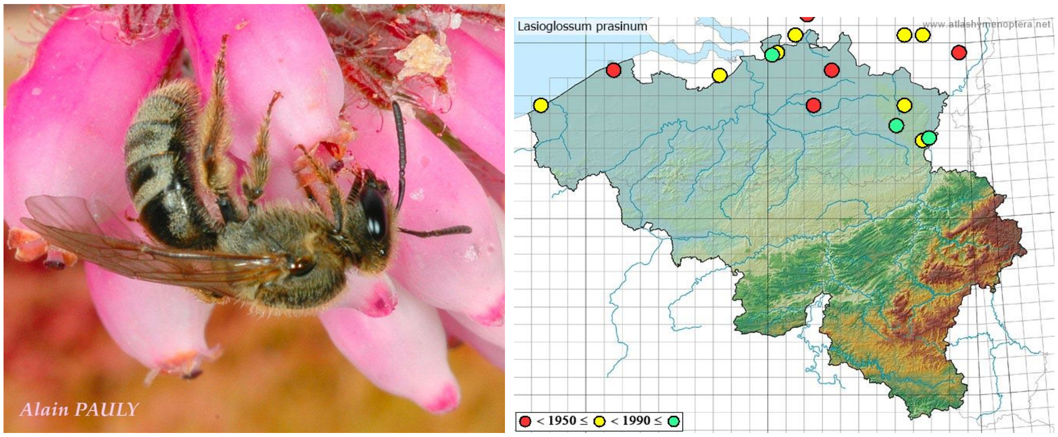


Figure 6. Distribution map of Lasioglossum prasinum in Belgium (A. Pauly)

Ten yellow pan traps were placed and collected every two weeks in 2017 (Figure 5). In total, 330 specimens of bees were collected only, which is due to bad meteorological condition problems (pan traps filled with sand following windy periods). Most interesting species are *Lasioglossum prasinum* (Figure 6) and *Bombus magnus* (Figure 7). A publication is being prepared to present the results.

Montagne-Saint-Pierre

An inventory of the wild bee community was performed by UMONS at Montagne Saint-Pierre during the 2016 summer (Buchet, 2017). Montagne Saint-Pierre is localized between Belgium and Netherlands and is considered as the location with the greatest biodiversity in Belgium. The samplings highlighted 61 wild bee species including 32 rare species with 8 protected species in Wallonia (*Coelioxys inermis, Colletes cunicularius, Dasypoda hirtipes, Eucera nigrescens, E. longicornis, Osmia bicolor, Panurgus calcaratus* and *P. banksianus*) and one new species, *Hylaeus annularis*. This last species has never been observed in Belgium before and is rather located in Netherlands. The most abundant bee family at the Montagne Saint-Pierre is the Apidae family due to a large number of collected *Bombus* specimens (1,496 specimens). The second family is the Melittidae followed in decreasing order by the Halictidae, Andrenidae, Colletidae and Megachilidae.

The past inventories of wild solitary bees at the Montagne Saint-Pierre showed that numerous species once occupied the area; 123 species between 1933 and 1937, and 230 species between 1922 and 1988. The solitary bee data of 1937 were compared to the current ones. Although the sampling effort was higher in 2016, the species richness showed that 67 species were present in 1937 against 47 species in 2016. The diversity of solitary bees would have then decreased since 1937.

4.2.6. WP2. Task 6. Trend analyses and IUCN Red List

European IUCN Red List

In the context of the assessment of the bee decline, UMONS published with its partners of the STEP project (http://www.step-project.net/) the IUCN RedList of European Bees (including Belgian species). Moreover, the first distribution analyses of wild bee species from Belgium were completed and they are published on www.atlashymenoptera.net. The three main unexpected results in this European Red List are: 1) the high number of species (2051 species); 2) the high number of species that are data deficient; 3) the role of climate change, in a direct way by moving the distribution of species toward the north, or indirectly e.g. by increasing the areas destroyed by wild fires, impacting so several endemic Mediterranean species.

Belgian IUCN Red List

The Belgian Red List is a review of the conservation status of Belgian species according to IUCN regional Red Listing guidelines (Drossart *et al.* 2018). It identifies those species that are threatened with extinction at a regional level, in order that appropriate conservation actions can be taken to improve their status. The Red List publication summarizes results for all bee species recorded once in Belgium (i.e. 399 species), the geographical scope being national-wide. Regional distributions were considered in the discussion as many nature conservation policies are organized at regional level in Belgium.

The status of all species was assessed using the IUCN Red List Criteria (IUCN 2012a), which are worldwide the most widely accepted system for measuring extinction risk. All assessments followed the Guidelines for Application of IUCN Red List Criteria at Regional Levels (IUCN 2012b). These assessments were compiled based on the data and knowledge from a network of national bee experts. The assessments were then completed and reviewed at six small workshops held in Mons (Belgium) as well as through email correspondence with relevant experts. Individual assessments are planned to be available on the website Atlas Hymenoptera (http://www.atlashymenoptera.net).

We recorded 399 species in Belgium. We did not evaluate 22 species for which we did not find clear evidence that a stable population ever occurred in Belgium. We assessed the 377 other species to the following categories: Data deficient (33 species), Least concern (162 species), Nearly Threatened (26 species), Vulnerable (34 species), Endangered (32 species), Critically Endangered (46 species), and Regionally extinct (44 species) (Figure 8). These results show that we lost already 12% of Belgian wild bee fauna within one century. For the 333 remaining species, we found that for 49% of them the populations seem stable or expanding. The threatened species (i.e. assessed as critically endangered, endangered or vulnerable) represent a third of the 333 remaining species. A further 8% of the remaining bees are considered Near Threatened. Compared to the European red list (9% of threatened species and any species regionally extinct), the Belgian one includes much more threatened species.

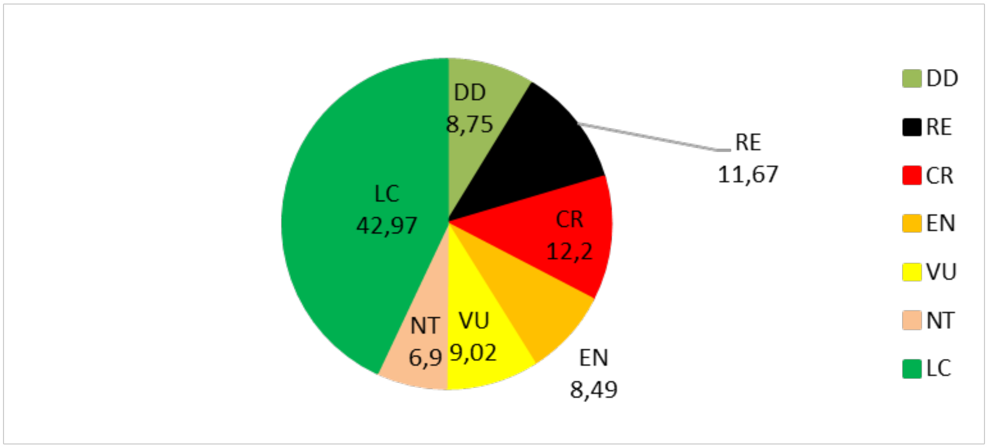


Figure 8. IUCN Red List status of bees in Belgium (n= 377 species) where DD= Data Deficient; RE= Regionally Extinct; CR= Critically Endangered; EN= Endangered; VU= Vulnerable; NT= Nearly Threatened; LC= Least Concern.

The species richness of bees increases from north to south in Belgium, with the highest species richness being found in the area of Rochefort and the Gaume. Local hot spot of diversities are found in particular habitats like calcareous grasslands and heatlands. The regions of Famenne and Gaume, in Wallonia, and Campine in Flanders, present a high diversity of threatened species. The main threats identified are habitat loss as a result of agriculture intensification (e.g., changes in agricultural practices including the use of pesticides and fertilisers), urban development and climate change.

4.3. WP3. Hypothesis testing

4.3.1. WP3. Task 1. Hypothesis 1: Food resource depletion

4.3.1.1. WP3. Task 1. Subtask. 3.1.1. Using the information about resource preferences by all possible methods: literature, notes from ancient collection, analysis of pollen loads

Variation in pollen diet and its impact on bee development

Impact on colony development and individual size

Experimental studies on the development of colonies fed on different pollen diet show that pollen has an impact on the total mass, the individual size and the mortality of the offspring (i.e. Vanderplanck *et al.*, 2014b; Moerman *et al.*, 2015). Pollen of *Salix* sp. represents a very efficient diet while *Cirsium* pollen is one of the poorest diets (Roger *et al.*, 2016). Pollen diets of *Trifolium* and *Salix* genera are more efficient in terms of quantity of collected pollen than those of *Rubus* and *Cistus* (Figure 9).

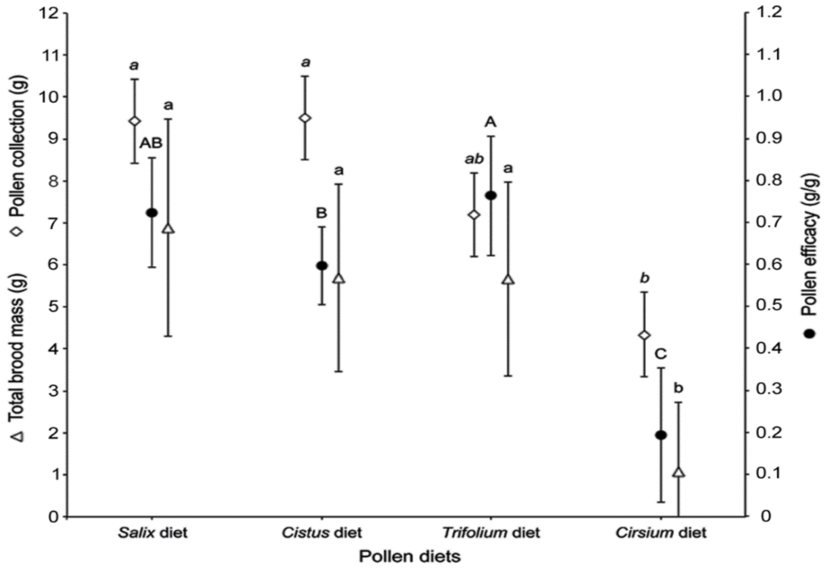


Figure 9. Efficacy of the different pollen diets with regards to pollen collection and brood mass at the end of bioassays (n = 10 per diet). Different letters indicate significant differences between diets (post hoc tests, P < 0.05) (Vanderplanck et al., 2016).

It has been also observed that bumblebee colonies development on *Salix* and *Cistus* pollen provides a large number of small larvae, whereas colonies fed with pollen of *Trifolium* and *Rubus* lead to a reduced number of larvae but with a higher mass (Roger *et al.*, 2016). This might be an ecological advantage as large larvae have a higher immunocompetence (Vogelweith *et al.*, 2013), lead to more efficient foragers (Spaethe & Weidenmuller, 2002) and to large queens that have a higher winter survival and reproductive success (Beekman *et al.*, 1998). Other pollen such as from *Helianthus* sp. might also have negative effects on the development of bumblebee colonies (Tasei & Aupinel, 2008) and honeybees (Schmidt *et al.*, 1995). Moreover when bumblebee colonies are feeding with a poor pollen quality diet, workers eject a fraction of larvae in order to obtain adequate nutrition from the other individuals (Tasei & Aupinel, 2008b).

Chemical analyses of pollen associated to measure of colony developments show that pollen efficiency (i.e. ratio between offspring mass and collected pollen) is probably related to the concentration of sterols and amino acids (De Groot, 1953; Rasmont *et al.*, 2005; Vanderplanck *et al.*, 2014b; Moerman *et al.*, 2015; Vaudo *et al.*, 2015; Moerman *et al.*, 2017). Other chemical elements could be taken into account to determine that the nutrient pollen quality is suitable. Generalist insects tend to prefer floral resources which also contain suitable sterols to improve nest growth and development (Nes *et al.*, 1997). Pollen of *Sorbus aucuparia* and *Cytisus scoparius* contain 24-methylenecholesterol and a high concentration of polypeptide and total amino acids favorable to the reproduction of workers and linked to a good health for the brood (Svoboda *et al.*, 1978; Svoboda *et al.*, 1983; Human *et al.*, 2007). The 24-methylenecholesterol, b-sitosterol and δ5-avenasterol (potentially involved in the metabolic pathway of *B. terrestris* and that could act as a phagostimulant) seem to be positively associated to the larval development of bumblebees (Vanderplanck *et al.*, 2014). Micro-colonies of bumblebees feeding with these two pollen plant species produced bigger larvae which are likely to become best reproductive partners and winter survivors (Vanderplanck *et al.*, 2014).*Cirsium* pollen contains a suitable concentration of amino acids for the development of bumblebee colonies but is rich inδ7-sterols (phytosterols) which could constitute a defense against a too high harvesting of pollen by insects (Vanderplanck *et al.*, 2016).

Studies on *Calluna vulgaris*, *Cistus* sp. and *Taraxacum* sp. pollen diet showed that they are not optimal for the development of *B. terrestris* colonies (Génissel *et al.*, 2002; Tasei & Aupinel, 2008b; Vanderplanck *et al.*, 2014a). While their chemical composition confirmed a low quality, it also showed a low digestibility (Human *et al.*, 2007). The structure of *Cirsium* pollen induces a difficult consumption of this pollen for the bumblebee workers, since requiring specific proteases (Vanderplanck *et al.*, 2016). This structural issue is also observed with honey bees fed with *Taraxacum* pollen (Peng *et al.*, 1985), *Chelostoma rapunculi* (oligolectic on *Campanula* sp.), *C. florisomne* (oligolectic on*Ranunculus* sp.) and *Hoplitis adunca* (oligolectic on *Echium* sp.) (Praz *et al.*, 2008).

Impact on health and immune system

The immune capacity of insects can be affected by the quality of their diet (Alaux *et al.*, 2010). The influence of three different pollen diets (*Salix*, *Cistus* and *Cirsium* genera) on the immune activity of *B. terrestris* has been studied by Roger *et al.* (2016). It has been shown that the basal immunocompetence (i.e. the capacity of an organism to produce an immune response) of bumblebees is the same from one kind of pollen to another. However once bumblebees are fed with *Cistus* or *Cirsium* pollen (poor nutrient quality), there is a decrease of the immune activity (established on the basis of the prophenoloxidase concentration or PO). Bumblebees fed with *Salix* pollen during the whole experiment have shown a significant difference between their total PO and their active PO. On the contrary, bumblebees fed from a *Salix* diet to a *Cistus* or *Cirsium* diet have shown the same rate between the total PO and the active PO (Figure 10). An explanation could be that in a poor diet, the concentration of prophenoloxidase produced is lower (total PO) considering that energy supplies are involved in other physiological processes (i.e. reproduction and detoxification). After a nutritive stress, bumblebees could be therefore more vulnerable to diseases and infections (Roger *et al.*, 2017) linked to pathogens like *Crithidia bombi* (Brown *et al.*, 2003). It has also been observed that bumblebees fed with a poor nutrient pollen quality increase their consumption of nectar and pollen to maintain their immune system performance (Roger *et al.*, 2017).

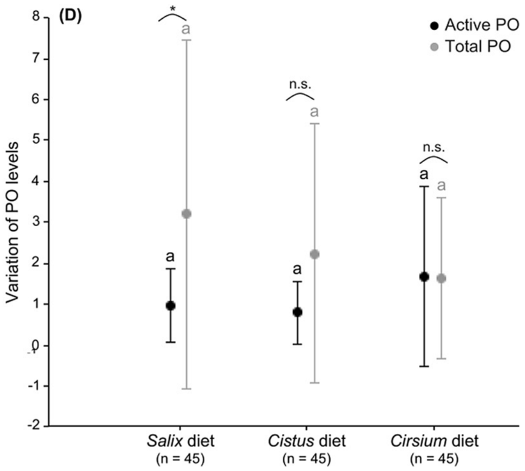


*Figure 10. Impacts of different pollen diets (*Salix*,* Cistus *and* Cirsium*) on variations in active and total phenoloxidase (PO) activities of workers after diet change (*Salix-Salix*,* Salix-Cistus*,* Salix-Cirsium*). Groups differing significantly from each other in post hoc tests are marked with different letters, with shared letters indicating a non-significant difference. Within one diet case, groups differing significantly from each other in t-tests are marked with the following code: n.s. = non-significant difference; *= p < 0.05 (Roger et al., 2017).*

Pollen toxicity

Aconitum septentrionale (Ranunculaceae) is a middle-toxic plant (Zinvrda *et al.*, 2000; Goncharg *et al.*, 2006) foraged by specialized bumblebee species (i.e. *Bombus consobrinus*) and generalist ones (i.e. *B. wurflenii*). Analyses of alkaloids contained in this plant were performed to localize the toxicity of the plant (Gosselin *et al.*, 2013). This alkaloids concentration (aconitine form) was higher in the pollen than the nectar of the plant (Figure 11) (Gosselin *et al.*, 2013).

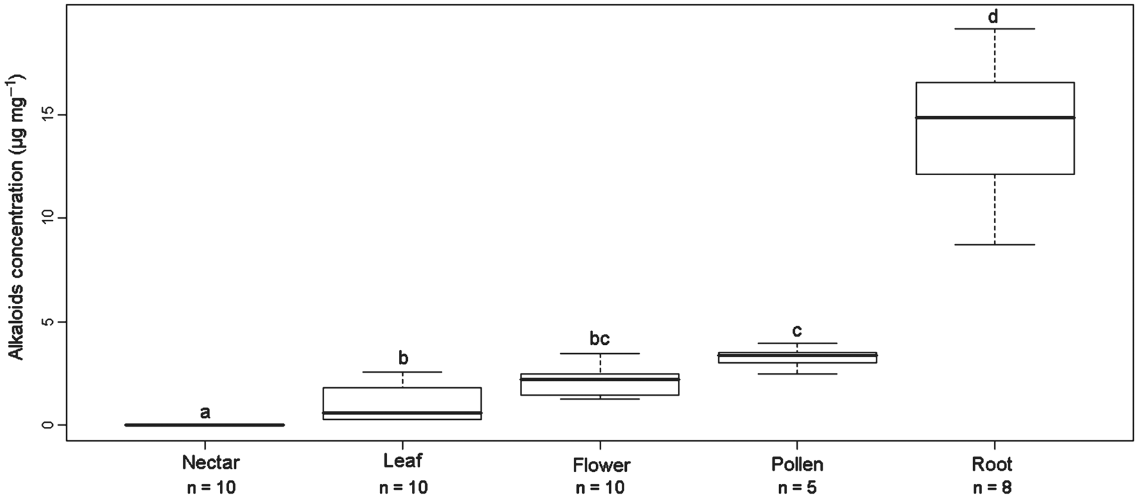


Figure 11. Total alkaloid concentration in the different parts of Aconitum septentrionale*. Letters indicate data significantly different (Gosselin et al., 2013).*

The lappaconitine, a toxic alkaloid (Ameri, 1998), is the most abundant alkaloid contained in the pollen and nectar of *Aconitum septentrionale* (Gosselin *et al.*, 2013). The potential toxicity of the pollen might be considered as a chemical defense to limit the losses caused by excessive pollen harvesting (Praz *et al.*, 2008). The low nectar toxicity is offset by a difficult access to nectaries hidden in flower's bottom. The high concentration of alkaloids (pollen) and the low accessibility of floral rewards (nectar) of *A. septentrionale* might discourage generalist foragers and could promote specialized behavior as well as for *Bombus consobrinus* (Gosselin *et al.*, 2013). The consumption of alkaloids by *B. consobrinus* could also protect this species against microbial and/or predators attacks (Elliot *et al.*, 2008; Manson *et al.*, 2010) and could be an advantage of low foraging competition (Duan *et al.*, 2009).

Interspecific variability

The relative development of bumblebee colonies on a specific pollen diet is variable among bumblebee species (Figure 12). In other words, there is an interspecific variation in the pollen efficacy in the *Bombus* genus. The development of *B. terrestris* requires twice less pollen and six times less nectar to produce the same brood mass than *B. hypnorum*. Workers of *B. terrestris* collect less pollen (less energy devoted to food research activity) to produce an equivalent mass of offspring. This high performance (high development of the colonies independently of the pollen diet) and its foraging behavior (polylectic with 20 foraged host plants) allow *B. terrestris* to easily incorporate new host plants in its diet (Moerman *et al.*, 2016). Consequently, a change in the range of host plants will probably have no significant impacts on its conservation. The development of *B. hypnorum* colonies is the same on any of three different pollen diets (*Cistus*, *Erica* and *Salix*). For *B. pratorum*, the number of produced larvae is higher with *Cistus* pollen than *Salix* pollen (Moerman *et al.*, 2016). The higher performance of *B. pratorum* on *Cistus* pollen could be explained by a higher concentration in 24-methylenecholesterol than in *Salix* pollen (Moerman *et al.*, 2016). This chemical compound is known to influence the molt and ovaries development in bees (Svoboda *et al.*, 1978; Svoboda *et al.*, 1983; Human *et al.*, 2007). These differences in species development depending on pollen diet could be explained by species physiological ability to deal with pollen characteristics (structure and chemical composition) (Moerman *et al.*, 2016).

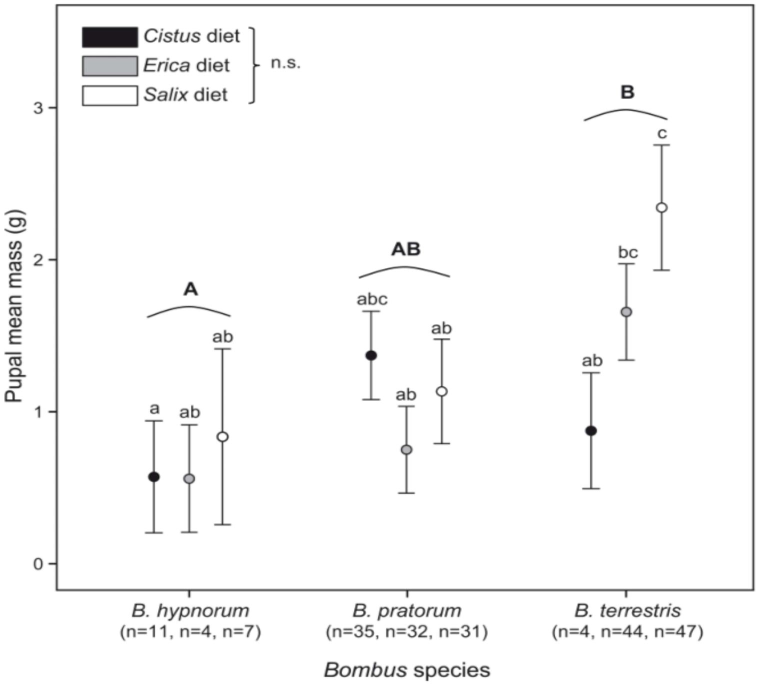


*Figure 12. Pupal mean masses from micro-colonies of three bumblebee species (*B. hypnorum, B. pratorum *and* B. terrestris*) fed with three different pollen diets. Uppercase letters indicate interspecific significant differences and lowercase letters indicate intraspecific significant (Moerman et al., 2016).*

Floral resources in Belgium

Factors of choices

Most bumblebees are flexible in their floral resource choices (polylectism). Bumblebees can distinguish a pollen with a high protein concentration (Ruedenauer *et al.*, 2015), a high amino acid concentration (Hanley *et al.*, 2008; Leonhardt & Blüthgen, 2012; Ruedenaeur *et al.*, 2015; Somme *et al.*, 2015; Kriesell *et al.*, 2016) and specific protein/lipid rate (Vaudo *et al.*, 2016). Therefore they choose plants with high pollen nutrient quality when they are exposed to a binary choice (Roberston *et al.*, 1999; Kitaoka & Nieh, 2008; Moquet *et al.*, 2015; Quiniet *et al.*, 2016). In the field, floral choices of bumblebees are less clear once they are exposed to a large range of plant species (Rasheed & Harder, 1997). Other factors than the chemical composition of the pollen could be involved in the floral resource choices of bumblebees. Accessibility and availability of floral resources should also be considered (Moerman *et al.*, 2017). In the Upper Ardennes, *Vaccinium uliginosum* (Ericaceae) is the most foraged plant by bumblebees although this plant is not considered as a suitable resource because of its low amino acids concentration (Vanderplanck *et al.*, 2014). This choice could be explained by the abundance of this Ericaceae species at this location. Workers increase their visits on dense floral patches (Waser, 1986; Kunin & Iwasa, 1996; Kamper *et al.*, 2016).

It has been shown that bumblebees are able to mix pollen from different floral resources to compensate the low nutrient quality of one pollen resource (Moerman *et al.*, 2017). The combination of several pollen sources would provide an optimal nutritional requirement by compensating a lack of nutrient or by diluting the toxicity of some pollen (Arnold *et al.*, 2014; Eckhardt *et al.*, 2014). This social detoxification system has been observed on *B. terrestris'* colonies fed with *Cirsium* pollen. Workers mixed the pollen with a large quantity of nectar (Vanderplanck *et al.*, 2016). Some bumblebee species supplement a pollen poor in 24-methylenecholesterol but rich on δ7-sterol (coming from *Vaccinium myrtillus*, *Erica tetralix* and *Calluna vulgaris*) with a pollen rich on 24-methylenecholesterol and campesterol (coming from *Filipendula ulmaria*, *Malus pumila* or *Rubus* spp.) (Moquet *et al.*, 2017). Monofloral pollen coming from *Cytisus scoparius* is known to induce mortality of the workers probably because of the presence of quinolizidine alkaloids (Sundararajan & Koduru, 2014; Moerman *et al.*, 2017). This negative effect is countered once this pollen is mixed to others. Development of *B. terrestris* colonies fed with pollen from *Cytisus scoparius*, *Erica* sp. and *Sorbus aucuparia* has been studied by applying a mono-, di- and tri-floral diet. Results showed that the colonies had potentially a greater development with a mixed pollen although a single pollen can be efficient as well depending on its nutrient quality. Monofloral diets of Fabaceae (*C. scoparius*) and Rosaceae (*Sorbus aucuparia*) pollen induce a greater colony development than a di-floral diet of several pollen of *Erica* sp. (Moerman *et al.*, 2017). The behavior of mixing several pollens could reduce the dependence of bee to specific plant hosts containing a favorable pollen. This could be an advantage in case of food shortage period or in a phenological delay between the blooming season of favorite hosts and flying period of generalist bees (Vanderplanck *et al.*, 2016). The behavior of mixed pollen is also observed with the solitary bee *Osmia cornuta* (Eckhardt *et al.*, 2014).

Pollen diet of four bumblebee species (*Bombus lapidarius*, *B. pascuorum*, *B. terrestris* and *B. hypnorum*) was investigated by Somme *et al.* (2015). Results showed that these species forage preferentially on plant species with a high pollen quality. *B. lapidarius* and *B. pascuorum* collect high-quality pollen (with a high concentration of essential amino acids and phytosterols) on *Comarum palustre* and *Trifolium pretense*. It has been shown that *B. terrestris* and *B. hypnorum* expand by 25% their pollen diet by integrating pollen resources of lower quality (i.e. *Cirsium palustre* and *Valeriana repens*). Analyses of pollen loads coming from bumblebees showed that bumblebees foraging flowers of *Comarum palustre* are very consistent during pollen collection. *B. lapidarius* and *B. pascuorum* are steadier than *B. terrestris* with almost 100% of the total pollen coming from *Comarum palustre*, *Lychnis flos-cuculi* or *Trifolium pratense*. Pollen of *Comarum palustre* and *Trifolium pratense* can be considered to have a higher chemical quality than the pollen of *Cirsium palustre* and *Valeriana repens* given the concentration of total amino acids (over 20%) and phytosterols. Pollen loads of *B. hypnorum* were principally composed of pollen coming from *Comarum palustre*, *Persicaria bistorta* or *Ranunculus acris*. Analyses of pollen loads coming from *B. terrestris* showed a larger range of pollen diet than in the three other species of bumblebees (Somme *et al.*, 2015). However, other studies showed that pollen diet of *B. lapidarius* can be the same or larger than the diet of other larger of concurrent species (Goulson & Darvill, 2004; Kleijn & Raemakers, 2008) and *B. pascuorum* can have a pollen diet larger than B*. terrestris* (Carvell *et al.*, 2006; Leonhardt & Blüthgen, 2012). These observations on *B. lapidarius* and *B. pascuorum* suggest that floral resources choices of these two species could be linked to local and temporal conditions rather than specific preferences (Roulston & Goodell, 2011). While *B. terrestris* can expend its pollen diet in conditions of strong competition for floral resources, this could be done to the detriment of the chemical pollen quality. *Bombus terrestris* was the only one of the four species to collect pollen of *Cirsium palustre* (up to 77% inside the pollen loads) although the nutrient quality of this pollen is considered as poor (low concentration of amino acids and high abundance of δ7-stigmastérol). Observations performed on colonies showed that larvae of *B. terrestris* and *B. hypnorum* seem to easily assimilate pollen with a poor nutrient quality (Somme *et al.*, 2015). Difference in strategy related to pollen's collection throughout bumblebee species can not be explained by the length of the colony cycle, the tongue length, or the size of the specimens, given that these characteristics are the same for *B. terrestris* and *B. lapidarius* (Goulson & Darvill, 2004; Benton 2006; Carvell *et al.*, 2006).

Floral resources in orchards

The production of nectar and pollen of four pear trees varieties (i.e. Concorde, Conférence, Doyenné du Comice, and Triomphe de Vienne) and five apple trees varieties (i.e. Braeburn d'or, cultivars de Reinders, Jonagored, Pinova and Wellant) commonly cultivated in Belgium has been studied (Quinet *et al.*, 2016). At the beginning of the spring, pear trees have flourished one week before the apple trees. Flowers of pear trees are approximately six times less foraged by insects and have less pollen grains from anthers than in apple trees (2,425-4,937 for pear trees against 3,284 - 7,919 for apple trees). In contrast, concentration of polypeptides, amino acids and phytosterols is higher in the pear trees' pollen (Quinet *et al.*, 2016). The 24-methylenecholesterol and campestrol are the most abundant compounds in the composition of sterols, and these sterols are the most essential in bee's metabolism (Human *et* *al.*, 2007; Vanderplanck *et al.*, 2014a). Cultivars of pear trees produce therefore less pollen grains by flower than the cultivars of apple trees, but the nutrient quality of the pear trees' pollen is higher (Quiniet *et al.*, 2016). The pollen of pear trees has similar concentration of polypeptides, amino acids and sterols than pollen of *Cytisus scoparius* (Fabaceae) and *Sorbus scoparius* (Rosaceae) which have a suitable nutrient quality for bumblebee colonies' development (Vanderplanck *et al.*, 2014b). Pear trees are most foraged for the pollen resources whereas apple trees are foraged for the nectar. Feeding behavior of insects is therefore better explained by the quality of the nectar and pollen than the quantity available. Conservation of abundant floral resources throughout the season in and around orchards should help the conservation of bee fauna after the massive blooming (Quinet *et al.*, 2016).

Floral resources in heatlands

Heatlands are opened habitats providing suitable resources for bumblebee species such as *Bombus jonellus*, a declining species in Belgium. In these heatlands, Ericaceae species are the most abundant plants providing pollen and nectar to bumblebees from the beginning of the spring to the end of the summer once the diversity of other plant species is low (Moquet *et al.*, 2017). Bumblebees are the main pollinators of the Ericaceae species located in heatlands except for *Calluna vulgaris* which is a generalist plant (Ritchie, 1955; 1956; Jacquemart, 1993; Mahy *et al.*, 1998; Mayer *et al.*, 2012). Ericaceae species are not all foraged for the same resources (i.e. pollen and/or nectar). *Vaccinium vitis-idaea* is principally foraged by bumblebees for pollen resources and *Calluna vulgaris* for nectar resources (Moquet *et al.*, 2017). The blooming period of *Vaccinium vitis-idaea* happens at the same time than bumblebee colonies development which requires pollen (Ribeiro *et al.*, 1998; Pelletier & McNeil, 2003). The blooming period of *Calluna vulgaris* matches with the mating phase of bumblebees which requires nectar to fed males and queens (Prys-Jones & Corbet, 1987).

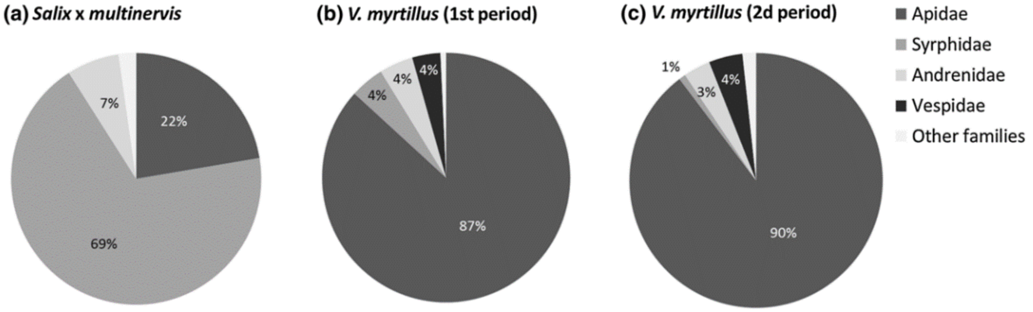


Figure 13. Visitor proportions of a Salix x multinervis *and b* V. myrtillus *during the first period (from early April to early May) and c* V.myrtillus *during the second period (mid-May) recorded in six sites in the Upper Ardenne, Belgium (Moquet et al.,2015).*

At the beginning of spring, only two floral resources are available in the Belgian heatlands: willows (*Salix* spp., Salicaceae) and blueberries (*Vaccinium myrtillus*, Ericaceae). On *S. x multinervis*, the main foragers recorded belong to the Syrphidae family (Diptera, 69%) and Apidae (Hymenoptera, 22%) with the genus *Bombus* (86%), *Apis* (12%), solitary bees (7% with especially *Andrena haemorrhoa*, *A. clarkella* and *A. praecox*) (Figure 13). The main foragers of *V. myrtillus* are belonging to Apidae (88%) with specimens of genus *Bombus* (96%), *Apis* (4%) and solitary bees (4%) with *Andrena apicata* and *A. haemorrhoa* and *Lasioglossum* spp. (Moquet *et al.*, 2015).

Chemical composition of pollen is significantly different between the two species. Pollen of *Salix x multinervis* has a concentration in polypeptides higher than the pollen of *Vaccinium myrtillus*. However, the abundance of nectar in the flowers of *V. myrtillus* is higher than *S. x multinervis*. During the blooming period of the two floral species, foragers seem to select the pollen with a higher nutrient quality and more accessible of *S. x multinervis* and forage on *V. myrtillus* for the nectar resources. An exception is the species *B. jonellus* which collects pollen of *V. myrtillus*. Analyses of 9 pollen loads from bumblebees caught on *Salix x multinervis* showed that 8 of them were composed of pure pollen of *Salix*.

After the blooming of *S. x multinervis*, the percentage of bumblebees foraging *V. myrtillus* to collect pollen increases significantly to reach 25%. *Vaccinium myrtillus* is then foraged for pollen resources. Pollen collected by bumblebees caught on *V. myrtillus* came largely from the flowers of *Vaccinium* genus (59% of the total pollen). Analyses of the pollen loads showed that the number of plant taxa varied between 1 and 4 plant species and was not significantly different among bumblebee species. From the total pollen loads collected on bumblebees, an average of 72% was mono-specific, 69% was constituted of *Vaccinium* pollen and 20% was constituted of *Salix* pollen. Other floral resources contained in the pollen loads were *Sambucus racemosa* (11%), *Vicia* sp. (3%), *Malus sylvestris* (3%), *Cytisus scoparius* (1%) and *Acer* sp. (0.5%).

Salix x multinervis and *Vaccinium myrtillus* are therefore complementary floral resources for the foragers during their life cycle inside heatlands (Moquet *et al.*, 2015). It has been shown that bees don't often collect simultaneously pollen and nectar but generally forage several plant species for each of the two kinds of resources (Brian, 1957). Pollen loads analyses showed that bumblebees are moving between different elements of the landscape mosaic (i.e. heatlands, bogs, grasslands and surrounding area) (Moquet *et al.*, 2017), and this highlights the importance of heterogenicity in the landscape mosaic (Westphal *et al.*, 2006; Goulson *et al.*, 2010; Somme *et al.*, 2015; Senapathi *et al.*, 2015).

Floral resources in urban area

The role of trees in the conservation of pollinator insect populations should deserve greater consideration as a single tree produces thousands of flowers (Somme *et al.*, 2016). The chemical composition of pollen and nectar of the 9 main ornamental tree species of Western European cities (*Acer pseudoplatanus*, *Aesculus carnea*, *A. hippocastanum*, *Robinia pseudoacacia*, *Tilia cordata*, *T. x euchlora*, *T. x europaea*, *T. platyphyllos* and *T. tomentosa*) have been analysed to determine the most effective tree species as bee floral resources.

Results showed that *Tilia* trees provide pollen with the lowest concentration of polypeptides, amino acids and phytosterols. Pollen of all the studied plant species contains more than 20% of total amino acids and all the essential amino acids. This confirms their potential use as a pollen resource. Concentration in phytosterols is particularly high in the pollen of *Acer pseudoplatanus* and *Robinia pseudoplatanus* (Somme *et al.*, 2016). The main phytosterols contained in the pollen of urban trees (β-sitosterol and δ5-avenasterol) seem to be positively associated to the larval development of bees (Vanderplanck *et al.*, 2014b). An increase of bee mortality seems to be linked to the presence of *Tilia cordata*, *T. tomentosa* (Crane, 1977; Pawlikoswski, 2010; Rasmont, 2014), *T. x euchlora* (Pigott, 2012) and *Aesculus hippocastanum* (Detzel & Wink, 1993), which could be explained by the presence of mannose (Crane, 1977) or nicotine in nectar (Singaravelan *et al.*, 2006; Naef *et al.*, 2004). The ornamental tree species studied here could be therefore considered in future plantations in cities (Somme *et al.*, 2016). *Acer* species are early-blooming trees (starting in April) and could be a precious floral resource during the early stages of social insect colonies development (Moquet *et al.*, 2015). *Acer pseudoplatanus* is one of the most important pollen resources and its production of nectar is between the other studied species (Somme *et al.*, 2016). After the spring blossom tree species, *Tilia* species could be foraged by pollinators during the maximal stage of colonies development from June to July. Although the *Tilia* pollen nutrient quality is lower than the others studied species, it could be an important resource of proteins and phytosterols for insects. Currently, only 4 native tree species (*Acer pseudoplatanus*, *Tilia cordata*, *T. platyphyllos* and *T. x europaea*) are generally planted in European cities (Somme *et al.*, 2016).

Floral choices in a changing world

Opportunistic behavior of bees

In a context of global changes, generalist bee species could adapt their diet by integrating new floral resources (Roger *et al.*, 2017). This drift in floral resources could modify the nutrient quality of the pollen diet of the bumblebees given that the pollen nutrient quality differs between plant species (Roulston & Goodell, 2011). The diet composition of five common bumblebee species (*Bombus hortorum*, *B. lapidarius, B. pascuorum, B. pratorum* and *B. terrestris*) in the North-West of Europe was studied between prior to 1950 and 2004-2005, and the study showed that one species (*B. lapidarius*) integrated more floral resources in its diet than the other species. Even if this observation is only significant for this bumblebee species, this trend to integrate new floral resources in the diet was observed throughout the four other species (Roger *et al.*, 2017). It could be explained by a morphological criterion of bumblebees which is the length of their tongue (Goulson & Darvill, 2004). *Bombus lapidarius* and *B. terrestris* possess a short tongue allowing them to foraged plants with short corolla. These plants are considered as in expanding distribution. Plants with a large corolla are not considered as in expanding distribution but are included in the diet of *B. hortorum* (i.e. *Trifolium pretense*). We estimated that half of the plants foraged by the five bumblebee species have expanded their distribution (especially plants belonging to *Pulmonaria, Rhododendron* and *Rubus* genera), the remaining half of the plants becoming less present in the diet of the bumblebees (especially plants belonging to *Stachys* and *Lotus* genera). In this context, bumblebee species with short tongue like *B. lapidarius* or *B. terrestris* could be considered as ecological opportunistic species given that these species integrate in their diet new plants species which are in expansion (e.g. *Trifolium repens*).

Although the chemical composition of the plants' pollen has changed during the two periods considered, the content in amino acids (total and essential) and the sterol profile of the pollen diet are not significantly different. However, plants foraged before 1950 possessed a median concentration of sterols higher than those foraged at the end of the 2004-2005 period. The total sterol content is lower in the recent diet and may consequently induces physiological issues (e.g. ovaries development) and an increase of pollen collection. Bumblebees may be able to exploit a large diversity of floral resources to compensate these modifications of pollen nutrient quality. The chemical quality of the pollen diet coming from entomophile plants seems to remain stable at a global scale thanks to a high diversity of pollen resources (Roger *et al.*, 2017).

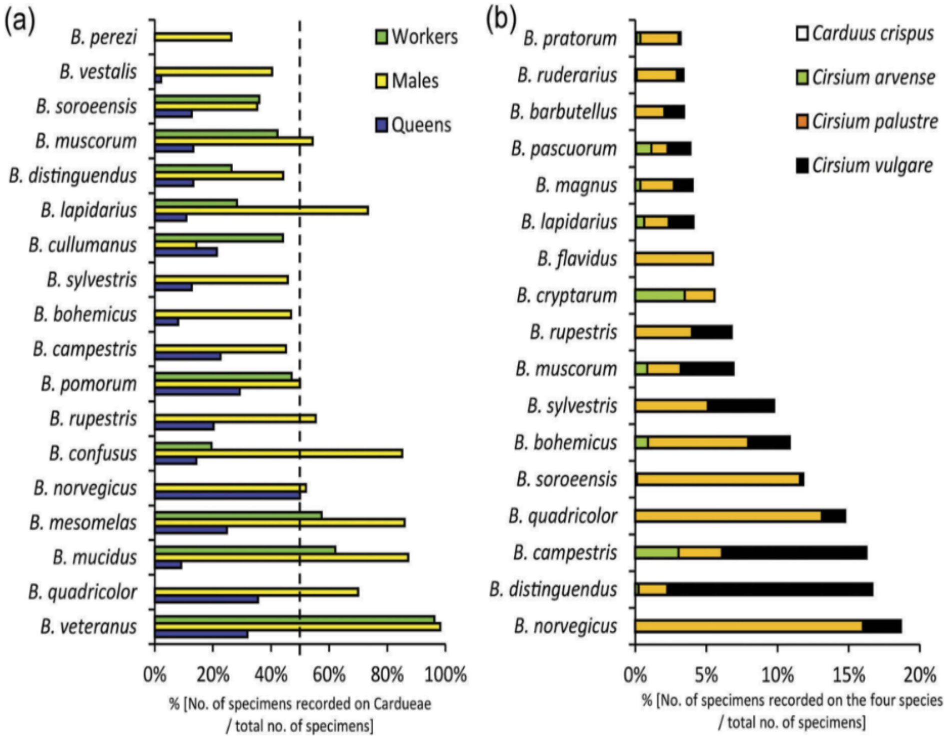


Figure 14. Proportion of bumblebee specimens observed on (a) Cardueae and (b) the four thistles species concerned by the regulations (number on Cardueae = 14,113 specimens; number on the four species = 3,016 specimens; total number = 88,974 specimens). Only species with a percentage higher than (a) 25% and (b) 3% (for all castes combined) are presented (Vray et al., 2017).

By combining several pollens, bumblebees are probably able to constitute an optimal diet (Eckhardt *et al.*, 2014). Bumblebees' diet could therefore remain rich in nutrient if the plant community is rich in species. A depopulated plant community could be composed of “extreme” elements leading to a potential unbalanced diet.

Importance of thistles for bumblebees

Our results reveal the great importance of thistles in the diet of male bumblebees (Figure 14) (Vray *et al.*, 2018). We show that a high number of bumblebee species, many of which are rare in Belgium and Europe, largely depend on four thistle species (*Cardus crispus, Cirsium arvense, C. palustre* and *C. vulgare*) 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 favor of alternative measures that reconcile the conservation of biodiversity and agricultural needs.

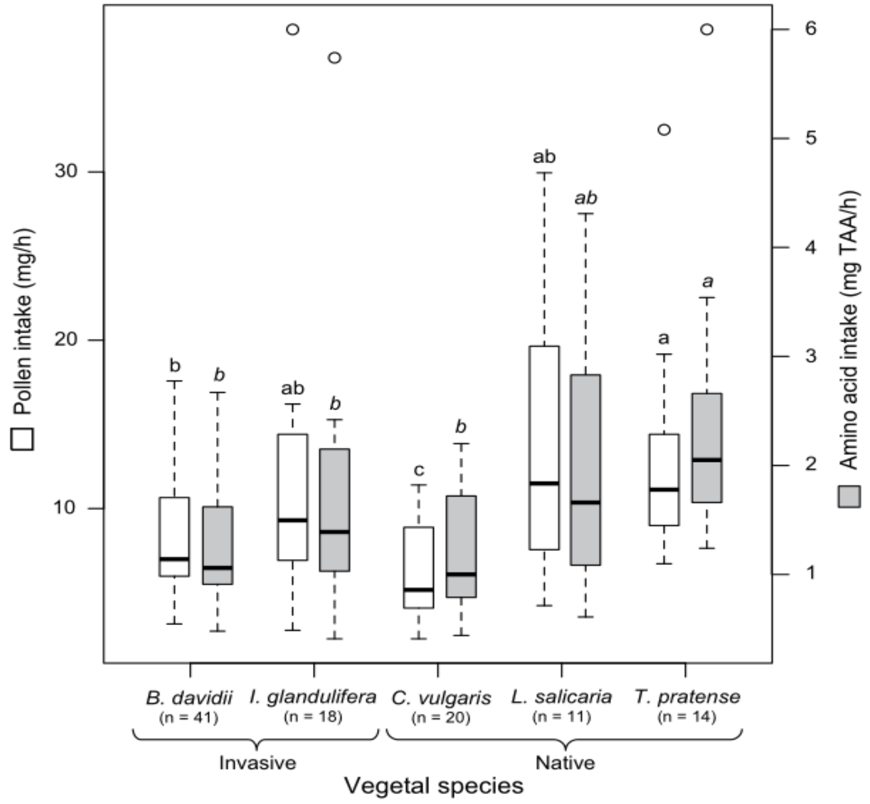


Figure 15. Foraging efficacy expressed by pollen intake (mg/h) and amino acid intake (mg TAA/h) depending on visited plant species. Species with the same letter are not significantly different (Drossart et al., 2017).

Invasive plants

Chemical analyses of the pollen of the two invasive plant species *Buddleia davidii* and *Impatiens glandulifera* highlight that there seems to have no differences in amino acids content except a lower concentration of proline than in the pollen of native plant species (Figure 15)

(Drossart *et al.*, 2017). Proline is an amino acid involved in the flight metabolism of bumblebees (Micheu *et al.*, 2000; Teulier *et al.*, 2006). Pollen of *I. glandulifera* contains a higher concentration of histidine than the native plant species. Despite the invasive behavior of these plants, bumblebees may forage them without changing their total pollen diet (Harmon & Kremen, 2015; Roger *et al.*, 2017).

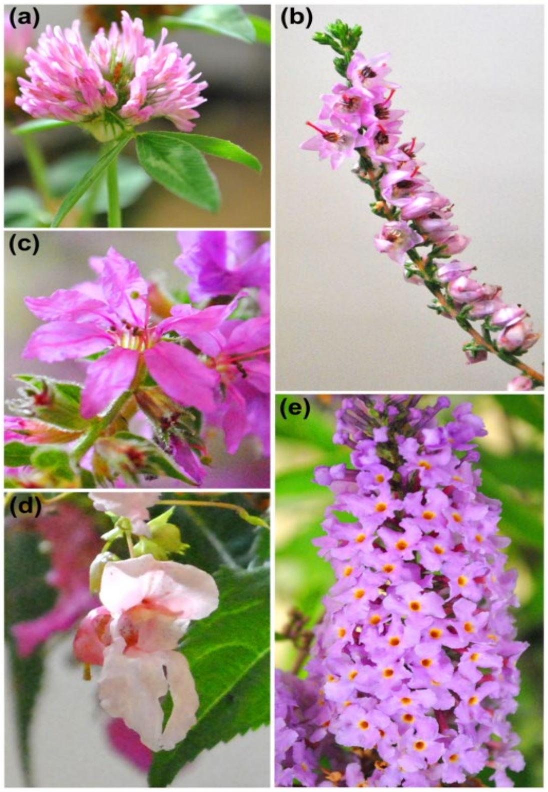


Figure 16. Photographs of studied plant species (a) Trifolium pratense*, (b)* Calluna vulgaris*, (c)* Lythrum salicaria*, (d)* Impatiens glandulifera *and (d)* Buddleia davidii *(Drossart et al., 2017).*

The behaviour of pollen collection in bees strongly depends on the floral symmetry of the host plant. Radial flowers are generally easier to manipulate than zygomorphic flowers (Goulson, 2010). The dense inflorescence of *Buddleia davidii*, *Calluna vulgaris*, and *Lythrum salicaria* allows foragers to feed them of a large number of flowers in a given time (Figure 16) (Drossart *et al.*, 2017). Other studies suggest that flowers of *C. vulgaris* placed in groups and the plant morphology allow workers to walk from flower to flower by enhancing their foraging time (Heinrich, 1979). These observations could be applied to *Trifolium pratense* which has a spherical and dense inflorescence. Despite the complex flower morphology of *T. pratense*, the flowers of Fabaceae are easily foraged by bumblebees and constitute precious host plants (Heinrich, 1979; Rasmont, 1988; Teper, 2005; Hanley, 2008). Pollen intake on *Trifolium* *pratense* provides the higher nutrient input considering the behavior of pollen research and the pollen chemical composition (Drossart *et al.*, 2017).

The foraging rate on *I. glandulifera* is low and suggests that the workers need more time to manipulate each flower. The energetic and temporal investment of workers is probably higher than for a dense inflorescence, by necessitating flight from flower to flower. The floral dimension could also reflect an abundance of resources which encourage workers to spend more time on a flower of *I. glandulifera*. The removal of these invasive plants could have detrimental effects on generalist bees in poor forage agro-environmental landscapes. However, the expansion of invasive plants might also have a negative impact on oligolectic bees which are closely dependant on native plants [e.g. *Bombus jonellus* on *Vaccinium*, and *Melitta nigricans* (Melittidae) and *Tetraloniella salicariae* (Apidae) on *Lythrum salicaria*)] (Drossart *et al.*, 2017).

4.3.1.2. WP3. Task 1. New subtask. Long-term dynamics of bee-plant interaction networks

To better understand the interactions and the long-term dynamics between plants and bees in Belgium, we used a network analysis approach. This network is composed of two kinds of nodes (plants and bees), where each link represents an interaction between a bee and a plant species. Several studies have demonstrated non-random structure of bee-plant networks (Thébault & Fontaine, 2010; Dupont & Olesen, 2009; Martín González *et al.*, 2012), as they tend to be made of subgroups of bee species connected to similar plants (modularity property) and to cover a broad spectrum of generalism among partners (Watts *et al.*, 2016).

Generalist bee species are recognized to be less vulnerable than specialist species. They visit a wide range of floral resources and may have an opportunist behaviour, i.e. shift on alternative resources. Because of this, we expected that over the last century (1) the frequency of generalist bee species increased over time in the interaction network; (2) species observed across time diversified their diet.

We used the *Banque de Données Fauniques de Gembloux & Mons* (Rasmont *et al.*, 2015) containing data about bee specimens collected by naturalists in Belgium since 1900. We focused on the continental bioclimatic region of Belgium, from which we had a more homogeneous distribution of historical data (Figure 17). Information about the plant species visited during sampling was available for almost 14,000 specimens in the study area. We splitted the database into two periods (1930-1969 and 1990-2009) corresponding to contrasted contexts, i.e. before and during agricultural intensification (Donald *et al.*, 2002; Kleijn & Sutherland, 2003). For each period, we built a binary bipartite network of observed bee-plant interactions (dimensions in TABLE III).

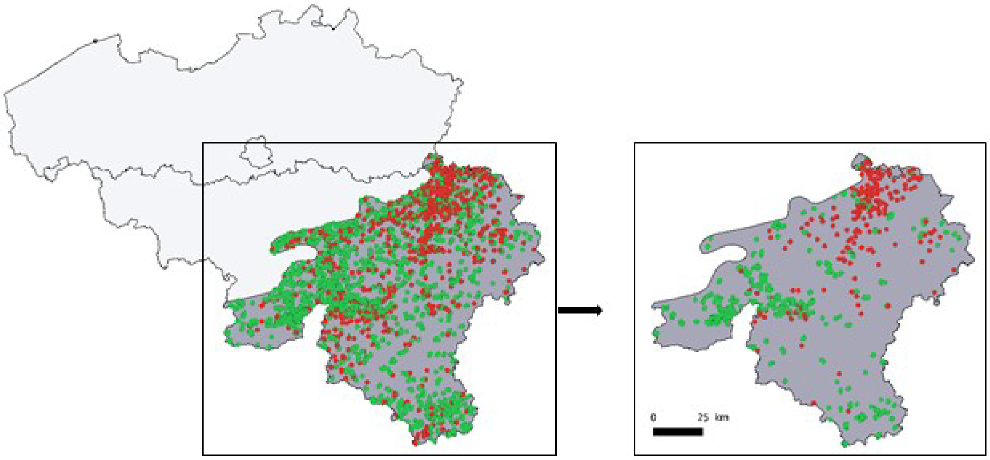


Figure 17. Distribution of data between 1930-1969 (red) and between 1990-2009 (green) in the continental biogeographical region (dark grey) in Belgium (extreme coordinates are 50°49'38" ; 5°37'00" North, 50°20'05"; 6°25'07" East, 50°08'08"; 4°08'16" West and 49°29'49"; 5°28'56" South). Left: data of bee specimens identified at the species level. Right: data of interactions between bee and plant species when at least two bee specimens were recorded per interaction per period.

Table III. Network dimensions according to historical periods (1930-1969 and after 1990-2009). The column "1930-2009" takes into account the number of species and interactions observed in both periods. The "total diversity" takes into account the number of unique species and interactions observed over time.

Dimensions	1930-1969	1990-2009	1930-2009	Total diversity
Number of bee species	132	126	68	190
Number of families	6	6	6	6
Number of genera	23	24	21	26
Number of plant species	202	206	92	316
Number of families	42	42	34	50
Number of genera	144	137	84	197
Number of interactions	541	795	66	1270

The modular structure of a network is related to the specialization of interacting species (Prado & Lewinsohn, 2004). Modules represent groups of species, such as the interaction density within modules is higher than between modules (Newman & Girvan, 2004; Olesen *et al.*, 2007). We computed it for the network of each period and characterized the position of species in the network structure based on their connectivity, i.e. number of interactions, inside and between modules. We calculated two statistics (Guimerà & Amaral, 2005a; Guimerà & Amaral, 2005b): the relative connectivity coefficient (*z*) and the participation coefficient (*c*), respectively. The higher the *z*, the more a species is associated to many partners of the same module. The higher the *c*, the more a species is connected to partners of other modules.

Based on these two coefficients, we assigned to each species one of four possible roles in the network topology statistics (Guimerà & Amaral, 2005a; Guimerà & Amaral, 2005b): within-module key species (i.e. module hubs with higher z-values and lower c), among-module key species (i.e. connectors with higher c-values but lower z), within-network key species (i.e. network hubs with both higher c and z), or peripherals (i.e. with both lower c and z values). A generalist species, taking into account its position in the network (in addition to the number of interactions in which it is involved), is a species with a significantly high c and/or z coefficient (Olesen *et al.*, 2007), compared to thresholds corresponding to the 90% quantiles of *c* and z coefficients of species of null models (Dormann & Strauss, 2014). These null models correspond to random networks obtained by permuting randomly the links of the observed matrix.

To test the hypothesis (i), we compared the distributions of *c*- and *z*-coefficients of bee species between periods. To test the hypothesis (ii), we focused on bee species observed at both periods.

We observed a significant decrease of bee species specialization at the network level over time, based on a decreasing modularity (M = 0.472, SES = 10.38 before 1970; M = 0.279 SES = 8.85 after 1990, p < 0.001) and an increasing *c*-coefficient (mean *c* = 0.30 before 1970; mean *c* = 0.43 after 1990, p-value < 0.001) while *z*-coefficient did not vary (p-value = 0.893) (Figure 18). Bee species diversified their diet by visiting more plants from other modules, i.e. by greater sharing of their resources.

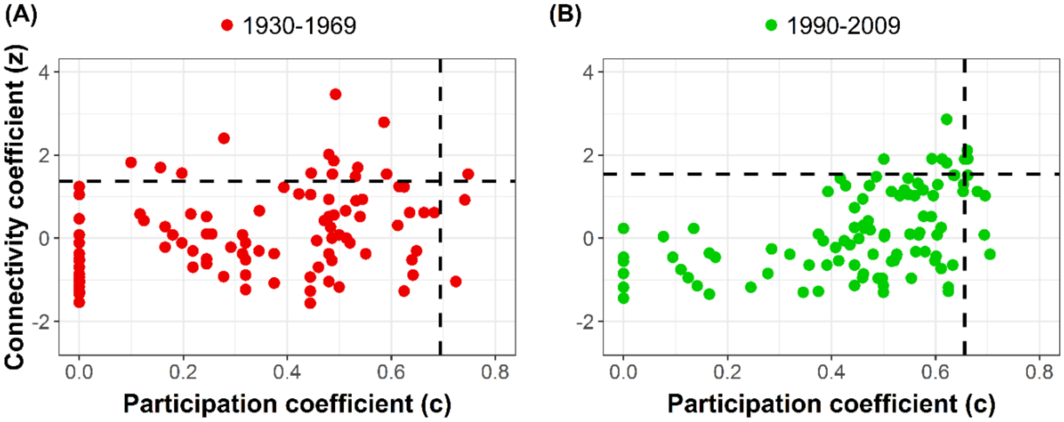


Figure 18. Biplot of connectivity coefficient of bee species (z, ordinates) and their participation coefficient (c, abscissa), during the periods 1930-1969 (left) and 1990-2009 (right). Vertical and horizontal dashed lines represent 90% quantiles of null model coefficients statistics (Guimerà & Amaral, 2005a; Guimerà & Amaral, 2005b; Guimerà et al., 2007), and delimit groups of species with different roles in network structure.

Likewise, the *c*-coefficient of the bee species observed in both periods significantly increased over time (mean values: 0.33 before 1970 and 0.45 after 1990, p-value = 0.001) (Figure 19). Some of them were able to shift their diet to alternative resources, or to modify the number of visited plant species (Roger *et al.*, 2017; Wood & Roberts, 2017). This opportunistic behaviour may have contributed to their maintenance in the network. It is however limited by the need to present the appropriate traits and learning abilities to balance costs and benefits of foraging of new plant resources (Heinrich, 1979; Rasheed & Harder, 2003; Drossart *et al.*, 2017).

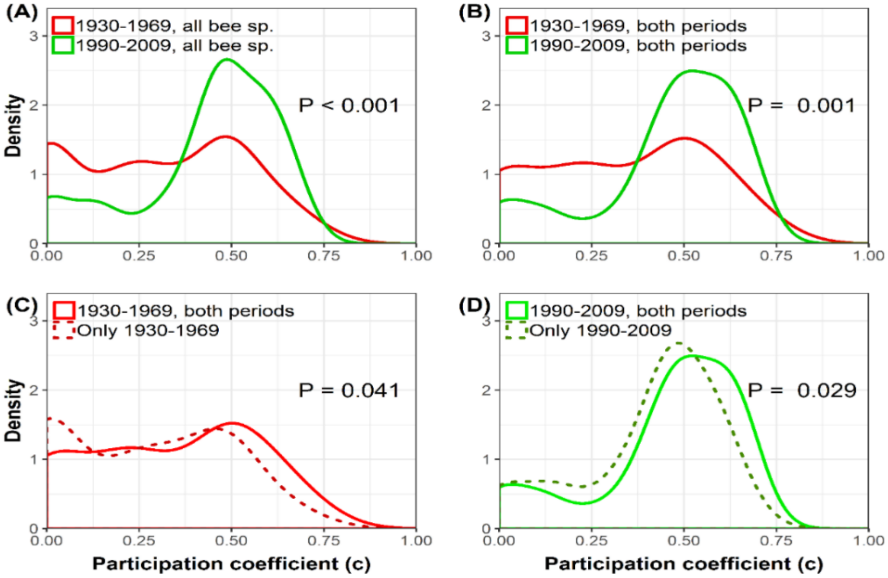


Figure 19. Distribution of participation coefficient (c-coefficient) of bee species (A) per period (red: 1930-1969; green: 1990-2009), (B) persisting in the network (i.e. observed during the two periods) per period (red: 1930-1969; green: 1990-2009), (C) that disappear of the network (dashed line) and persisting species (solid line) from 1930 to 1969, (D) that appear in the network (dashed line) and persisting species (solid line) from 1990 to 2009 (Guimerà & Amaral, 2005a; Guimerà & Amaral, 2005b; Guimerà et al., 2007).

Species that disappeared/appeared over time in the network tended to be more specialist (mean *c* = 0.263 for species disappearing, p-value = 0.041; mean *c* = 0.406 for species appearing in the network, p-value = 0.029) (Kleijn & Raemakers, 2008), plausibly because they were less able to switch their diet and therefore more sensitive to habitat fragmentation and changes in plant community composition (Ashworth *et al.*, 2004; Burkle *et al.*, 2013; Ollerton *et al.*, 2014; Roger *et al.*, 2017). Some specialist bee species may persist or appear in the network by interacting with generalist plants around which interactions become increasingly concentrated (Poisot *et al.*, 2015) or because their host plants are not negatively affected by anthropic disturbances (Scheper *et al.*, 2014). The greater sensitivity of specialized species at entry and exit from the network and their lesser detectability (Cirtwill *et al.*, 2018) partly explain the increase in the frequency of generalists in the networks.

Generalist bee species had a central position in the networks and are surrounded by a large number of peripheral species (Biella *et al.*, 2017). We identified 13 module hubs, one network hub and two connector species before 1970. After 1990, we found five module hubs, three network hubs and five connectors (TABLE IV). Five species retained a key role in the networks at both periods (e.g. *Bombus terrestris*).

TABLE IV. Key bee species identified by comparing their c-z coefficients with thresholds corresponding to the 90% quantiles of c-z coefficients of null models (Dormann, & Strauss, 2014): module hubs had higher z-values and lower c, connectors had higher c-values but lower z and network hubs had both higher c and z (Guimerà & Amaral, 2005a; Guimerà & Amaral, 2005b). $^{\alpha}$ = species that disappeared from the network after 1990; $^{\beta}$ = species that became peripheral after 1990; $^{\gamma}$ = periphal species that became key species after 1990; $^{\delta}$ = species that appeared in the network after 1990 as a key species; underlined = species that had a key role during both periods; in bold = species that kept their key role when we used the 95% quantiles of c-z coefficients of null models). Plant species with which they interacted the most before 1970 and after 1990 were the 10 species that have the maximum degree with key species.

	1930-1969	**1990-2009**
Module hubs	*Andrena bicolor*$^{\beta}$, *A. cineraria*$^{\alpha}$, *A. coitana*$^{\alpha}$, *A. flavipes*, *A. haemorrhoa*, *A. labiata*$^{\alpha}$, *A. minutula*$^{\alpha}$, *A. sabulosa*$^{\beta}$, *Eucera longicornis*$^{\beta}$, *Halictus rubicundus*$^{\beta}$, *H. tumulorum*, *Lasioglossum calceatum* and *Osmia rufohirta*$^{\alpha}$	*Bombus lucorum*$^{\delta}$*, B. pascuorum, B. terrestris*$^{\gamma}$*, Lasioglossum fulvicorne*$^{\gamma}$*, L. pauxillum*$^{\gamma}$
Network hubs	*Bombus pascuorum**	*Andrena flavipes, A. haemorrhoa****, Lasioglossum morio*$^{\gamma}$
Connectors	*Andrena proxima*$^{\alpha}$ and *Osmia bicornis*$^{\beta}$	*Ceratina cyanea*$^{\gamma}$*, Chelostoma rapunculi*$^{\gamma}$*, Halictus tumulorum, Lasioglossum calceatum, L. pallens*$^{\delta}$
Plant species (degree)	*Salix caprea* (5), *Tussilago farfara* (5), *Glechoma hederacea* (4), *Prunus spinosa (4), Bryonia dioica* (3), *Heracleum sphondylium* (3), *Hypochaeris radicata* (3), *Lotus corniculatus* (3), *Prunus cerasus* (3) and *Ranunculus acris* (3)	*Centaurea jacea* (6), *Echium vulgare* (6), *Origanum vulgare* (6), *Potentilla neumanniana* (6), *Ranunculus bulbosus* (6), *Thymus pulegioides* (6), *Cirsium arvense* (5), *Eupatorium cannabinum* (5), *Knautia arvensis* (5) and *Picris hieracioides* (5)

*Considered as a connector before 1970 based on 95% quantiles of c-z coefficients of null models.

**Considered as a module hub after 1990 based on 95% quantiles of c-z coefficients of null models.

Using a trait-based approach, we showed that the most generalist species had a larger body size (Figure 20), which can be associated to longer foraging distance (Greenleaf *et al.*, 2007), and a longer flight period than species limited to their module. Thanks to these characteristics, these species can have access to higher resource diversity/quantity than other specialized bees (Bommarco *et al.*, 2010; Wright *et al.*, 2015), even in a context of vegetation change and habitat fragmentation.

To ensure the viability and the functioning of plant-pollinator networks in the long-term, we recommend setting up a monitoring of key generalist species (Newman & Girvan, 2004; Biella *et al.*, 2017; Fortuna *et al.*, 2010). This is all the more important as their larger pollen quantity requirement threatens them (Müller *et al.*, 2006; Bartomeus *et al.*, 2013; Scheper *et al.*, 2014). However, these roles being quite variable in time, additional studies are needed to determine if this shift of key species ensures the maintenance of network functioning or if we are currently facing a "network-functioning debt".

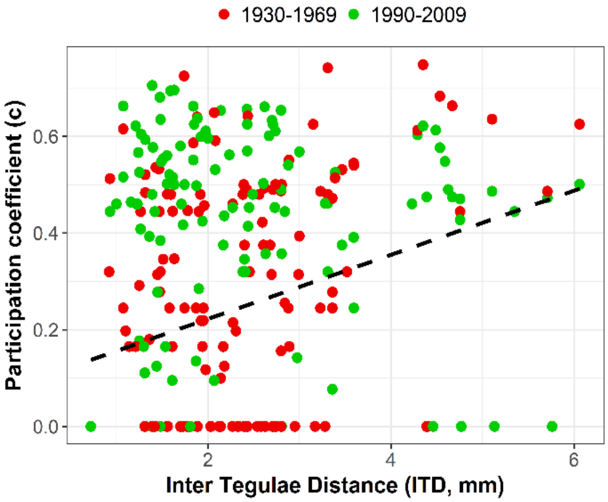


Figure 20. Distribution of inter tegulae distance (mm) of bee species according to their participation coefficient (c) by period (red: 1930-1969; green: 1990-2009). The dashed line is a 30% quantile regression line.

4.3.1.3. WP3. Task 1. Subtask 3.1.2. Dynamic of occurrences of the main food resources using Flora Atlases from Belgium

The study of dynamics of floral resources in Belgium firstly involved compiling floristic data in Belgium. We gathered almost 7 million floristic data in the country and checked their taxonomy. To limit the risk of misidentification and because of the heterogeneity of data sampling methods and of their intensity over time and over the country, we worked on taxa identified at the genus level.

We splitted the dataset into two historical periods (1930-1969 and 1970-2017) and synthetized the data by 4*4 km squares. For each period, we built a contingency table (squares in rows, plant genus in columns, and number of records as values). For the analysis, a single record represented one or more observations of a studied genus on a specific year at a particular square. We focused on squares with extensive sets of observations before and after 1970. Based on Biesmeijer *et al.* (2006), we selected squares containing at least 150 records of genus per period, a records-to-genus ratio of at least 1.5 in each of the two time periods and less than 10-fold difference in numbers of records between periods. This produced a total of 1,857 cells in Belgium.

To test the link between floral resources dynamics and pollinator decline in Belgium, we established a list of plant genera visited by ten bee species during last century: five declining bumblebees (*Bombus humilis, B. jonellus, B. ruderatus, B. soroeensis* and *B. sylvarum*), and five stable bumblebee species (*Bombus hortorum, B. lapidarius, B. pascuorum, B. pratorum,* and *B. terrestris*) (Kleijn & Raemakers, 2008). Like all species in the *Bombus* genus, these species are eusocial, polylectic and long-tongued. In addition to the data used for network analysis (see 4.3.1.2. and Rasmont (1988)), we compiled data of pollen load analysis (Kleijn &

Raemakers, 2008; Moquet, 2017; Roger *et al.*, 2017) to establish a list of plant genera visited by each of these bee species.

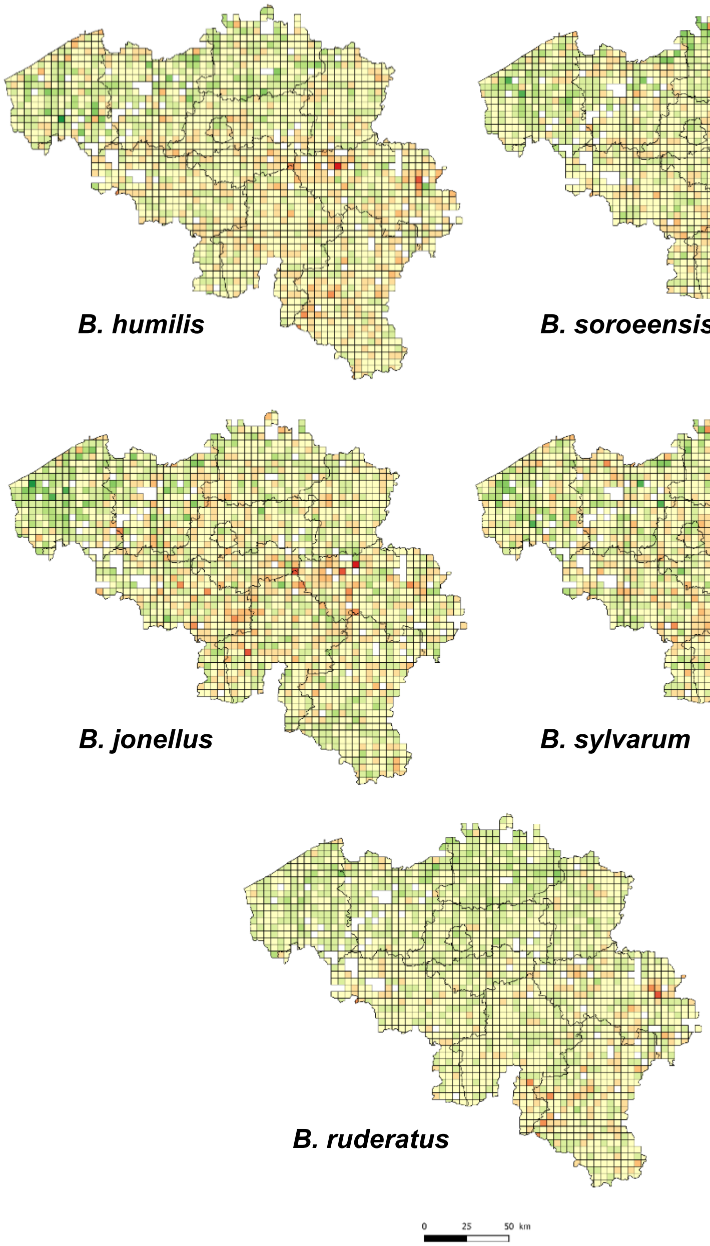


*Figure 21. Rate of change of floral resource genus diversity of declining bumblebees per square of 4*4km, between the periods 1930-1969 and 1970-2017.*

We crossed these lists of plant-resource genus with the flora database and built a matrix resource-genus*IFBL square for each studied bee species. Because sampling intensities and methods were not the same over periods, we applied rarefaction methods following Hurlbert method (Hurlbert, 1971) on each of these matrices to compare the diversity of plant-resource genus for each bee species before and after 1970. This approach allowed valid comparisons between time periods and regions (Biesmeijer *et al.*, 2006). We rarefied randomly 50 times the matrix of each period per region using the sample size of the minimum size of sample across periods (i.e. number of records) to calculate a mean number of plant-resource genus per square.

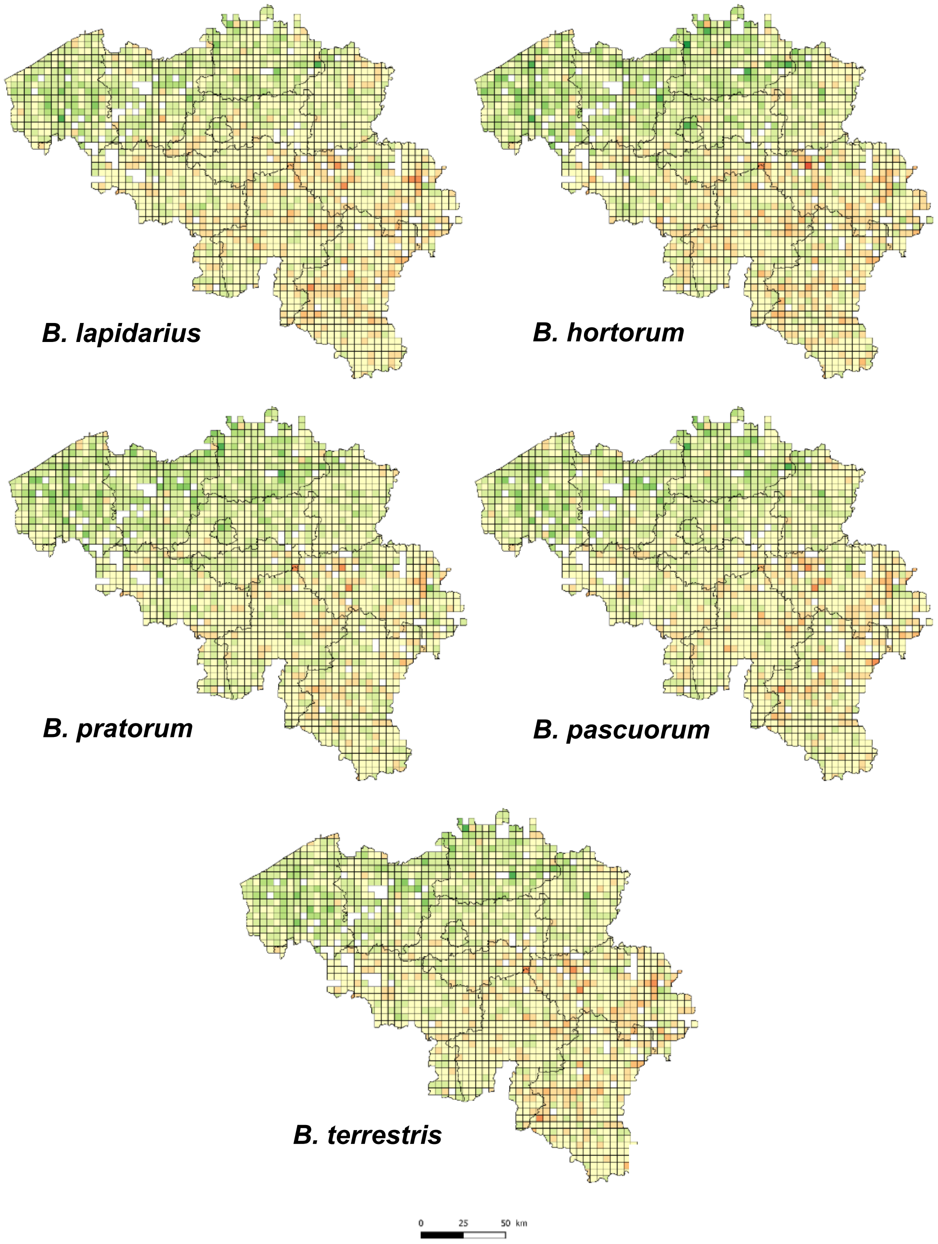


*Figure 22. Rate of change of floral resource genus diversity of stable bumblebees per square of 4*4km, between the periods 1930-1969 and after 1970-2017.*

For each targeted bumblebee, we calculated the shifts of plant-resource genus diversity as (div2-div1)/(div2+div1) with div1 and div2 = number of genera visited before and after 1970, respectively (Figure 21). Then we compared distributions of plant-resource genus diversity between periods (Wilcoxon tests, paired = T) per bee species and distributions of rate of change between declining and stable bee species (Wilcoxon test).

We compared these results to those obtained for stable generalist species (*Bombus hortorum*, *B. lapidarius*, *B. pascuorum*, *B. pratorum*, and *B. terrestris*) by applying the same method (Figure 22).

The diversity of floral resources was significantly lower after 1970 for all bumblebees (p-value < 0.001). The floral resource diversity of declining bumblebees decreased significantly more than resources of stable bumblebees (p < 0.001). This ties in with the conclusion made previously (see 4.3.1.2.) that generalist species seem less sensitive to dynamics of their resources.

The observed decrease in resource diversity is consistent with the drastic land use changes observed in Belgium during the last century (Hance *et al.*, 2010). However, sampling biases may persist despite rarefaction of our plant*IFBL square matrices. This could be partly responsible of the increasing diversity in the Flemish region.

Although the effect of floral resource dynamics on bee populations is combined with other factors (e.g. pesticides, diseases), the decline in resource diversity is parallel to that of the studied bee species. However, the work is still in progress: shifts of resource diversity being probably continuous, we would like to verify these shifts on shorter periods. We would also test the use of other indices (e.g. abundance) than diversity of genus-resources. The lists of plants used as resources by the studied bees will also be implemented by complementary data (e.g. GBIF). Land use maps could be introduced in the analysis in order to interpret the context of all these shifts.

4.3.1.4. WP3. Task 1. New subtask. Impact on pollination service of Belgian crops

Our evaluation of the pollination service is based on a methodology established on a global scale by Gallai *et al.* (2009). The economic value of the pollination service is estimated by the contribution of pollinators to the market value of Belgian crop production intended for human consumption (Klein *et al.*, 2007; Gallai *et al.*, 2009). All pollinators are considered here without distinction. Nevertheless, it is widely recognized that wild bees are major pollinators of entomophilous plants (Ollerton *et al.*, 2011).

This calculation involves data on production price (P, €/ton from FAOSTAT, 2010), quantity (Q, tons) from Belgian Federal Public Service of Economy (2010) (Function 1) and dependency on pollinator insects (D, % from Klein *et al.*, 2007) (Function 2) of a crop $i \in [1, I]$ in a region $i \in [j, J]$ (TABLE V). The dependency ratio D reflects the contribution of pollination to food production and corresponds to the quantitative relative loss of agricultural production that would be induced by the disappearance of pollinators. For example, cereal production dependency on entomophilous pollination is null (D = 0%) but it is essential to ensure the production of cucurbits (very high dependency, D = 90-100%) (Klein *et al.*, 2007). The ratio between these two values quantifies the rate of vulnerability of crops to the disappearance of pollinator insects (Function 3).

$$PEV = \sum_{i=1}^{I}\sum_{j=1}^{J} P_{ij} * Q_{ij}$$

Function 1. Total production economic value (€).

$$IPEV = \sum_{i=1}^{I}\sum_{j=1}^{J} P_{ij} * Q_{ij} * D_i$$

Function 2. Insect pollination economic value (€).

$$RV = \frac{IPEV}{PEV}$$

Function 3. Rate of vulnerability of crops (%) to pollinator insect disappearance.

TABLE V. Total value of crop production (PEV, €), pollination service (IPEV, €) and vulnerability (RV, %) of crop categories used as human food in Belgium in 2010.

Crop category	**Total value of crop production** (PEV, million €)	**Total value of pollination service** (IPEV, million €)	**Vulnerability index** (RV, %)
Cereals	476.88	0.00	0.00
Fruits	320.41	205.27	64.06
Oilcrops	6.06	1.82	30.00
Pulse	1.57	0.17	10.97
Roots and Tubers	409.12	0.00	0.00
Stimulant crops	0.26	0.00	0.00
Sugar crops	119.21	0.00	0.00
Vegetables	992.50	44.37	4.47
Total	**2,264.70**	**251.62**	**11.11**

Based on this method, it appears that the productivity of main crops in Belgium does not depend on pollinators (eg. cereals, roots, tubers, sugar crops) (Figure 23). This suggests that crop yield might be little sensitive to ongoing decline in pollinators. However the answer is more contrasted at local scale since the crop diversity varies a lot across areas. These findings highlight the importance of conservation programs maintaining floral resources essential to pollinator survival outside main crop areas, ensuring other crop and wild plant pollination.

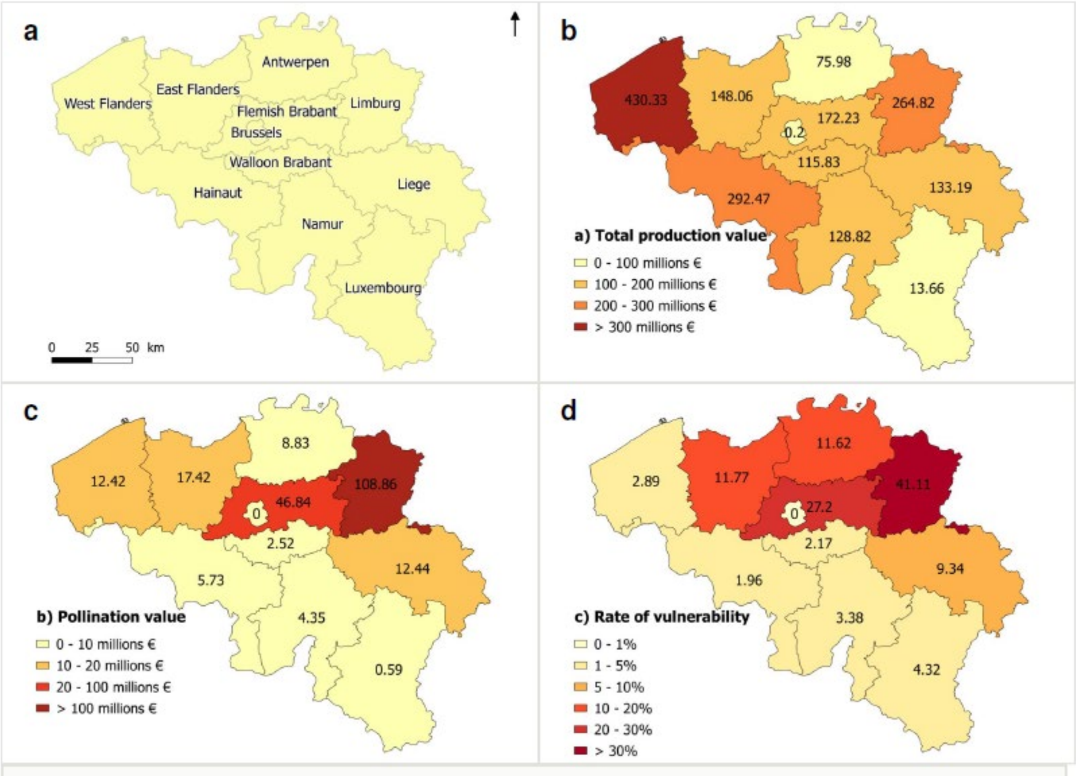


Figure 23. Major crops whose products are used directly for human food per Belgian province in 2010 (a: Belgian provinces; b: total production value; c: total value of insect pollination; d: rate of vulnerability to the decline in pollinators).

The rate of vulnerability of crops used for human food is about 11.1% at the national scale and ranges from 0.0% (Brussels Capital) to 41.1% (Limburg) at the provincial one. Even if these values of the pollination service heavily depend on our knowledge of pollination requirements which may vary between varieties and areas (Gallai *et al.*, 2009), they have the advantage of giving an overview of the spatial heterogeneity of pollination demand in agriculture. This huge spatial variability is mostly due to the concentration of fruit crops in the northern provinces of Belgium (e.g., Limburg and Flemish Brabant where the RV > 20%). Some provinces are highly productive but are less dependent on pollinators (e.g., Hainaut, West Flanders) because they are dominated by cereal crops. Unfortunately, the lack of historical price data prevents us from comparing the 2010 results with the past situation. At this stage, it does not allow us to establish a trend at the national level.

It should be noted that products of some large Belgian crops are not entirely used for human consumption (Delcour *et al.*, 2014). However, it is very difficult to isolate the part of the production that is used as food, feed, fuel or fiber. This distinction could not be made in this study, leading to an overestimation of the proportion of food production that depends on pollinators.

Estimates of potential production loss would also be more realistic if considering the rate of decline of pollinators and their substitutability, but also changes in other factors than only pollinator decline. Currently this caveat cannot be considered in the production function proposed by Gallai *et al.* (2009).

The hypothesis of total disappearance of the pollinators which is behind these calculations is binding or even unrealistic. Although local extinction is feasible, it is unlikely to occur on a larger scale. Nevertheless, the mapping of these indices highlights the geographical distribution of the preservation issues of pollinators and shows the importance of preserving pollinating activity to ensure the sustainability of Belgian agricultural production. In this sense, it could constitute a tool to aid decision to prioritize conservation measures of pollinators on Belgian territory, including the implementation of agri-environmental schemes (e.g., sown wildflower strips, high biological value meadows). They generally enhance species richness and abundance of major pollinator groups but not rare and/or declining species. Thus, they preserve the crop pollination service but their role in the conservation of threatened pollinator species is limited (Albrecht *et al.*, 2007; Haaland *et al.*, 2011; Scheper *et al.*, 2013).

4.3.2. WP3. Task 2. Hypothesis 2: Habitat fragmentation and genetic pauperization

4.3.2.1. WP3. Task 2. Subtask 3.2.1. To assess historical and current samples of different bee species: occurrence of genetic bottlenecks, changes in gene flow and genetic diversity

First, UG2 selected 16 microsatellite loci: four microsatellite markers (B11, B100, B126, B132) developed by Estoup *et al.*, (1993); four loci (BL02, BT04, BT08, and BT10) developed by Reber-Funk *et al.* (2006), five loci (BL13, BT02, BT05, BT23, BT24) were developed by Reber-Funk *et al.* (2006) and three loci (0294, 0304 and 0810) by Stolle *et al.* (2011), and developed 4 microsatellite multiplexes (4 loci per multiplex). These multiplexes were tested on representatives of almost all in Belgium living bumblebee species, this to enable the selection of a ‘core set’ of microsatellites. After DNA extraction, PCR amplification with these 16 microsatellite markers, and visualization with capillary electrophoreses on an ABI-3730xl sequencer (Applied Biosystems) performed with the methods described in Maebe *et al.* (2015) it became clear that all multiplexes could be reliable amplified in all species and were thus retained for all further analyses.

As published in Maebe *et al.* (2017), we then genetically investigated multiple bumblebee species, and hypothesized that (i) widespread bumblebee species have larger effective population sizes than sympatric restricted bumblebee species; and (ii) genetic diversity in the potentially smaller populations of restricted species should decrease over time by the influence of genetic drift.

Therefore, 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. 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 (Figure 24).

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. These five locations 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 biogeographical units of the country. Historical bumblebee specimens were collected from the Hymenoptera collection of the RBINS. In these five locations (Francorchamps, Moorsel, Nieuwpoort, Trivières, and Torgny; Figure 24), bumblebees were collected within a 5 x 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 VI), 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 VI).

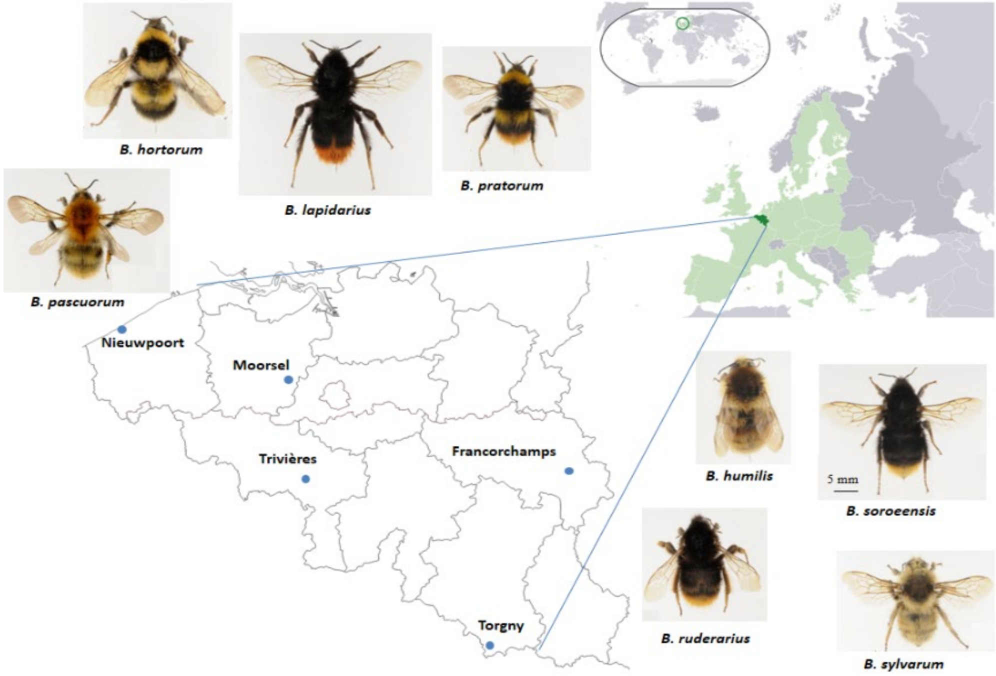


Figure 24. Overview of the bumblebee species sampled at five locations in Belgium. Specimens for each species were collected at the same five locations in Belgium in 1913–1915 and 2013–2015.

All bumblebee species belong to only one genus, *Bombus*, but are divided in different subgenera. The eight bumblebee species selected here belong to five different subgenera: *Kallobombus*, *Megabombus*, *Melanobombus*, *Pyrobombus*, and *Thoracobombus* (see TABLE VIII).

The 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, 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 with R package lme4 version 1.1-10 (LMM, *t*-test, $t = 6.721$, $p < 0.001$; TABLE VII) (original data from Rasmont *et al.* 1993; 2005); (ii) the presence and/or absence of the species at each location during the historical and recent bumblebee foraging season (TABLE VII): 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 (IUCN 2015; TABLE VII).

TABLE VI. 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.

		Historical					**Recent**				
Species	**Subgenera**	**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

Loci amplification and data validation

DNA extractions, PCR, capillary electrophoresis, manual scoring of microsatellite profiles and genetic analyses of +/- 1,100 samples were performed and finalized with the 16 microsatellite loci as mentioned above. Some of the genotyped specimens were excluded prior to data analyses, after applying several validation steps following Maebe *et al.* (2015). 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 (Wang 2004) and Kinalyzer (Ashley *et al.,* 2009). Furthermore, genotypic linkage disequilibrium, deviations from Hardy-Weinberg equilibrium (HW), and evidence of null alleles were tested using the programs FSTAT 2.9.3 (Goudet 2001), GENALEX 6.5 (Peakall & Smouse 2006) and MICROCHECKER (Van Oosterhout *et al.,* 2004) respectively.

TABLE VII. 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 (IUCN 2014); (ii) the number of bumblebee specimens within the RBINS collection before 1950 and between 1955–1993 (Rasmont et al. 1993; 2005); 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.

Species	European Population Trend	Belgian collection data		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

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 VIII). 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. When a full-sib pair within one population (specimens from the same location and time period) was detected, only one random selected specimen per sibship was kept for further analysis (TABLE VIII). After these two exclusions steps, 357 out of 566 historical and 436 out of 533 recent specimens remained in our dataset, which we used to estimate the different genetic parameters of all populations of each species (TABLE VIII). 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 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 (Kalinowski 2005) calculated and normalized on 10 diploid specimens for all populations, and Nei's unbiased expected heterozygosity (H_E; Nei 1978]) calculated with the program GENALEX 6.5.

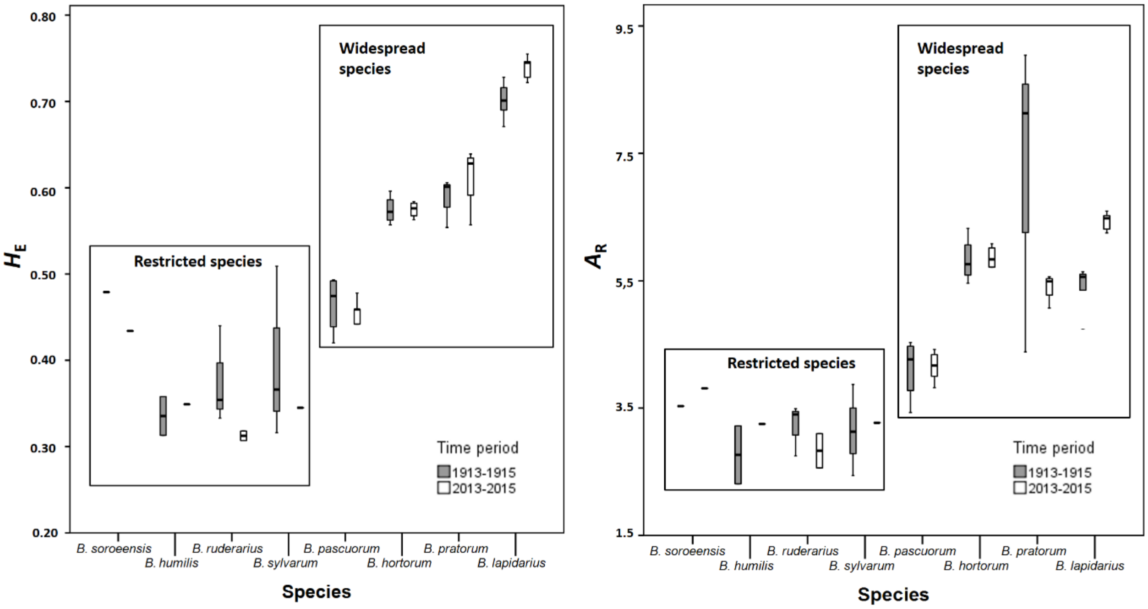


Figure 25. Comparison of the historical and recent genetic diversity within each Bombus *species. Box-plots of HE and AR for each species and for both time periods 1913–1915 and 2013–2015.*

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 VIII).

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; Figure 25). 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 VIII and Figure 25).

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. 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 subgenus), and species distribution (widespread or restricted, see explanation above and in TABLE IX). 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 (Barton, 2015). As described in Maebe *et al.* (2015), 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 subgenus 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 (Darvill *et al.,* 2010; Goulson *et al.* 2011). 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, subgenus was included in the LMM. The best LMMs were run in R studio with R package lme4 version 1.1-10 (Bates *et al.* 2015).

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 IX). 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 VIII). 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 VIII), time period was included in the best LMM models for A_R (delta = 0.000, TABLE IX). However, the effect of time period was not significant (LMM, P = 0.766, TABLE X), neither with the interaction of "distribution" and the different "subgenera" (LMM, P = 0.910, and P = 0.054–0.820, respectively; TABLE X). 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.

TABLE VIII. 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.*

			Historical time period (1913-1915)					***Recent time period (2013 - 2015)***				
	Species	**Location**	***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 IX. Selection of best fitting model explaining the genetic diversity in Bombus. *Of all possible models run under MUMIn (Barton 2015) using species distribution, species subgenus, 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	**(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

The models (delta < 2) which best fitted the observed pattern of the genetic diversity variable H_E, 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 IX). 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 IX). Although model (M12) showed a similar significant result for A_R (delta = 0.648, weight = 0.181; TABLE IX), 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 IX). Therefore, model M12 and model M48 were selected and performed as best fitting models for H_E and A_R, respectively (TABLES IX and X).

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 X), 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 X) and in *Thoracobombus* a lower H_E (LMM, *t*-test, $t = -3.348$, $p < 0.001$; TABLE X).

TABLE X. 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.*

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

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$_e$ (Wang & Whitlock, 2003). This method recently became recommended for the estimation of N_e in natural populations under both ideal and migration scenarios (see Gilbert & Whitlock, 2015). 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 one year was used, as most bumblebee species have one life-cycle per year.

The effective size of each population, with data from two time points available, were measured with MLN$_e$ (TABLE XI). Within one species our estimations of N_e varied remarkably depending on the population. Although these complexes the comparison of N_e between species, we can clearly 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 XI). Furthermore, the estimates also showed large population sizes for *B. hortorum*. *B. pascuorum*, *B. pratorum* and *B. lapidarius* showed similar values in N_e as those observed in *B. ruderarius* and *B. humilis* populations (TABLE XI), 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.

To conclude, our analyses showed that the genetic diversity of stable and declining bumblebee species remained stable over 100 years. Furthermore, our results also showed that recent populations of widespread bumblebee species have a higher genetic diversity than observed in the populations of restricted bumblebee species, this difference being already present 100 years ago. These results suggest that no genetic bottleneck has occurred during the 1950-60s due to the agricultural intensification. The alleged drivers are thus not directly linked with the genetic variation of currently declining bumblebee populations. Furthermore, our results showed that species distribution (being widespread or being restricted) could also explain the observed pattern of genetic diversity. Therefore, future sampling in the entire distribution range of these species should infer if the observed link between low genetic diversity and population distribution on the Belgium scale correlates with species decline on a global scale.

TABLE XI. 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	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

4.3.2.2. WP3. Task 2. Subtask 3.2.2. To assess the current population fragmentation by studying the genetic structure of different bee species in correlation with geographic distance

In general, no population structure under the small geographic distances in Belgium nor patterns of inbreeding were observed for the four stable species (*B. pascuorum, B. lapidarius, B. hortorum* and *B. pratorum*). For the four declining bumblebee species (*B. ruderarius, B. sylvarum, B. humilis* and *B. soroeensis)*, unfortunately too few locations of these species were still present in Belgium to allow accurate population structure measurements. Therefore, enlarging the scale from a country scale (Belgium) to a continental scale (Europe) should allow for more meaningful population structure measurements in these species.

Thus here, we compared the population structuring between populations of seven bumblebee species from Belgium and Estonia. Specimens of three declining species: *B. ruderarius*, *B. soroeensis* and *B. sylvarum, versus* four more abundant species: *B. hortorum*, *B. hypnorum*, *B. lapidarius*, and *B. pascuorum.* Specimens (*N* = 20-25) were sampled from each of the seven locations (Figure 26), five in Belgium (Moorsel, Trivières, Francorchamps, Nieuwpoort and Torgny) and two in Estonia (Harjumaa and Põlvamaa) during the bumblebee foraging seasons of 2013-2016. After all specimens were genotyped and analysed with 16 microsatellite loci (following the method described by Maebe *et al.,* 2015, and explained above see 4.2.2.1.), the population structuring and link with both the short and long geographic distances between these European populations were investigated.

Figure 26. Overview of the sampling locations in Belgium and Estonia for population structuring.

Population structure within each *Bombus* species was inferred, based on a Bayesian clustering method. The software Structure v. 2.3.3 (Pritchard *et al.,* 2000) was used to perform a Bayesian approach to determine the number of populations in each species' dataset separately. In this analysis, the number of populations (K) was estimated from 1 to 10 for all species separately. Each K-value was calculated with a burn in of 1,000,000 iterations and 2,000,000 MCMC data collecting steps, and was repeated 9 times. The open source program Structure Harvester v. 0.6.93 (Earl & vonHoldt 2012) was used to determine the best value of K (Evanno *et al.,* 2005), and the program Distruct v.1.1 (Rosenberg 2004) was used for graphical visualization of the population structure.

The Evanno method identified ΔK = 2, which is the best value of K (or number of populations) that fitted our data, for almost all bumblebee species (*B. hypnorum*, *B. lapidarius*, B*. pascuorum*, *B. ruderarius* and *B. sylvarum)*. Furthermore, the best K-value for *B. hortorum* and *B. soroeensis* were identified as K = 4 and K = 3; respectively. These structure results are shown in Figure 27, for each species separately. As each specimen of four bumblebee species (*B. sylvarum*; *B. soroeensis*, *B. hortorum, B. hypnorum* and *B. ruderarius)* belonged for approximately 50% to both groups (Figure 27) and as the Evanno method is not able to calculate K = 1, we therefore changed K to 1, highlighting that no structuring is retrieved in these species. So, the Belgian and Estonian populations could not be genetically distinguished from each other. However, the results for *B. lapidarius* and B*. pascuorum* represent a structuring of the specimens following the original sampling country.

The latter results should be investigated in more detail, as it could be a small but clear difference in allele composition of a few or multiple loci between the Belgian and Estonian bumblebee populations within these species, but it could also be the results of a more limited gene flow between these populations on the European scale. The discrimination between both hypotheses will be important to understand the impact of these results for the conservation of these wild bee species.

The more detailed investigation of population structuring between the Belgian and Estonian populations of *B. pascuorum* and *B. lapidarius* revealed only small differences between the populations allelic composition. Furthermore, pairwise *F*st-values performed in GENALEX 6.5 (Peakall & Smouse, 2006) were only low (*F*st = 0.037-0.088) but significant ($p < 0.05$) (TABLE XII), while also the performed Mantel tests (Mantel, 1967), in which the geographic and genetic distances of the populations were compared, showed a clear trend but no significant structuring or isolation by distances ($p > 0.05$) so that spatial processes are not strongly driving population structure within these species (Figure 28). Thus, in general no strong population structuring was found in the seven selected bumblebee species on a European scale.

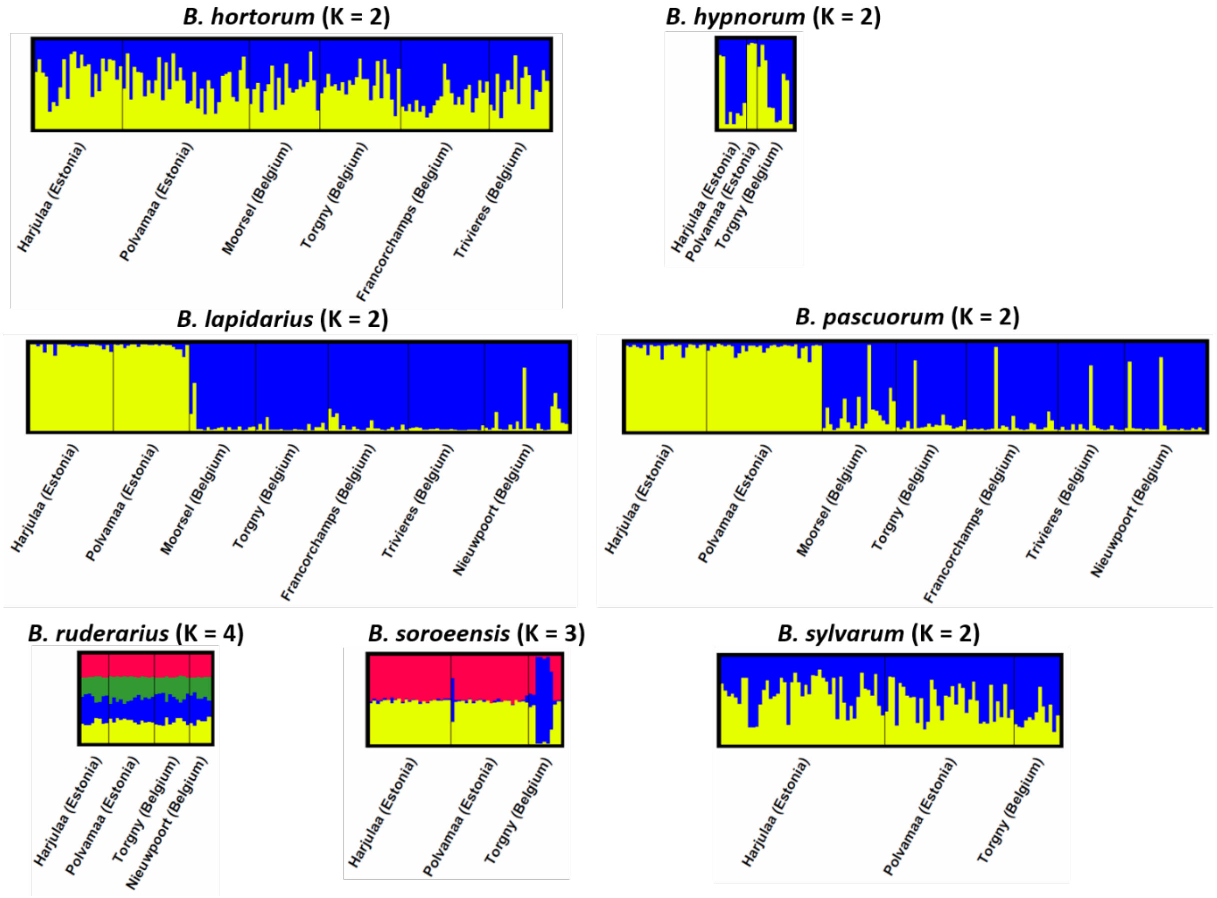


Figure 27. Bayesian clustering of the populations of each bumblebee species. Each vertical line stands for an individual specimen, while the colors are indicative for the proportion a specimen belongs to a certain group. Each cluster of specimens represents their 'original' population.

TABLE XII. Estimation of population structuring (F_{st}) between both Belgian and Estonian populations for B. lapidarius and B. pascuorum

Species	**Locations**							
	Estonia		***Belgium***					
	Harjumaa	Põlvamaa	Moorsel	Torgny	Francorchamps	Trivières	Nieuwpoort	
B. lapidarius	-	0.056	0.001	0.001	0.001	0.001	0.001	Harjumaa
	0.015	-	0.001	0.001	0.001	0.001	0.001	Põlvamaa
	0.042	**0.050**	-	0.237	0.176	0.249	0.090	Moorsel
	0.050	**0.058**	0.016	-	0.528	0.762	0.103	Torgny
	0.047	**0.053**	0.016	0.012	-	0.563	0.013	Francorchamps
	0.047	**0.054**	0.016	0.012	0.012	-	0.054	Trivières
	0.037	**0.043**	0.016	0.015	**0.017**	0.016	-	Nieuwpoort
	Harjumaa	Põlvamaa	Moorsel	Torgny	Francorchamps	Trivières	Nieuwpoort	
B. pascuorum	-	0.296	0.001	0.001	0.001	0.001	0.001	Harjumaa
	0.011	-	0.001	0.001	0.001	0.001	0.001	Põlvamaa
	0.055	**0.050**	-	0.200	0.070	0.121	0.001	Moorsel
	0.085	**0.074**	0.016	-	0.001	0.001	0.001	Torgny
	0.068	**0.065**	0.016	**0.033**	-	0.496	0.001	Francorchamps
	0.070	**0.062**	0.016	**0.026**	0.011	-	0.001	Trivières
	0.088	**0.082**	**0.033**	**0.046**	**0.023**	**0.030**	-	Nieuwpoort

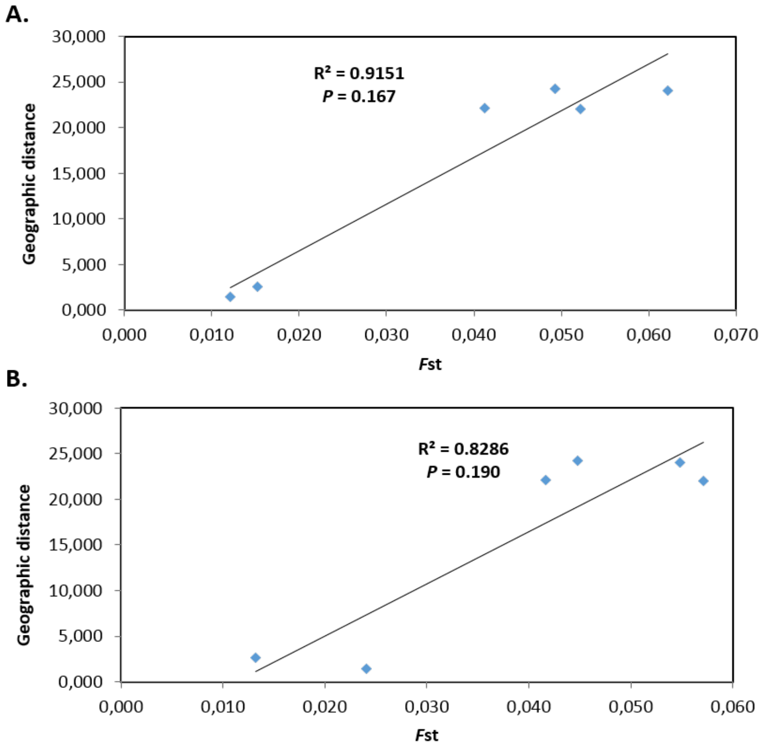


Figure 28. Mantel tests performed on populations of: A. B. lapidarius *and B.* B. pascuorum

4.3.2.3. WP3. Task 2. Subtask 3.2.3. To assess the effects of landscape changes on wild bee species

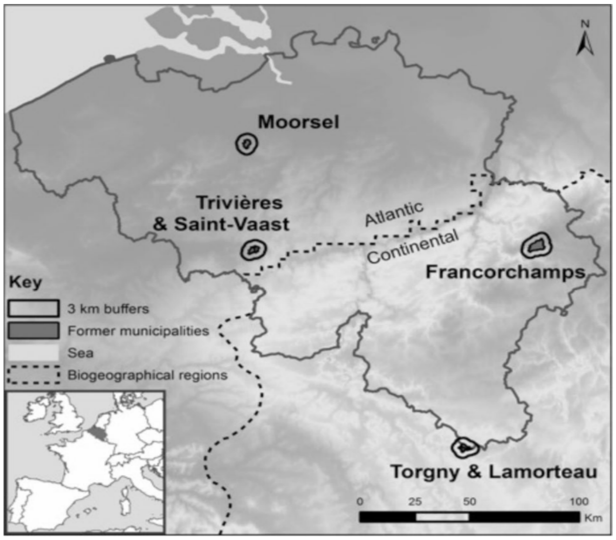


Figure 29. Localization of the four sampling localities consisting in former municipalities (red zones) and their 3 km buffer (black circles). Trivières and Saint-Vaast are merged in "Trivières", and Torgny and Lamorteau are merged in "Torgny" in analyses (from Vray et al. 2019).

To qualify and quantify the changes in bumblebee communities in relation to landscape changes that occurred during the last century (i.e. largescale agricultural intensification and urbanisation), here, we performed a comparative analysis based on landscape composition and bumblebee occurrence records in the early twentieth century (1910–1930) and the contemporary period (2013–2015) in four localities representative of Belgium (Figure 29; see Vray et al. 2019).

We used the *Banque de Données Fauniques de Gembloux & Mons* (Rasmont *et al.*, 2015) containing data about bee specimens collected by naturalists in Belgium for the old period (1910-1930) and we resampled same sites during 2013-2015. To assess the compositional dissimilarity of bumblebee communities between localities and periods, we computed the Bray–Curtis dissimilarity coefficient based on the logarithm of species abundances (i.e. number of specimens). 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 $\chi 2$ distance in Correspondence Analysis. 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.

Landscape composition was analysed at a highresolution in each period and each locality (Figure 30). Based on the foraging range of bumblebees, we considered 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", obtained from the actual "Institut Géographique National" (IGN) of Belgium. For the recent period (P2), we used the "Top10Vector" from the IGN of Belgium for 2012. Because Torgny is at the border of France, we used aerial photographs from 1930 and orthophotos of 2012 from the French IGN in order to obtain land cover information for the French part of the buffers of 1 and 3 km. Then, we manually classified land cover into eight types: 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, and others (water, marsh and swamp, rock, quarry, and sand) (Figure 30, TABLE XIII). All geographic analyses were performed using ArcGIS 10 software (ESRI 2011).

TABLE XIII. Landscape composition of the four localities in hectares (and in percentage of the considered buffer) of the 8 land cover types in the 1-km and 3-km buffers in the past period (P1: 1910-1930) and the present period (P2: 2013-2015) (from Vray et al. 2019)

	Francorchamps		**Torgny**		**Moorsel**		**Trivières**	
	P1	**P2**	**P1**	**P2**	**P1**	**P2**	**P1**	**P2**
Land cover area in 1km buffer (%)								
Cropland	14.6	0.1	35.6	21.0	71.4	28.9	67.0	37.8
Gardens	0.1	3.8	1.3	3.0	4.1	17.2	3.6	18.5
Grassland	12.7	21.0	30.6	37.9	10.8	33.7	8.8	15.4
Heathland	11.6	4.8	/	/	/	/	/	/
Orchards	0.1	/	0.5	0.5	2.3	1.9	1.5	0.1
Settlement	3.6	4.7	4.4	3.2	6.2	8.2	14.5	14.7
Woodland	57.2	65.0	26.6	33.1	5.0	9.9	1.6	11.0
Other	0.01	0.4	0.9	1.2	0.2	0.2	3.1	2.4
Land cover area in 3km buffer (%)								
Cropland	15.0	0.2	36.8	25.3	64.1	25.3	65.2	36.8
Gardens	0.2	4.5	1.1	1.9	4.8	20.5	4.3	18.6
Grassland	11.1	20.6	26.8	31.5	12.5	28.1	8.1	14.5
Heathland	13.5	6.3	/	0.1	/	0.1	/	/
Orchards	0.2	0.1	0.4	0.2	3.4	1.8	2.2	0.1
Settlement	4.0	5.1	3.1	2.9	8.4	13.4	15.3	17.9
Woodland	55.8	62.8	31.1	37.2	5.7	10.0	2.4	9,00
Other	0.2	0.5	0.7	0.9	1.0	0.8	2.5	3,00

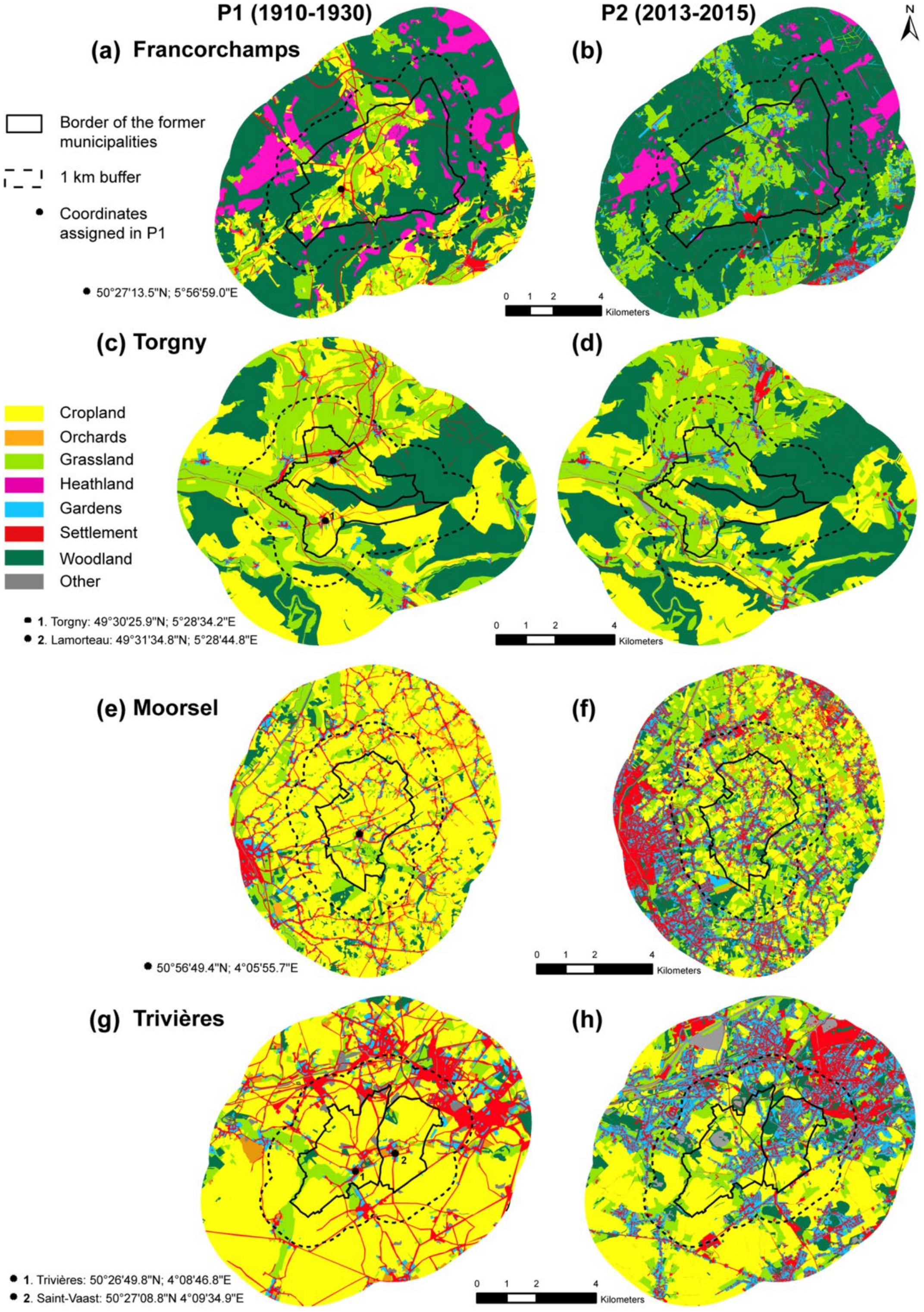


Figure 30. Land cover in a 3 km buffer around the former municipalities of Francorchamps in P1 (a) and P2 (b), Torgny (and Lamorteau) in P1 (c) and P2 (d), Moorsel in P1 (e) and P2 (f), and Trivières (and Saint-Vaast) in P1 (g) and P2 (h) (from Vray et al. 2019)

Changes and intensification of land-use during the last century

Land use drastically changed between the contemporary period and a century ago in most localities, with a similar pattern at the regional and national scales, as well as the international scale as seen between 1930 and 2007 in England. Landscapes of a 100 years ago were dominated by low productivity crops, grasslands with low livestock density, orchards, and woodland sometimes associated with heathland. Settlements and gardens covered a relatively small percentage of the landscape. During the last century, we observed a strong increase in grassland area (Figure 30, TABLE XIII). This pattern is also highlighted at the global scale, as the share of global pasture grew from 2% of the world's surface area in 1700 to 24% in 2000 (34 million km^2). However, not only grassland areas (including pastures and mown grasslands) increased, their management is also more intensive than in the past, with higher livestock densities and the addition of fertiliser to increase animal production, which decreases grassland quality. Generally, the remaining grasslands are usually turned into intensive pastures or meadows mown early in the season for ensilage, which removes floral resources.

Cropland drastically decreased, probably to be partly converted to mown and grazing grassland (in agreement with the previous trend), as observed in previous European studies, with 21% of all land use changes caused by the conversions of cropland to grassland. However, the types of crops changed and the intensity of their use strongly increased in comparison with the early 20th century, with a threefold increase of yield per hectare. In 1910, croplands in Trivières and Moorsel were dominated by low yielding cereal crops, leguminous plants (e.g. *Trifolium pratens* with 70 ha and 30 ha in Moorsel and Trivières respectively), potatoes (170 ha in Moorsel) and sugar beet. They are now dominated by cereals, potatoes, and sugar beet with high productivity, but without value for pollinators populations, and with the total abandon of leguminous crops, which can provide a very valuable resource for bumblebees. Moreover, orchards have almost disappeared today (e.g. in Trivières), probably resulting from the Mansholt Plan in the 1970s, which strongly encouraged the felling of most orchards.

However, a part of the strong increase in the surface of grasslands in the landscape could be due to changes in grassland classification between pre-1930 maps and current ones. Maps of the past period (1910-1930) combined "artificial grasslands" (e.g. mown grasslands) with the crop fields into only one identification class called "cultivated fields and artificial grasslands", while recent maps separate these artificial grasslands and other field crops. Thus, for the recent present period (2013-2015), it is therefore possible to sum mown grasslands with permanent grasslands (e.g. pastures), decreasing the percentage of crop areas to the detriment of grassland areas. But this affects neither the results nor the observed trends, as shown by the agricultural statistics at the restricted scale of the commune. Overall, there has been a large decline in crops (notably cereals), hayfields, clover crops, alfalfa, with an increase in overgrazed pastures, and probably urbanized areas in highly urbanized areas. Residual permanent elements, such as hedges, slopes and field margins in the late mowing, remain abundant in Francorchamps and Torgny. The hedgerows between pastures and hayfields is still well developed. However, heathland areas in Francorchamps decreased by half, as observed in England.

Finally, urbanisation, accompanied by an increase in population density, was the most intense in Moorsel and Trivières, with the highest expanding of settlement areas and gardens. On the other hand, Torgny is the least affected by land cover and land use changes and its human population density barely increased.

Saptio-temporal changes in bumblebee assemblages

Our results show that observed bumblebee species richness globally decreased, 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. Decreases of species richness and shifts in pollinator communities were also observed in 79% of the studied localities in England between 1930 and 2007. In our results, the most severe decline is observed in the two western localities, Moorsel and Trivières (with an expected richness decreasing from 17.2 and 14.1 in P1 to 6.6 and 7 species in P2, respectively), whereas the two eastern localities, Francorchamps and Torgny, tend to have, respectively, a stable or even a higher expected richness than in the past (from 10.7 to 10.1 species in Francorchamps and from 11.6 to 15.4 species in Torgny). 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' assemblage composition during a century.

As several previous studies, we observed a higher bumblebee species richness and expected richness in landscapes dominated by grassland than in landscapes dominated by settlement, gardens, and intensive crops. Francorchamps and Torgny are the two localities with most grassland and woodland and harbour the highest species richness today. The high proportion of woodland in these two localities could explain the presence of some species preferring wooded habitats, such as *B. norvegicus, B. lucorum* and its parasite, *B. bohemicus*. However, regions highly dominated by woodland may be detrimental for species preferring open lands, such as *B. ruderarius, B. rupestris, B. sylvarum* and *B. veteranus*, associated to grasslands. The highly wooded locality of Francorchamps harbours indeed less expected species than Torgny, and it 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*). Previous studies have shown that landscapes too closed by woodland do not seem 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.

Species sensitivity to land use change tend to be influenced by their ecological traits (Figure 31). Generalist species such as *B. hypnorum, B. lapidarius, B. pascuorum* and *B. terrestris*, tend to be less sensitive to agriculture intensification and to urbanisation. These species are indeed among the only ones still observed in Moorsel and Trivières in the recent samplings. Conversely, species specialized in specific habitat or plants declined or disappeared when this indispensable resource decreased. This is the case of *B. cryptarum, B. jonellus*, and *B. magnus* specialized on heathland. Hundred year ago, they were more abundant in the only locality presenting heathland (i.e. Francorchamps) than in the others. 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 changes at large scale.

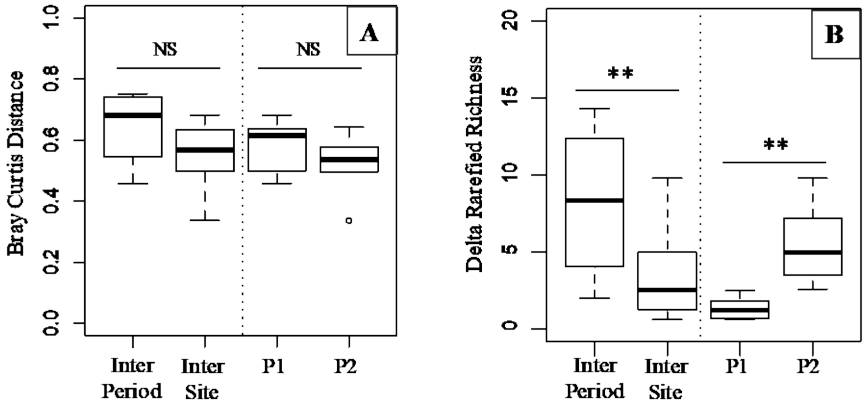


*Figure 31. Bray Curtis dissimilarity coefficients (BCd) (a), and difference in expected species richness in a sample of 796 specimens (b) between the two periods. Inter-period : BCd or Richness difference between P1 and P2 for each locality (n = 4). Inter-site : BCd or richness difference between each pair of localities in a same sampling period (n = 12). P1 : BCd or richness difference between each pair of localities during P1 (n = 6). P2 : BCd or richness difference between each pair of localities during P2 (n = 6). Mean value comparisons was performed by tests of Student (NS not significant ; *: 0.05 > p-value > 0.01; **: 0.01 > p value > 0.001; ***: 0.001 > p value) (from Vray et al. 2019).*

4.3.3. WP3. Task 3. Hypothesis 3: Disease emergence

4.3.3.1. WP3. Task 3. Subtask 3.3.1. Metagenomic survey of microbes in target bee species

Two metagenomic surveys were carried out in a total of nine bee species (*Andrena cineraria, A. fulva, A. haemorrhoa, A. vaga, Bombus cryptarum, B. pascuorum, B. terrestris, Osmia bicornis,* and *O. cornuta*) over five localities in Belgium (Ghent, Moorsel, Trivières/St-Vaast, Francorchamps and Torgny/Lamorteau). The first metagenomics survey was carried out in the proximity of an apiary stand in Ghent (Schoonvaere *et al.*, 2016). The second metagenomic survey extends on the first survey by including more geographical regions and doubling the number of bee species (Schoonvaere *et al.*, 2018). We found a large diversity of parasites of wild bees, but no significant associations of honeybee-specific pathogens. In fact, the study revealed that wild bees carry their own set of viruses which are either related to known arthropod infecting viruses or a previously unknown group of viruses ("negative-sense single-stranded RNA or –ve ssRNA viruses") that are demonstrated to infect bees (Schoonvaere *et al.*, 2016). The genomes of two viruses in particular, Ganda bee virus and Scaldis River bee virus, were better characterized (the genome sequence is illustrated in Figure 32). This will facilitate future research on the biological role of –ve ssRNA viruses in bees. Currently the role of these viruses is unknown. Apart from our observation, evidences suggest that the viruses are able to infect bees (Remnant *et al.*, 2017).

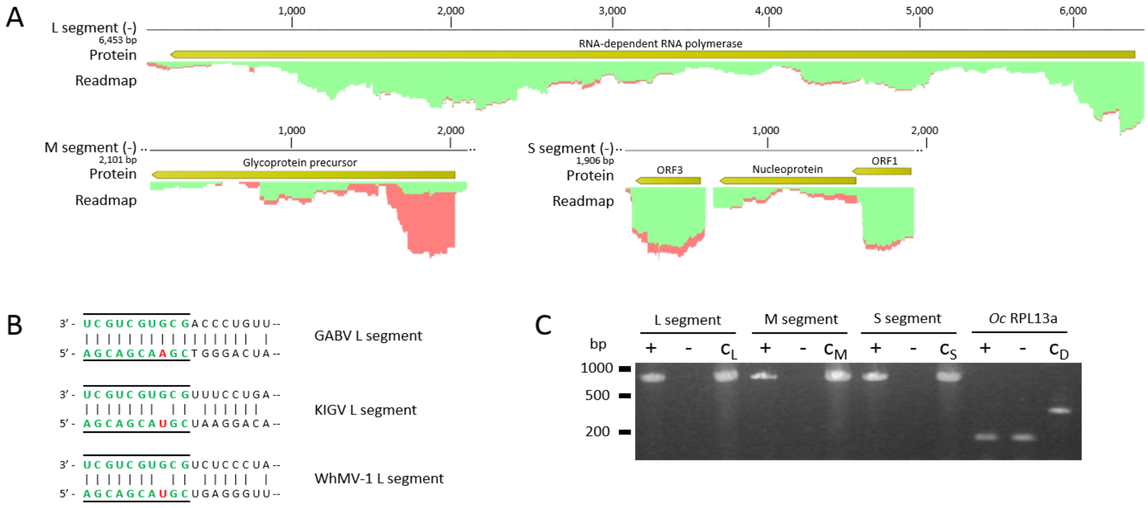


Figure 32. The segmented genome characterization of Ganda bee virus, a novel negative-sense single-stranded RNA virus infecting Osmia cornuta. *(A) the organization of the genome showing three segments L, M, S making up the total genome. Each segment encodes a specific set of proteins. This tripartite genome organization is typical for viruses in the family Peribunyaviridae. (B) Conserved terminal repeats of the L segment of Ganda bee virus compared to related insect viruses. (C) PCR-based detection of each of the L, M and S segments in GABV-infected (+) or –free (-) female individuals.*

In Schoonvaere *et al.* (2018), we report for the first time in Europe a microsporidian parasite of the genus *Tubulinosema*, a novel *Apicystis* sp. that likely has been mistaken previously for *Apicystis bombi* to which it is related, and the association of *Crithidia pragensis* or a related trypanosome species with *Osmia*. Further, we observed that *Nosema thomsoni*, a recently distinguished microsporidian bee parasite, is able to infect bee species of the genus *Andrena*. The parasites *Tubulinosema* sp. (MF998087) and *Apicystis* sp. (MF998086) were also further studied.

Over 20 different viruses were associated with wild bees among which known and novel viruses (Figure 33). Three bee pathogenic viruses (black queen cell virus, sacbrood virus, varroa destructor virus 1) were found, although always in low abundance and thus not representing a clear infection. Three viruses related to known bee pathogenic viruses (bee iflavirus 1, bee macula-like virus 2, an unclassified virus related to chronic bee paralysis virus) were present as infections, evidenced by a high abundance in the bee. In the first metagenomic survey (Schoonvaere *et al.*, 2016), we characterized the genome of two -ve ssRNA viruses that infect bees (Ganda bee virus, GABV, KY053854-6 and Scaldis River bee virus, SRBV, KY053857). One year later, related –ve ssRNA viruses were discovered in honeybees (Remnant *et al.*, 2017) and this also initiated a global prevalence study of bee rhabdovirus-1 (Levin *et al.*, 2017)**.** Very recently, a global metatranscriptomic survey was published confirming our earlier findings that the viral diversity in bees is highly underestimated (Galbraith *et al.*, 2018)**.** The –ve ssRNA viruses are highly prevalent (Levin *et al.*, 2017, Schoonvaere *et al.*, 2018)**,** widespread and can invoke an antiviral immune response in bees, which suggests a closer biological role within bee hosts.

Two unknown DNA viruses that reached a high level of replication in the host were discovered. *Osmia cornuta* nudivirus (OcNV) occurred in both *O. cornuta* and *O. bicornis* but was not detected in other bee species. The most related virus, *Oryctes rhinoceros nudivirus*, is a serious pathogen of palm beetles and is used as a biological agent for pest control. Densoviruses were found in *Bombus terrestris* and *B. cryptarum*. This group of viruses are notorious pathogens of insects. For example, *Acheta domesticus* densovirus (AdDNV) can be responsible for high mortality rates in mass rearing facilities of the house cricket (Liu *et al.*, 2011)**.** *Osmia* spp. and *Bombus* spp. are commercial pollinators that are often shipped to areas where the species does not occur naturally. In such scenario, if exported colonies are infected by viruses, there is always the risk of pathogen spillover to natural pollinators. Current diagnostics and health certificates are focusing on known pathogens including American foulbrood and *Nosema bombi* but do not consider unknown and potentially dangerous pathogens. Further research is needed to investigate what is the impact of nudi- and densoviruses to bee hosts. Our study revealed that the viruses circulate in natural populations (and bee hotels of *Osmia* spp.). We stress caution on possible risks for accidental introduction or pathogen spillover events until the viruses remain better characterized.

Finally, multiple unknown insect-specific viruses were found, of which some related to mosquito-infecting negeviruses. Evidence is accumulating that negeviruses are insect-specific viruses although they share a common origin with plant viruses (Kondo *et al.*, 2017). Notably, the co-occurrence of one particular nege-like virus species with one particular bee species, repeatedly observed in different geographical locations, indicates that these viruses are not just accidental visitors in bees. Next to this, bees were commonly associated with plant pathogenic viruses suggesting that bees mediate vectoring of plant viruses in a plant-pollinator community. This observation was also done in a recent metatranscriptomics study in honey bees (Galbraith et *al.*, 2018).

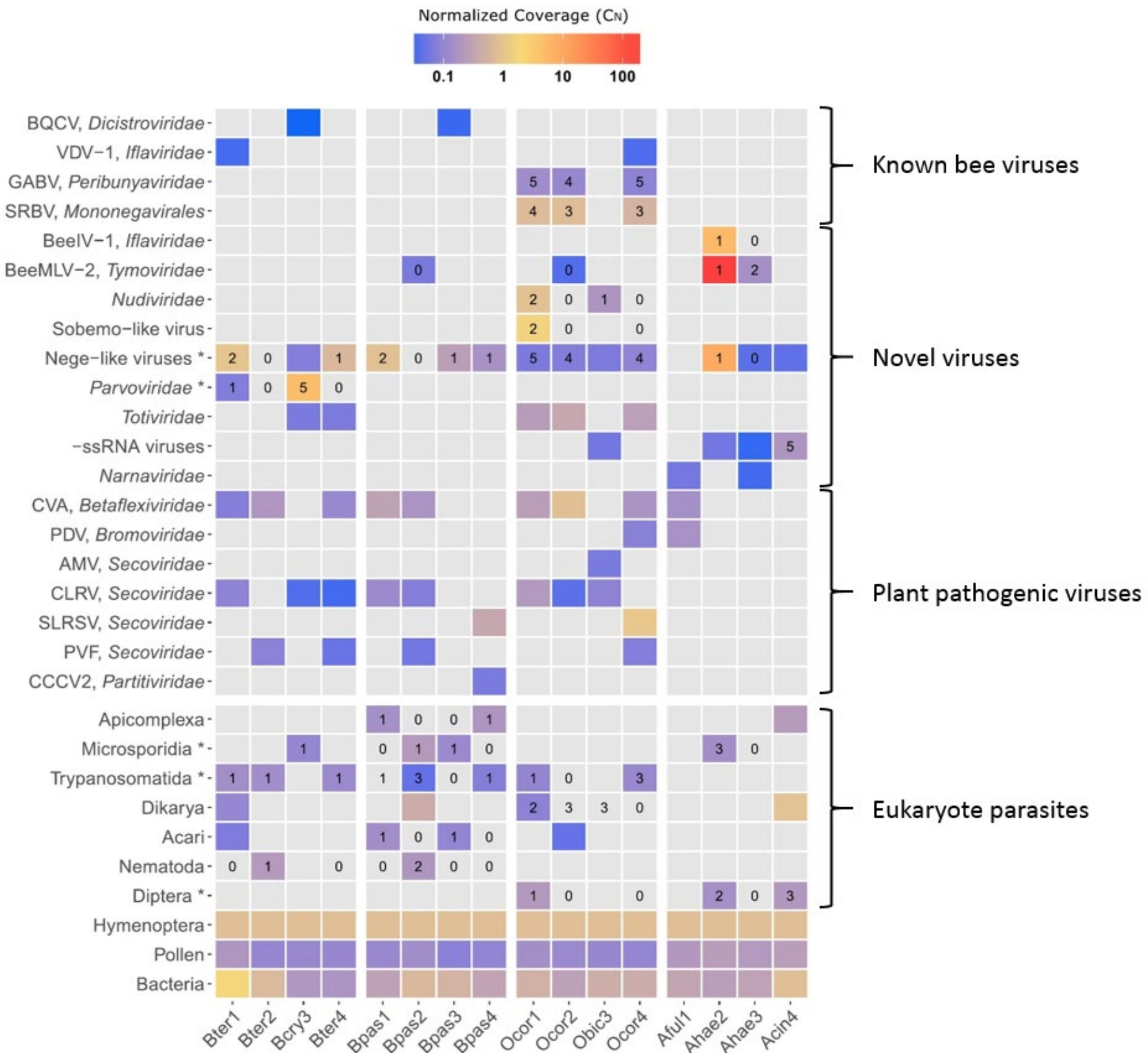


Figure 33. Second metagenomic survey conducted in wild bee species of Belgium. The heatmap illustrates the abundance of each association (row) in a wild bee species sample per locality (column, e.g. "Bter1" = Bombus terrestris *from locality 1). The abundance is normalized for the host taxon Hymenoptera (C_N = 1). An asterisk indicates that the taxon includes more than 1 species, e.g. "Microsporidia*" includes* Nosema bombi*,* Nosema thomsoni *and* Tubulinosema *sp .*

4.3.3.2. WP3. Task 3. Subtask 3.3.2. To trace pathogens in bee samples from past and present

Current diagnostic molecular methods allow us to rapidly assess the presence or absence of parasites in bees. First, high quality DNA is extracted from a biological sample. Second, the DNA is used as a template for Polymerase Chain Reaction (PCR)-based qualitative detection with target-specific primers. PCR is used for its high-throughput, reliability and widely applicability to a diverse set of bodies including viruses, protozoa, fungi and metazoans. Although the lab is competent in routine diagnostic methods in fresh (contemporary) or well-preserved bee samples, a robust method lacked for high quality DNA extraction from bad-preserved such as pinned insect museum specimens. Generally, DNA of old museum specimens is fragmented ("degraded") up to sizes less than 250 base pairs (bp) (Strange *et al.*, 2009). Moreover, museum specimens are valuable records in natural history and a non-destructive DNA extraction method is necessary to preserve morphological features of pinned insects in a collection. To reliably and routinely detect bee pathogens in past bee samples we first had to tackle these obstacles.

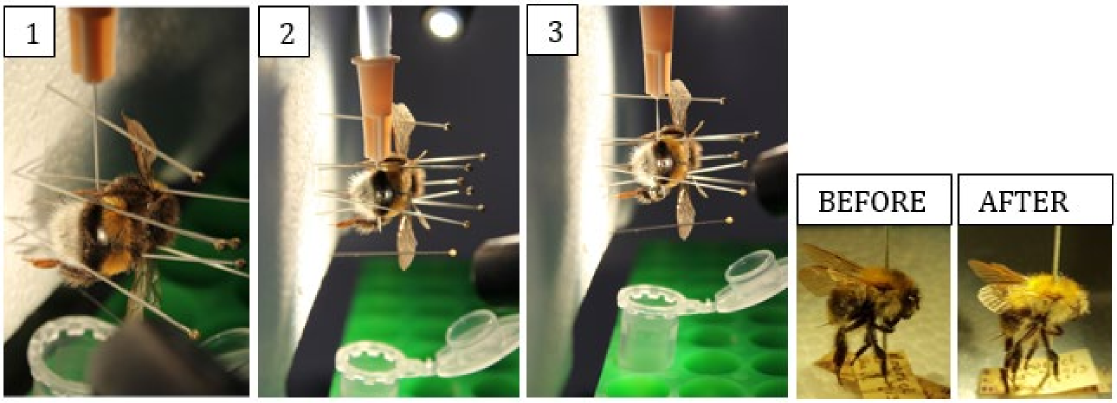


Figure 34. Non-destructive DNA extraction method from pinned bee museum specimens. A fine needle was inserted in the abdomen of an immobilized specimen. A volume of aqueous buffer was next pressed under high-pressure through the abdominal cavity and collected in a vial. This procedure theoretically rinsed the interior of the abdomen while collecting cellular debris from host and foreign bodies (e.g. spores, cysts, viral particles, …). The flow-through liquid was used for subsequent DNA extraction using phenol-chloroform extraction. The damage inflicted to the specimen was limited to two narrow wholes at the lateral sides of the bee abdomen.

A non-destructive DNA extraction method was designed and tested on pinned bumble bee museum specimens (Figure 34). The method proved successful in the extraction of PCR-mable DNA. Subsequently, we tested the sensitivity of this method for a common bee parasite, *Nosema ceranae*. After injecting dried bumble bee specimens with a dilutional series of known amounts of *N. ceranae* spores, we found that a minimum of 8.3×10^4 spores had to be present in order to reliably detect *Nosema* spores in dried bumble bee specimens (Figure 35). This is already a moderately high spore load for fresh bee specimens and especially for preserved specimens over 100 years old. DNA extraction methods other than phenol-chloroform were subsequently tested to ameliorate spore destruction. The lithium-chloride method specifically developed for *Nosema* diagnostics was even less sensitive than phenol-chloroform (results not shown).

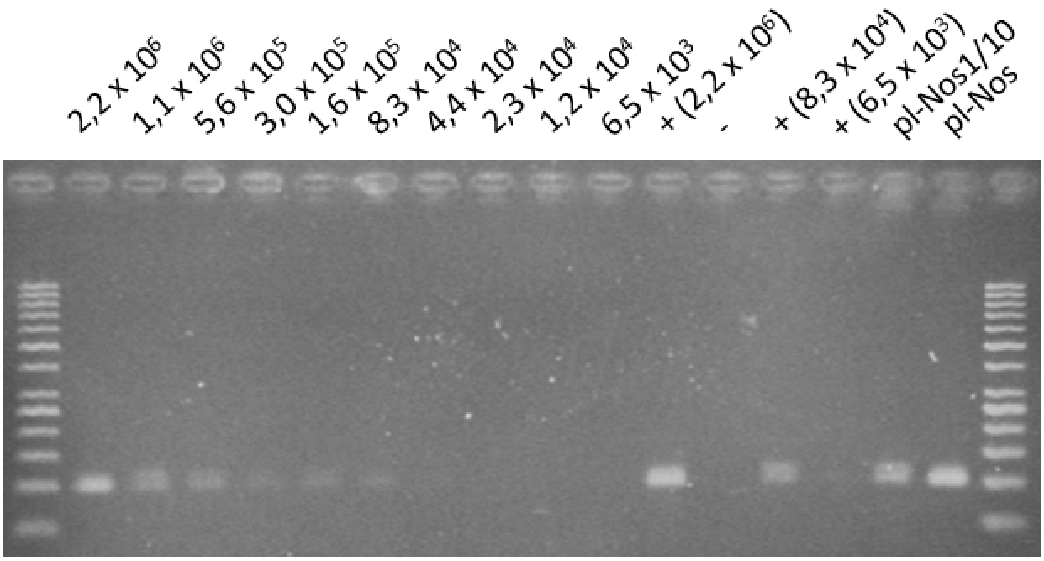


Figure 35. Sensitivity experiment for the detection of Nosema ceranae *using a non-destructive DNA extraction method and PCR. The amplicon size for N. ceranae was 90 bp as this template size is expected to readily amplify from highly degraded museum specimen DNA. Dried bumble bees were inoculated with a dilution series of known spore amounts of the parasite. DNA was extracted using the method described in Figure 31. In left part of the agarose gel, it is clear that signal is lost below 8,3 x* 10^4 *injected spores. In the right part of the agarose gel are experimental controls (+/-, DNA extraction controls of the original spore samples without injection; pl-Nos, PCR control). Notice that the signal around 6500 spores is very faint.*

The results of these experiments indicated that the molecular detection of parasites in museum specimens was insensitive and therefore unreliable. Nonetheless, we tested the method for *Nosema bombi* (a traditional bumble bee parasite with ~ 10% prevalence) in real museum specimens of the RBINS museum collection. We collected 52 pinned individuals of a dispensable species because it is abundantly present in the collection, *Bombus pascuorum*. More specifically, of the period 1911-20 we collected #10 specimens, 1921-30: #6, 1931-40: #6, 1941-50: #6, 1951-60: #2, 1961-70: #1, 1971-80: #3, 1981-90: #6, 1991-2000: #6, and in 2001-10: #6. We started with the most recent specimens, 1971 until now and a total of 21 individuals. Seen the prevalence of *N. bombi* in natural populations, we expected at least 2 positive specimens. None of the specimens turned out to be positive for *N. bombi,* i.e. none contained at least 83k spores. The specimens were subsequently homogenized and DNA was extracted with the procedure for *Nosema* diagnosis in fresh samples, but again, none were positive. Either none of the sampled bumble bees carried *Nosema bombi* or the infection rate was below the sensitivity of the method. To test this, a fraction of the aqueous buffer flow-trough was microscopically examined for *Nosema* spores (partner UG2) and 1 spore was found in 1 of the 21 samples analyzed (Ivan Meeus, *pers. comm.*). We therefore concluded that the negative molecular detection of parasites in museum specimens can be attributed to the low sensitivity of the non-destructive method used.

4.3.3.3. WP3. Task 3. Subtask 3.3.3. To trace *Nosema spp.* (including *N. bombi*) in past and present samples of pollen

We have abundantly prospected in the old wild bee collections but the quantity of pollen loads available on the specimens wasn't sufficient to perform this analysis.

4.3.4. WP3. Task 4. Hypothesis 4: Pesticide development

4.3.4.1. WP3; Task 4. Subtask 3.4.1. To identify temporal and spatial dynamic of pesticide use by literature review and to select the most pertinent chemicals for laboratory analysis

Four contemporary insecticides (alpha-cypermethrin, imidacloprid, indoxacarb, thiamethoxam) were selected for study based on bibliographic review of their application in bee-attractive crops and potential hazard to bees in Belgium. Nowadays, the most effective insecticides on the market are neurotoxins, i.e. molecules targeting the nervous system of insects causing paralysis and death. Their use in bee-attractive crops is widespread in Belgium although many formulations have been removed from the market since the 21st century (expert side effects and collaborator Guido Sterk, IPM-Impact).

Alpha-cypermethrin belongs to the class of synthetic pyrethroids, which are sodium channel modulators based on IRAC's Classification (group 3). We used the formulation FASTAC® EC (50 g/L alpha-cypermethrin) manufactured by BASF for toxicity testing in bumble bees. In Belgium, FASTAC® is registered for use in herbaceous crops, potatoes (against aphids and *Leptinotarsa decemlineata*) and oilseed (against beetle pests). BASF's 2018 spraying schedule advises the use of FASTAC® during crop stages BBCH 5-14 (germination-leaf development) and 57-69 (flowering). FASTAC® is the only formulation based on alpha-cypermethrin that is registered for use in Belgium. Alpha-cypermethrin, together with cypermethrin and dimethoate, was found to negatively influence honey bee colony health in a recent spatially-explicit study (De Smet *et al.*, unpublished).

Imidacloprid belongs to the class of neonicotinoids (IRAC group 4A), which function as nicotinic acetylcholine receptor (NAChR) competitive modulators, and which are amongst the most potent neurotoxic insecticides. For imidacloprid, we used the raw active ingredient for pilot toxicity testing in honey bees. Together with clothianidin and thiamethoxam, imidacloprid was restricted by EU legislation since 2013 for its use in bee-attractive crops during flowering. Eighteen formulations based on imidacloprid are currently registered for use in Belgium. For example, BAZOOKA® (200 g/L imidacloprid) is registered for use in apple orchards, *Brassica oleracea* var. gemmifera and horticulture.

STEWARD® WG is a formulation of 30% indoxacarb manufactured by DuPont. Indoxacarb belongs to the class of oxadiazines, which are voltage-dependent sodium channel blockers (IRAC group 22). These insecticides have the same biochemical target though different binding site as synthetic pyrethroids. Indoxacarb is highly effective against lepidopteran pests. In Belgium, STEWARD® is registered for use against leaf rollers and caterpillars in fruit crops (cherry, apple, and pear), solaneous fruit (tomatoes, aubergine, and paprika), berries, cabbage, oilseed and herbs. Notably, Bayer’s 2018 spraying schedule advises the use of STEWARD against leaf rollers in cherry, apple and pear cultivation during flowering (crop stages BBCH 60-69).

Thiamethoxam belongs to the class of neonicotinoids (IRAC group 4A). We used the formulation ACTARA® WG (25% thiamethoxam) manufactured by Syngenta for toxicity testing in bumble bees. In Belgium, ACTARA® is currently only registered for use in potatoes after flowering (against aphids and *Leptinotarsa decemlineata*) and pear after harvest (against aphids and *Psylla pyri*). Two other formulations based on thiamethoxam are registered in Belgium and mainly applied in seed production.

4.3.4.2. WP3. Task 4. Subtask 3.4.2. To trace pesticides previously selected in past and recent samples and comparison of residues

Following a consultation process with pesticide experts, we decided to consider this task from another point of view which will provide us more interesting results. Therefore, Subtask 4.3.4.2. is replaced by Subtask 4.3.4.3.

4.3.4.3. WP3. Task 4. New subtask. Identification of bio-markers for pesticide intoxication in wild bees

The use of synthetic agrochemicals for crop protection has changed considerably since their introduction in the 1950s (Sparks, 2013). Today, most controversial insecticides are no longer used or their application rate/dosage are minimized to avoid acute poisoning of beneficial insects (Blacquiere *et al.*, 2012). Despite of this positive evolution, scientific evidence irrevocably demonstrates that contemporary insecticides impair bee health, mediated by and commonly referred to as (chronic) sublethal effects (Desneux *et al.*, 2007). It is demonstrated that field-realistic concentrations (i.e. sublethal dose range) of imidacloprid impair colony development and reproduction in the bumble bee *Bombus terrestris* (Whitehorn *et al.*, 2012). Also other xenobiotics in combination with other stressors act differently in the decline of wild bee populations (Goulson *et al.*, 2015)

Instead of tracing residues in past and present samples, we adopted a more practical approach to study pesticide development in field-exposed bees. In this approach, the central entity is biomarkers indicative for intoxication. A biomarker is a biological characteristic which alteration in state is indicative for a certain (normal or) stress-related process, for instance chronic insecticide poisoning. Examples of such biomarkers in bees are decreased hypopharyngeal gland size (Heylen *et al.*, 2011), increased detoxification enzyme activity (Badiou-Beneteau *et al.*, 2013) and differential gene expression (Shi *et al.*, 2017). In this work package, we aimed at identifying gene expression biomarkers indicative for chronic sublethal exposure of different classes of neurotoxic insecticides by adopting a dual omics approach.

A pilot study was conducted to assess different context-dependent effects (field vs. cage, dosage, and exposure time) of imidacloprid exposure in honeybees (De Smet *et al.*, 2017). We observed a general immunosuppression of caged bees following chronic exposure to imidacloprid. Remarkably, the expression of genes involved in detoxification processes (cytochrome P450 enzymes, glutathione S-transferases and carboxylesterases) was upregulated but not significantly. This result was unexpected since these enzymes are the bee's first line of defense against intoxication by xenobiotics. However, bees that were exposed to imidacloprid in field-conditions showed a significant upregulation of CYT P450 and CYP9Q3 after 20 days (Figure 36). Interestingly, both genes were also found to be involved in xenobiotic detoxification in earlier studies (Derecka *et al.*, 2013, Mao *et al.*, 2011). Caged bees, however, showed a significant downregulation of CYP9Q3 (Figure 36). It is suggestive that bees need time to induce their detoxification mechanisms, especially in stressful conditions such as caging. Based on this pilot study we can conclude that gene expression biomarkers for chronic insecticide exposure are relevant and exist at least in honeybees, and that biomarkers can be applied to monitor imidacloprid intoxication although depending on the context.

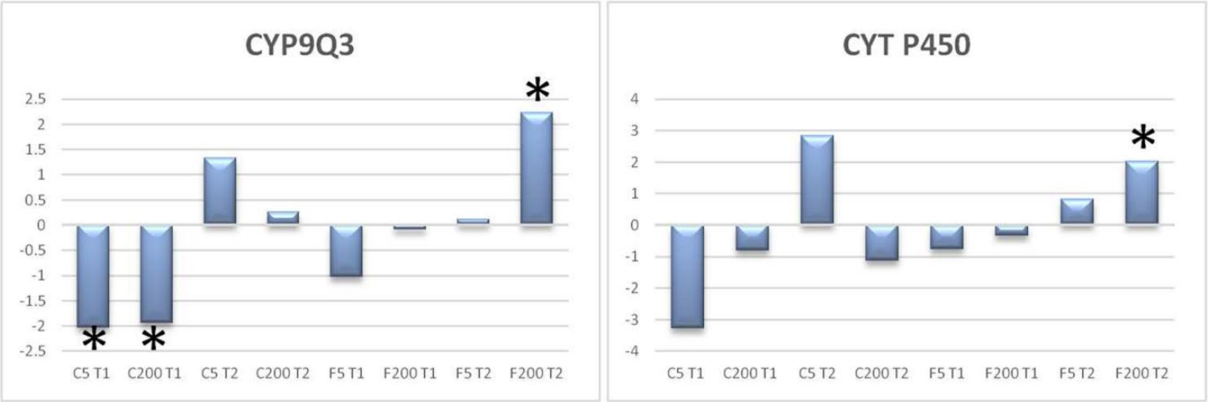


Figure 36. Subset figure abstracted from De Smet et al. 2017 Fig 6B. Expression profile of detoxification genes. Each bar represents the up- or downregulation of either CYP9Q3 (left) or CYT P450 (right) in a specific context (bottom) versus control. Different contexts include: C=caged; F=field; 5=imidacloprid-5ppb; 200=imidacloprid-200ppb; T1=10 days exposure; T2=20 days after exposure.

A follow-up study to the pilot study was launched with a specific focus on gene expression biomarkers in bumblebees. Although the way how bees tolerate insecticides differs significantly between species (Cresswell *et al.*, 2012, Uhl *et al.*, 2016), we assumed that a common detoxification mechanism exists. Indeed, although lower than other insects, the number of detoxification genes in the thus far sequenced bee genomes is comparable across species (Xu *et al.*, 2013). Experimental evidence however indicates that residual levels of imidacloprid are different between honey bees and bumblebees, suggesting a different efficiency of the detoxification mechanisms (Cresswell *et al.*, 2014). We aimed at identifying key genes in the detoxification mechanism in bumblebees by adopting a dual omics approach. More specifically, we searched for differentially expressed genes at the transcriptional and protein level following administration of different sublethal doses of alpha-cypermethrin (ACM), thiamethoxam (TMX) and indoxacarb (IDC).

The exposure study with bumble bees was done in cooperation with a side-effects specialist who was involved in several recent toxicological studies. We opted for insecticide formulations instead of pure compound. The survival rates of bumblebee micro-colonies (n=10, triplicate) for the three different formulations were experimentally assessed in 10-fold dilutions starting from the Maximum Field Recommended Concentration (MFRC). The MFRC is the maximum threshold dosage that is good-practice and assumed to be used by farmers. The survival rate started to decline at 4 mg active ingredient (a.i.)/kg (ppm), 10 ppm and 81 µg a.i. /kg (ppb) for ACM, IDC and TMX, respectively (Figure 37).

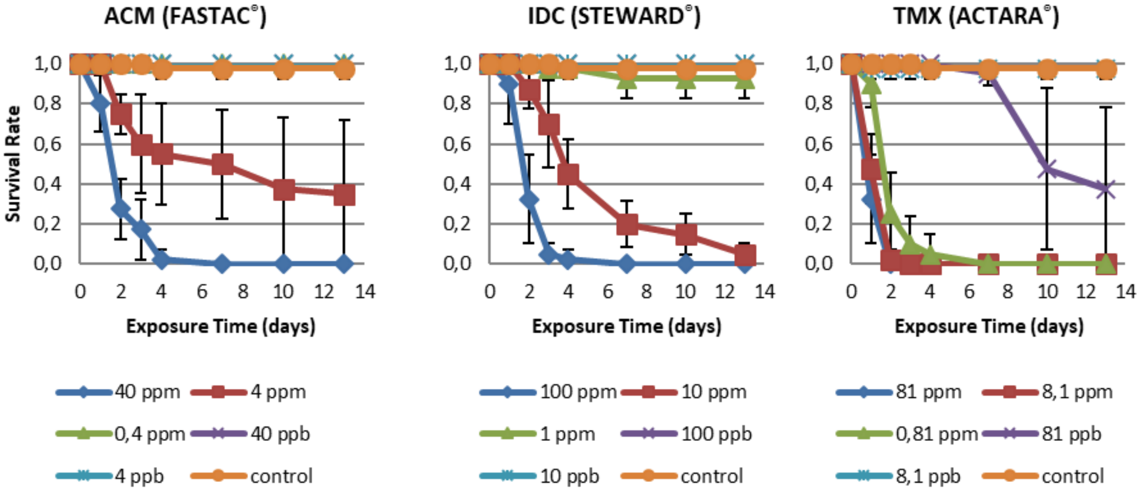


Figure 37. Survival rates of bumblebee micro-colonies exposed to different doses of insecticides. Each micro-colony consisted of 10 worker bees. Survival rate was calculated as the average ratio of surviving workers in 4 micro-colonies (quadruplicate). The dose is expressed as mg (ppm) or µg (ppb) active ingredient per kg sugar water. The highest dose of each insecticide corresponds to the Maximum Field Recommended Concentration used by farmers during spraying.

Exposure trials were repeated using macro-colonies (rightful queen + workers) especially constructed for R&D (Koppert). All workers were assumed to have the same age and genetic background at the start of the experiment. Colonies were administered a sugar-solution spiked with either a high or low sublethal dose of each formulation; 4 ppm and 160 ppb of ACM, 2 ppm and 80 ppb of IDC, 81 and 9 ppb of TMX. Exposure was continued for 10 days after which gene expression was quantified by RNA-Sequencing (transcriptomics). Each biological sample originated from a single worker (pool of head and abdomen). The number of differentially expressed genes (DEGs), and thus candidate transcript biomarkers, was overall very low (FDR < 0.05). For ACM-2ppm, top DEGs included genes encoding for mucin-3B, salivary glue protein Sgs-3 and probable salivary secreted peptide. All appear to have a role in the digestion of food and this result can be linked the study by Tasei and coworkers (Tasei *et al.*, 1994) who found that deltamethrin-exposed bumblebees suffer from a 50% reduced food uptake. Overall, we observed high variation in gene expression profiles among workers. Two workers had an elevated immune response, indicated by a high expression of anti-microbial peptides. However, when we compared our gene expression profiles with those of an earlier study (Harrison, 2015) we found a significant discontinuity between so-called “reproductive” and “non-reproductive” workers. The top gene LOC105665921, referred to as REPRED, encodes for a protein with unknown function. It is significantly higher expressed among reproductive workers versus non-reproductive workers (Figure 38).

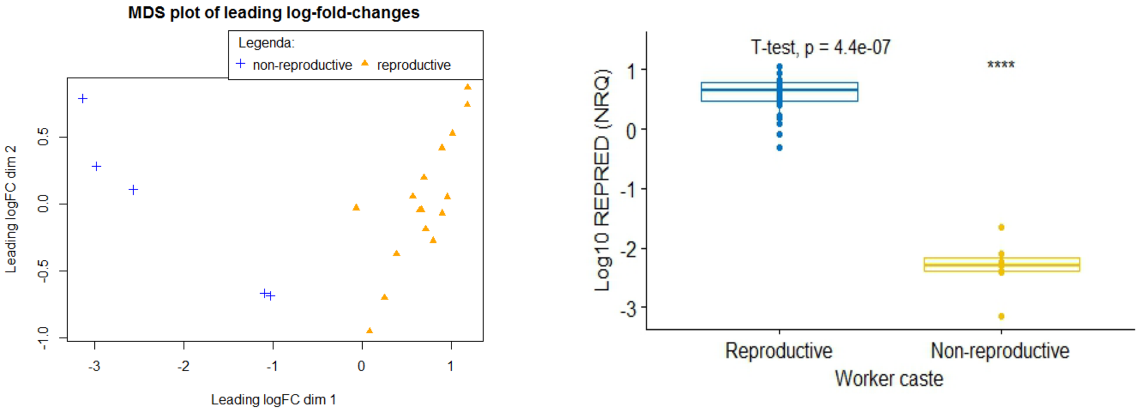


Figure 38. Caste-specific variation in gene expression profiles. <u>To the left</u>: multi-dimensional scaling (MDS) plot of Bombus terrestris *workers based on RNA-seq gene expression. The first dimension (x-axis) clearly separates reproductive workers (negative values) from non-reproductive workers (positive values) based on the highest variation in pair-wise comparison of gene expression between samples. <u>To the right</u>: expression of* B. terrestris *LOC105665921 (REPRED) in reproductive versus non-reproductive workers. RNA-seq expression was validated by qPCR and expression values of the latter are used in the plot (log10 Normalized relative quantities, NRQ). The expression of REPRED in non-reproductive workers was close to zero.*

The high variation in gene expression limited the study power to discover differentially expressed genes in insecticide-exposed bees versus control bees. As shown in the MDS-plot in Figure 35 (left panel) the best explanatory variable is the worker caste and thus we conclude that caste-specific gene expression overrules detoxification-induced gene expression in the bumblebee *Bombus terrestris*. If the caste-specific gene expression is driven by a particular tissue (for instance well-developed ovaries in reproductive workers), then it would be possible to rule out this kind of variation by dissecting the animals and narrow down the biological material. We launched a second experiment that will work further on the biomarker hypothesis and anticipates the problem of the worker caste phenomenon in *B. terrestris*.

Next to the gene expression biomarkers at transcript level, our goal was to discover biomarkers at the protein level (proteomics). The practical work on this topic are planned after the end of the BELBEES project and results will be communicated in a joint publication with transcriptomics part.

4.3.5. WP3. Task 5. Hypothesis 5: Climate change

Impact of increase of mean annual temperature

While bumblebees are recognized as providing a vital ecosystem service, they are sensitive to climate as cold-adapted animals (Heinrich, 1979; Ollerton *et al.* 2011; Potts *et al.*, 2016). It is therefore expected that climate change affects bumblebee distributions across Europe (Rasmont *et al.*, 2015). In this context, this atlas, considering the likely effects of climate change on bumblebees in Europe, is therefore a timely and vital work. Over one million bumblebee records from all over Europe have been collated. Based on data from 1970 to 2000 we modelled the current climatic niche for almost all European species (56 out of 69) and projected future climatically suitable conditions based on three climate change scenarii (SEDG, BAMBU, GRAS) for the years 2050 and 2100 (IPCC, 2013; Rasmont *et al.*, 2015). The general aims of this atlas are: (i) to inform the broader public about the potential risks of climate change for the future fate of European bumblebees; (ii) to aid biodiversity conservation managers and policy makers; (iii) to provide background knowledge for critical discussions about the sustainable provision of pollination services in the light of food security. In 2100, depending on the scenario of climate change, up to 36% of the European bumblebees are projected to be at a high climatic risk, 41% will be at risk. Only three species are projected to benefit from climate change and can potentially enlarge their current distributions in Europe (i.e. *B. argillaceus*, *B. haematurus*, and *B. niveatus*). As expected the three scenarios considered provide different projections for 2100 (Rasmont *et al.*, 2015). We have seen that the expected species loss due to climate change increases with decreasing latitudes (south Europe will be most affected by the loss of important pollinators). Climate risks for bumblebees can be extremely high, depending on the future development of human society, and the corresponding effects on the climate, strong mitigation strategies are needed to preserve this important species group and to ensure the sustainable provision of pollination services, to which they considerably contribute.

During last decades, mean annual temperatures have strongly increased (IPCC, 2013). Animals can display different responses to these changes through geographical shifts, plastic or adaptive modifications to avoid extinction (McCarty et al., 2001). Phenotypic modifications related to temperature increase have been mainly explored along latitudinal gradient but relatively understudied at a temporal scale (Gardner et al., 2011). We question how bumblebees' size has evolved since the last century throughout climate change (i.e. smaller mean body size in warmer conditions). We measured the evolution of queens mean body size of four bumblebee species (i.e. *Bombus balteatus*, *B. jonellus*, *B. pascuorum* and *B. pratorum*) in two geographical areas experiencing different intensities of global warming (i.e. Belgium and in Scandinavia above the arctic circle) during three time periods (i.e. from 1900 to 1945, from 1946 to 1989 and from 1990 to 2016). While body size of the two bumblebee species (i.e. *B. pascuorum* and *B. pratorum*) measured in Belgium increased during the last century, there was no significant differences in the three bumblebee species (i.e. *B. balteatus*, *B. jonellus* and *B. pascuorum*) measured in northern Scandinavia. Moreover, while specimens of *B. pascuorum* were significantly larger in northern Scandinavia than in Belgium during the two first time periods, there was no significant differences between specimens from the last period (i.e. 1990 and 2016) due to body size increase of *B. pascuorum* specimens from Belgium. Contrary to our hypothesis, temperature does not seem to be the main driver of bumblebee body size variation during the last century. We hypothesize that habitat fragmentation could be an alternative mechanism that shape body size clines. Indeed, bee body size is related to foraging distance; the increase of habitat fragmentation in Belgium could have induced a selective pressure promoting larger body size while northern Scandinavian bumblebee habitat remained roughly constant.

Marshall *et al.* (2018) have compared the roles of dynamic land use and land cover change (LULC) and climate change on the projected distribution patterns of 48 European bumblebee species for three change scenarios until 2100. We focussed our study at the scales of Europe, and Belgium, Netherlands and Luxembourg. Three types of models have been investigated: (i) only climate covariates, (ii) climate and static LULC covariates, and (iii) climate and dynamic LULC covariates. Model performance, range gain/loss and the shift in range limits for all bumblebees have been analyzed. Integration of LULC covariates improved modelisations. Dynamic models projected less range loss and gain than climate-only projections, and greater range loss and gain than static models. According to the models, species responses varied considerably, and effects were more significative at the BENELUX scale. Under the extreme growth climatic scenario (GRAS), most species were predicted to lose considerable range (mean: 64% ± 34). Besides, local extinctions and considerable range loss are expected at the BENELUX scale (mean: 56% ± 39). Our results suggest to consider species-specific modelling in order to understand how LULC and climate interact in future modelling with high spatial resolution.

In the context of assessment of impact of climate change on pollinator communities and their ecological networks, Denis Michez and Pierre Rasmont with funds of BELSPO have participated to a study to understand how damage of climate change on individual species propagates through ecological communities (Schleuning *et al.*, 2016). Species distribution models with ecological network analyses have been combined to test potential impacts of climate change on 4,700 plant and animal species in pollination and seed dispersal networks from central Europe. Results show that animal species which are associated to a low diversity of plant species have narrow climatic niches and are most sensitive to climate change. Modelisations incorporating different scenarios of species coextinction and capacities for animal-plant couple switches show that projected plant extinctions under climate change are more likely to trigger animal coextinctions than the opposite. These results highlight that effects of climate change can be amplified via extinction cascades from plants to animals in ecological networks.

Impact of extreme event (heat waves)

While during the last decade climate change such as the slow and progressive modification of climatic parameters (e.g. increase of annual temperature) has been largely investigated, little is known concerning the impact of heat waves on pollinator fauna (Martinet *et al*., 2015). The frequency and intensity of these extreme events will increase in the next few years (Meehl & Tebaldi, 2004). Since heat waves are suspected to dramatically increase insect mortality, there is an urgent need to assess their potential impact (Kingsolver *et al*., 2013). In this context, we developed a new experimental standardized device available in the field to assess the hyperthermic stress resistance of insects through their time before heat stupor (THS). In this protocol, insects are exposed to an extreme temperature (40°C). We applied this approach on different arctic, boreo- alpine and widespread bumblebee species (Martinet *et al.*, 2015). Our results show that bumblebees are very sensitive to heat stress. Their resistance seems to be species-specific: heat resistance of species with an arctic-centred distribution is weaker than the one of the boreo-alpine species with a larger distribution, itself lower than the one of the ubiquitous species. In two other studies, we tested the hyperthermic stress resistance of eight Belgian bumblebee species including sub-boreal taxa (e.g. *Bombus jonellus*) (Zambra *et al.*, in prep) (Figure 39) and the intra-specific variation of heat resistance inside a common bumblebee subgenus (*Bombus ss.*) (Martinet *et al.,* in prep.).

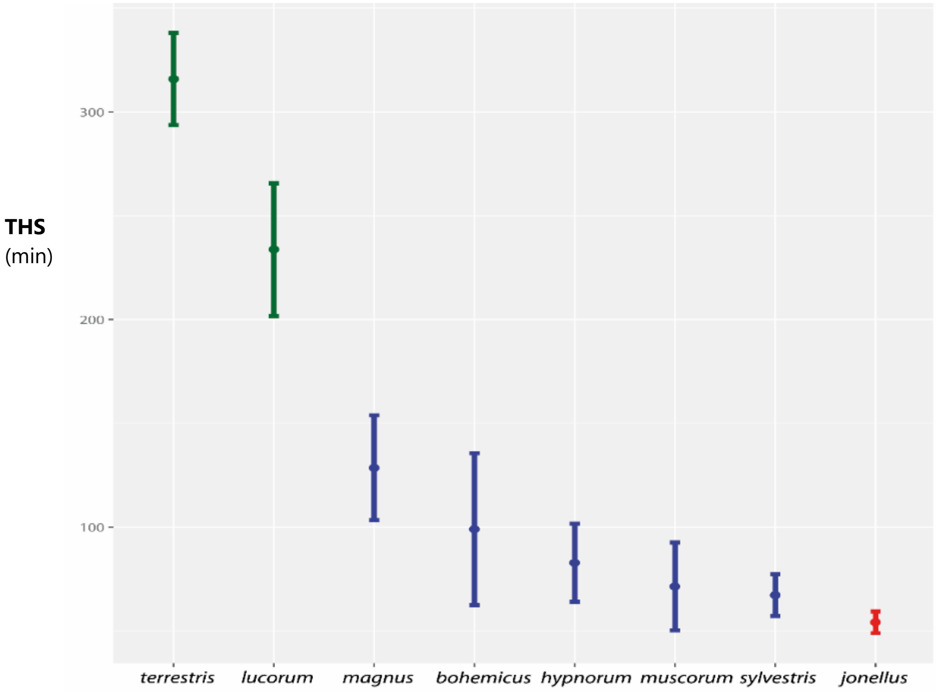


Figure 39. Resistance to heat stress of eight Bombus *species recorded in Belgium:* terrestris, lucorum, magnus, bohemicus, hypnorum, muscorum, sylvestris *and* jonellus*). THS= Time before Heat Stupor.*

While pollinators are experiencing declines globally, well-known drivers of such decline include climatic and nutritional stresses, i.e. change of diet resources due to degradation of habitat quality itself due to human activities (Goulson *et al.,* 2005). Understanding potential synergies between these two important drivers is needed to improve predictive models on future effects of climate change on pollinator decline (IPBES, 2016). Here we performed bioassays on 117 colonies of *Bombus terrestris* to evaluate the potential for interactive effects of heat stress, loss of resource quality and colony size. One third of the colonies was reared under normal temperature (26°C, control). The remaining colonies were divided in two groups and exposed to thermic stress (33°C) during five (short stress) or ten (long stress) days (Vogt, 1986). All colonies were fed during 28 days with one of the three pollen diets: *Salix* pollen (assumed as highly suitable), *Cistus* pollen (assumed as suitable), or *Taraxacum* pollen (assumed as unsuitable) (Figure 40) (Herbet, 1992). When acting alone, a nutritional as well as climatic stress changed the colony development. More specifically, investment in male production was substantially reduced. Additionally, when acting together climatic and nutritional stresses led to synergetic reductions in colony development but a high-quality diet buffered climatic stress. Small colonies were much more sensitive to heat and nutritional stresses than large ones, possibly because numerous workers help maintaining homeostasis. Overall, our study suggests that in the context of current global warming, ensuring access to high quality flower resources could reduce impacts of climate change on bee decline (Vanderplanck *et al.*, submitted)

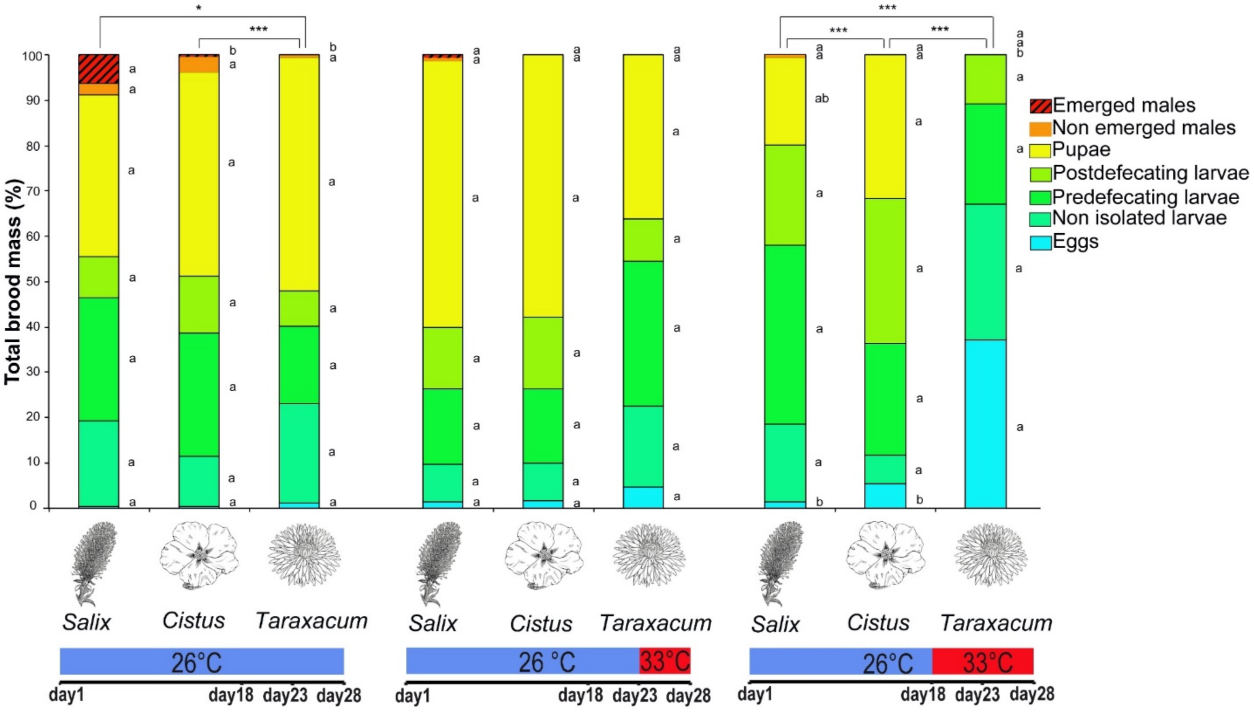


*Figure 40. Colony dynamics. Brood composition with the different developmental stages expressed as percentage of total brood mass (i.e. dynamics of micro-colony development) for small colonies exposed to different levels of nutritional and climatic stresses. Asterisks indicate significant differences in brood composition between micro-colonies fed imposed diet of pollen (pairwise perMANOVAs; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). Different letters indicate significant differences in proportions of brood stages among bioassays (post-hoc tests, $p < 0.05$).*

4.4. WP4. Meta-analysis and modelling

4.4.1. WP4. Task 1. Meta-analysis

4.4.1.1. WP4. Task 1. Subtask 4.1.1. A century of spatio-temporal variations in bumblebee communities at the Belgian scale

We performed comparative analyses based on a 100-year dataset of Belgian bumblebee populations using an original specimen-level database compiled in the *Banque de Données Fauniques de Gembloux et Mons* (BDFGM; Rasmont *et al.,* 2015).

We measured 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). 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), *B. quadricolor* (2 records in 1943), and *B. wurflenii* (3 records, last one in 1979). As sampling effort and amount of data differed greatly between years, we subdivided the final dataset (composed of 173,716 bumblebee records) into three time-periods: (i) 1910-1930 (P1; 60,498 bumblebee records) which corresponds to the time before the use of agricultural moto-mechanization and chemical fertilizers, occurring mainly from 1950 to 1970; (ii) 1970-1989 (P2; 23,854 bumblebee records) which corresponds to the beginning of the application of the Mansholt Plan, leading to fast intensification of agriculture; and (iii) 1990-2016 (P3; 89,364 bumblebee records) with 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.

For each of the three time-periods, 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. We chose this minimum value of 30 specimens by grid-cell because it is the theoretical minimum number of individuals that we need to encounter the 28 bumblebee species observed at the national scale during the last century (Figure 41, TABLE XIV). We chose the 10 km spatial resolution, which was the best compromise between the amount of data per grid cell (i.e. minimum 30 specimens) and the low spatial resolution of the oldest data (corresponding to the resolution of 5x5 km grid-cells). We estimated the range size of each species by counting the number of 10x10 km grid-cells where each species was recorded in each time period. 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).

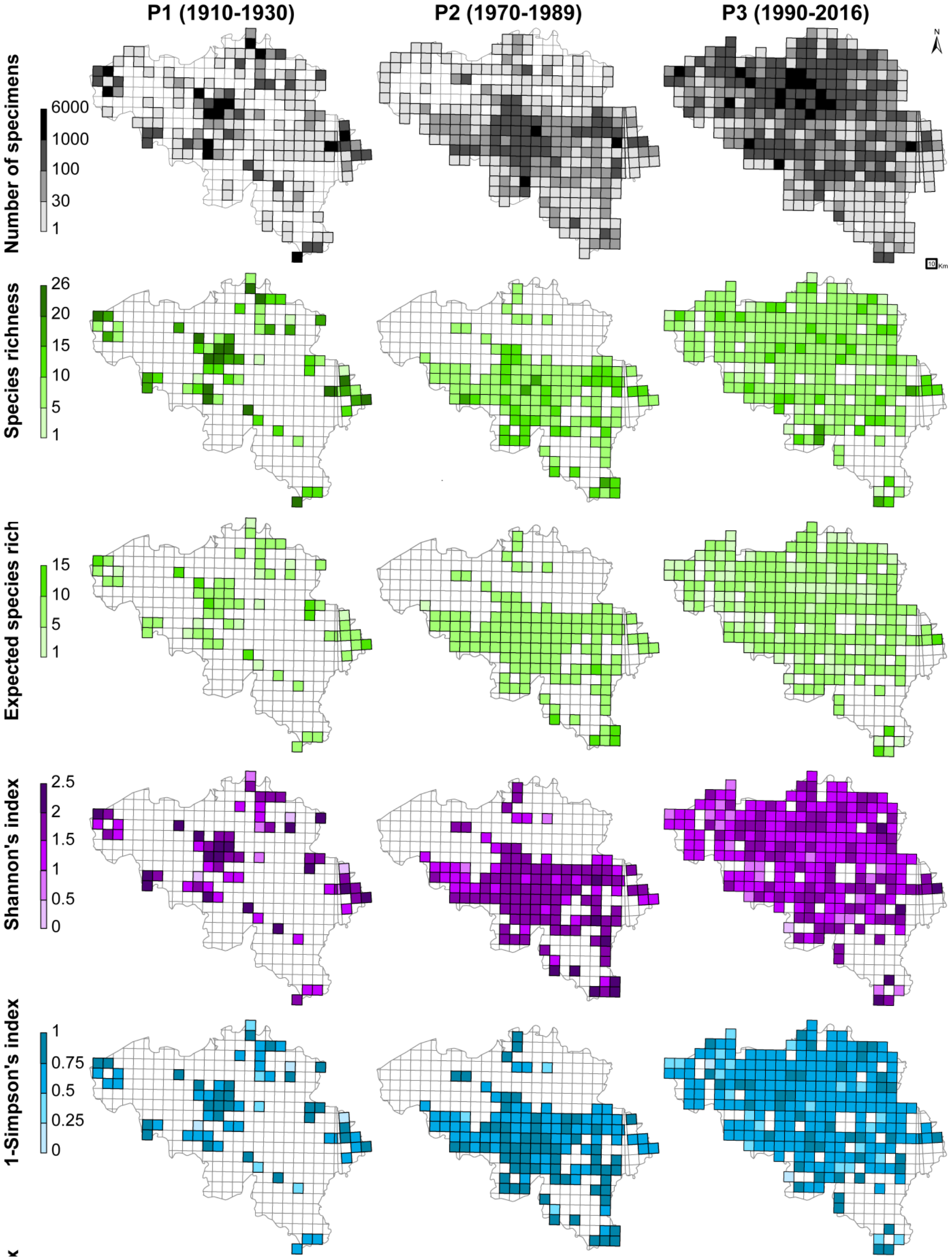


Figure 41. Abundance (i.e. number of specimens), species richness, Shannon's index, 1-Simpson's index, and Berger-Parker's index in grid-cells of 10x10 km for each time-period.

TABLE XIV. Species richness, expected species richness (i.e. Hurlbert's index) in a sub-sample of 100 specimens (see Table 2 for other sub-sample sizes), 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).

	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)
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)

Larges scale changes in bumblebee communities over the last century

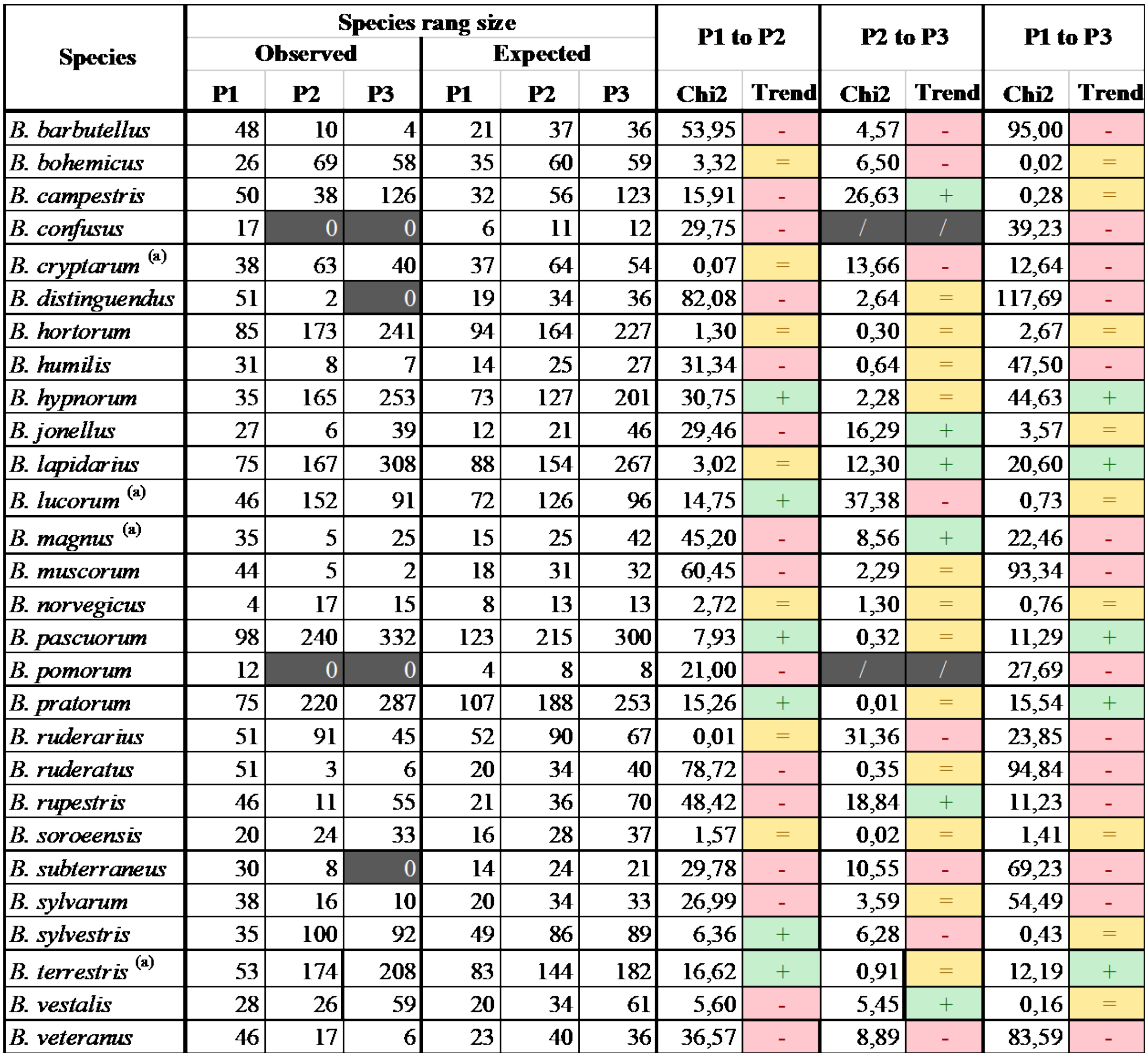
We show that Belgian bumblebee communities are experiencing a strong decline in their species richness and diversity, and that numerous species are decreasing in terms of abundance and distribution, since the last century in Belgium, especially between 1930 and 1990 (TABLE XV). Species richness decreased from 28 to 24 species, with the disappearance of *B. confusus, B. distinguendus, B. pomorum*, and *B. subterraneus* (TABLE XIV). Furthermore, the expected species richness decreased continually across the time-periods, from 19 in 1910 to only 10 species after 1990 (TABLE XIV). This decline in richness and diversity is general at the country scale but shows strong variations at a regional scale. We observed some exceptions, such as in the southeast of Belgium where communities remained relatively rich (Figures 41). This decrease in species richness goes along with a decrease in evenness and shifts in communities' composition (TABLE XV and XVI). While the dominant species (i.e. *B. pascuorum*) remains the same in the three time-periods, relative abundance and relative range size of several species changed significantly. Many species once abundant and widespread dramatically decreased between 1930 and 1990, while some others increased in their relative abundance and/or relative range size. However, some species with a decreasing or stable trend in their range size had an increasing trend in their abundances (*B. bohemicus, B. lucorum, B. norvegicus,* and *B. terrestris*).

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 (Kuussaari *et al.*, 2009). This time-delayed but deterministic extinction can also affect dominant species. The phenomenon of extinction debt has already been suggested for pollinators in south-eastern Sweden (Bommarco et al., 2012) and the Netherlands (Aguirre-Gutiérrez et al., 2016), 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,* and *B. veteranus*), they could still become extinct due to past drivers that weakened their populations, even if these impacts have ceased or regressed today.

TABLE XV. Species abundance trends at the country scale, estimated with the Stroot & Depiereux (1989) method between P1 (1910-1930) and P2 (1970-1989), P2 and P3 (1990-2016), and between P1 and P3. Signs "-", "=" and "+" represent respectively, negative (red cell), stable (yellow cell) and positive (green cell) significant trends at the p-value of 0.05 (Chi2 = 3.8), 0.01 (Chi2 = 6.6), and 0.001 (Chi2 = 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 abundance (rank abundance)**			**Expected abundance**			**P1 to P2**		**P2 to P3**		**P1 to P3**	
	P1	**P2**	**P3**	**P1**	**P2**	**P3**	**Chi**	**Trend**	**Chi**	**Trend**	**Chi**	**Trend**
B. barbutellus	577 (17)	14 (21)	10 (22)	424	167	19	196	-	20	-	818	-
B. bohemicus	198 (25)	225 (11)	476 (9)	303	120	553	129	+	51	-	34	+
B. campestris	1215 (10)	79 (12)	519 (8)	928	366	472	314	-	22	+	635	-
B. confusus	90 (27)	0 (/)	0 (/)	65	25	0	35	-	/	/	133	-
B. cryptarum (a)	517 (20)	1141 (6)	312 (13)	1189	469	1147	1343	+	2885	-	167	-
B. distinguendus	1192 (11)	3 (25)	0 (/)	857	338	2	463	-	11	-	1761	-
B. hortorum	5479 (3)	1119 (7)	3430 (6)	4732	1866	3591	417	-	34	-	1652	-
B. humilis	794 (14)	13 (22)	25 (20)	579	228	30	283	-	4	-	1089	-
B. hypnorum	920 (12)	1076 (8)	7154 (4)	1432	564	6496	646	+	316	+	2816	+
B. jonellus	249 (24)	29 (17)	401 (10)	199	79	339	44	-	53	+	1	=
B. lapidarius	10260 (2)	1954 (5)	21684 (2)	8760	3454	18658	908	-	2330	+	903	+
B. lucorum (a)	567 (18)	2743 (3)	2055 (7)	2374	936	3787	4864	+	3760	-	383	+
B. magnus (a)	478 (21)	42 (15)	349 (12)	373	147	309	105	-	25	+	104	-
B. muscorum	1398 (8)	7 (24)	5 (24)	1008	397	9	535	-	10	-	2048	-
B. norvegicus	7 (28)	28 (18)	30 (19)	25	10	46	46	+	26	-	7	+
B. pascuorum	19913 (1)	7561 (1)	37411 (1)	19705	7769	35497	8	-	490	+	755	+
B. pomorum	170 (26)	0 (/)	0 (/)	122	48	0	67	-	/	/	251	-
B. pratorum	3942 (4)	4843 (2)	8268 (3)	6301	2484	10349	3122	+	1985	-	331	+
B. ruderarius	1414 (7)	281 (10)	170 (17)	1216	479	356	114	-	461	-	1573	-
B. ruderatus	2798 (6)	3 (26)	7 (23)	2009	792	8	1096	-	0	=	4109	-
B. rupestris	602 (16)	15 (20)	182 (15)	443	174	155	203	-	21	+	432	-
B. soroeensis	369 (22)	68 (13)	226 (14)	313	124	232	35	-	1	=	116	-
B. subterraneus	302 (23)	12 (23)	0 (/)	225	89	9	93	-	45	-	446	-
B. sylvarum	519 (19)	30 (16)	69 (18)	394	155	78	141	-	5	-	560	-
B. sylvestris	914 (13)	377 (9)	375 (11)	926	365	594	1	=	382	-	499	-
B. terrestris (a)	1267 (9)	2127 (4)	6005 (5)	2434	960	6419	1979	+	127	-	1591	+
B. vestalis	729 (15)	45 (14)	179 (16)	555	219	177	193	-	0	=	601	-
B. veteranus	3618 (5)	19 (19)	22 (21)	2608	1029	32	1382	-	16	-	5268	-

TABLE XVI. Trends based on species range size (number of 10x10 km grid-cells with at least one individual) estimated with the Stroot & Depiereux (1989) method between P1 (1910-1930) and P2 (1970-1989), P2 and P3 (1990-2016), and between P1 and P3. Signs "-", "=" and "+" represent respectively, negative (red cell), stable (yellow cell) and positive (green cell) significant trends at the p-value of 0.05 (Chi2 = 3.8), 0.01 (Chi2 = 6.6), and 0.001 (Chi2 = 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	**Species rang size**						**P1 to P2**		**P2 to P3**		**P1 to P3**	
	Observed			**Expected**								
	P1	**P2**	**P3**	**P1**	**P2**	**P3**	**Chi2**	**Trend**	**Chi2**	**Trend**	**Chi2**	**Trend**
B. barbutellus	48	10	4	21	37	36	53,95	-	4,57	-	95,00	-
B. bohemicus	26	69	58	35	60	59	3,32	=	6,50	-	0,02	=
B. campestris	50	38	126	32	56	123	15,91	-	26,63	+	0,28	=
B. confusus	17	0	0	6	11	12	29,75	-	/	/	39,23	-
B. cryptarum (a)	38	63	40	37	64	54	0,07	=	13,66	-	12,64	-
B. distinguendus	51	2	0	19	34	36	82,08	-	2,64	=	117,69	-
B. hortorum	85	173	241	94	164	227	1,30	=	0,30	=	2,67	=
B. humilis	31	8	7	14	25	27	31,34	-	0,64	=	47,50	-
B. hypnorum	35	165	253	73	127	201	30,75	+	2,28	=	44,63	+
B. jonellus	27	6	39	12	21	46	29,46	-	16,29	+	3,57	=
B. lapidarius	75	167	308	88	154	267	3,02	=	12,30	+	20,60	+
B. lucorum (a)	46	152	91	72	126	96	14,75	+	37,38	-	0,73	=
B. magnus (a)	35	5	25	15	25	42	45,20	-	8,56	+	22,46	-
B. muscorum	44	5	2	18	31	32	60,45	-	2,29	=	93,34	-
B. norvegicus	4	17	15	8	13	13	2,72	=	1,30	=	0,76	=
B. pascuorum	98	240	332	123	215	300	7,93	+	0,32	=	11,29	+
B. pomorum	12	0	0	4	8	8	21,00	-	/	/	27,69	-
B. pratorum	75	220	287	107	188	253	15,26	+	0,01	=	15,54	+
B. ruderarius	51	91	45	52	90	67	0,01	=	31,36	-	23,85	-
B. ruderatus	51	3	6	20	34	40	78,72	-	0,35	=	94,84	-
B. rupestris	46	11	55	21	36	70	48,42	-	18,84	+	11,23	-
B. soroeensis	20	24	33	16	28	37	1,57	=	0,02	=	1,41	=
B. subterraneus	30	8	0	14	24	21	29,78	-	10,55	-	69,23	-
B. sylvarum	38	16	10	20	34	33	26,99	-	3,59	=	54,49	-
B. sylvestris	35	100	92	49	86	89	6,36	+	6,28	-	0,43	=
B. terrestris (a)	53	174	208	83	144	182	16,62	+	0,91	=	12,19	+
B. vestalis	28	26	59	20	34	61	5,60	-	5,45	+	0,16	=
B. veteranus	46	17	6	23	40	36	36,57	-	8,89	-	83,59	-

Recent stabilisation in the bumblebee trends

Our analyses show a reduced number of species declining between P2 (1970-1989) and P3 (1990-2016), compared to the trends between P1 (1910-1930) and P2 (TABLE XV and XVI). Variations in species abundance and geographic range size seem to be less marked between P2 and P3 than between P1 and P2, like if there occurred a stabilisation in bumblebee population trends at the national scale. We can suppose that the environmental drivers of bumblebee decline occurring during the first part of the century (P1 and P2) were stronger than between P2 and P3. Between P1-P2, agriculture deeply changed with the beginning of massive agricultural intensification in the 1950s (and the Mansholt Plan in the 1970s), characterized by changes in crop rotations (simpler and faster), landscape homogenisation, mechanisation of practices, and use of external chemical inputs (fertilizers and pesticides). All these new practices led to the transformation of a traditional countryside, constituted of small mixed crops with a lot of hedges and trees, toward intensive and homogeneous landscapes, with large monocultures, intensive pastures and hayfields in early mowing (Christians, 1998; Mazoyer and Roudart, 2006). This simplification of agricultural landscapes reduced nest sites availability for bumblebees as well as the composition and the spatial and temporal availability of floral resources (Goulson, 2010).

Moreover, conservation measures, more and more widespread across the country since the last decades, could also partly explain these lower variations in species abundance and geographic range size between P2 and P3 than between P1 and P2 (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 (e.g. Pywell et al., 2006). However, some legislation should still be reviewed, such as the ones requiring the destruction of thistles (i.e. *Carduus* and *Cirsium* spp.) (Vray et al., 2017).

4.4.1.2. WP4. Task 1. Subtask 4.1.2. Multi-stressor analysis of spatio-temporal shifts of wild bee communities

As recommended by Potts et al. (2010), it is necessary to address the multiple effects of drivers as interactions to evaluate the supposed role of non-additive effects. Species distribution models are increasingly used to predict species distribution shifts under scenarios of future change of environmental conditions. As previously mentioned in this report, various studies showed the synergetic effect of multi-stressor causes at different spatio-temporal scales and land use intensification and climate change are ones of the main causes threaten biodiversity and ecosystem services. In this context, this study attempts to provide elements of understanding on how wild bee species respond to environmental changes (both of land use and climate) occurring suring the last century in Belgium (using landscape unit of 10x10km UTM), to facilitate decision-making with regards to these species' conservation.

Wild bee species and floral resources data

As in the previous Subtask 4.4.1.1, we used the *"Banque de Données Fauniques de Gembloux et Mons"* (BDFGM; Rasmont *et al.,* 2015) to extract wild bee species and floral resources datasets for the three time-periods 1910-1930 (P1), 1970-1989 (P2) and 1990-2016 (P2). We considered only plant-bee interactions between taxa identified at the species level to create our data of floral resource. We selected plant species for which we had at least 2 bees recorded (2 interactions) to focus on plants with interest as a food resource for bees. Unfortunately, plant-data are available only for the second and third periods. For each UTM square, we calculated the number of plant species recorded, and the number of these plants known to be foraged by bees. We also calculated the ratio between the number of available plant species and the number of plant species known to be used by bee species. This ratio can be considered as a proxy of the usefulness of these plants as key floral resources for bees in each surface unit.

Land-use and climatic data

Land-use data were obtained from the "Historic Land Dynamics Assessment" (HILDA) version 2.0, and 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), grasslands (including natural grassland, wetlands, and pastures), water, and other lands (including sparsely vegetated areas, beaches, bare soil). We worked at the Belgium scale, using the resolution of 10 x 10 km (UTM squares) and by calculating the proportion of each land use type inside each grid cell.

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"). These data consist in monthly minimum and maximum temperatures as well as monthly precipitation for each year. We extracted these data at the 10 x 10 km resolution and aggregated them as an average for each period.

Analyses of species richness and species assemblage similarity

The bee species database constains validated species records collected at different times, by different recorders and according different standardized sampling methods. These sources of variation give unequal sampling between grid cells and between periods. To reduce this source of variability and compare our dataset in space and time, we used estimates of richness for each grid cell at each period, as used in previous studies (Colwell *et al.* 2012; Carvalheiro *et al.* 2013). We calculated the estimated richness for the three periods for every grid cell (n) where we had sampling data ($X_1[n]$, $X_2[n]$, $X_3[n]$) and calculated relative richness chan ge between P1 and P2 and then P2 and P3 as $\frac{X_2[n]}{X_1[n]}$ and $\frac{X_3[n]}{X_2[n]}$.

To evaluate changes in the wild bee species assemblages (and thus spatial and temporal turnover; Beta-diversity), we investigated the similarity or dissimilarity between every grid cell with wild bee sampling data. To correct the unequal sampling effort between grid cells and periods, we used an individual-based estimator (see also Chao *et al.* 2005; Carvalheiro *et al.* 2013). We computed the Bray–Curtis dissimilarity coefficient based on the logarithm of species abundances (i.e. number of specimens) at every cell and at the country scale.

We then used general linear-mixed models (GLMM; with grid cell identity as random factor and time period, land use and climatic variables as fixed effects) to evaluate the effects of land use and climatic variations on changes of wild bee species richness and composition occuring during the last century in Belgium.

4.4.1.3. WP4. Task 1. Subtask 4.1.3. Relative importance of the environmental and climatic factors to explain recent variations in wild bee assemblages

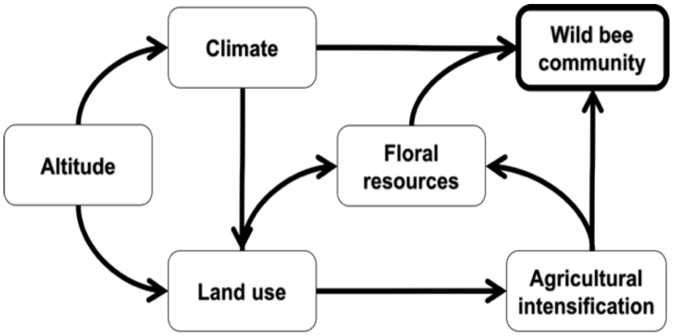


Figure 42. Conceptual model of environmental drivers affecting wild bee distributions

As previously seen, various environmental factors can drive changes in bee communities, such as floral resources, agricultural practices, land use, climate, genetic diversity, pathogens, etc. However, it is worthwhile to know the relative importance of their effects in order to target and prioritize agricultural policies, to develop the most efficient landscape that would mitigate in the short- to medium -term the global pollinator crisis. The objective of this study was to analyse the relative importance of the key factors of bee populations, checking the major hypothetical causes of their decline listed before. Here, we performed statistical multivariate analysis, using as in the previous Subtasks 4.4.1.1 and 4.4.1.2, the dataset “Banque de Données Fauniques de Gembloux et Mons” (BDFGM; Rasmont *et al.,* 2015), both to extract wild bee species data for the recent period 1990-2016, and for the floral resources data. However, analyses were performed without data from genetic variability of bee species or pathogens infection due to the high lack of data and knowledge about these variables for wild bees, and they focused on available environmental databases available for Wallonia (Figure 42).

Landscape datasets

We used data obtained from GIS data provided by SIGeC (Système Intégré de Gestion et de Contrôle), HILDA (“HIstoric Land Dynamics Assessment” version 2.0) and the TOP10Vector (IGN data) land cover data set from the Belgian National Geographic Institute (NGI, www.ngi.be) to characterize the landscape composition influencing the bee community composition at each UTM square. In this study, we reclassified the land-use in ten classes of interest: (i) crop, (ii) favourable grassland, (iii) intensive grassland, (iv) clearcutting, (v) deciduous tree forest, (vi) resinous tree forest, (vii) unused area, (viii) road, (ix) built area, and (x) water. “Land use” variable was defined as the proportion of each UTM cover used by each class of interest.

Concerning agricultural practices, we defined eight crop classes: (i) favourable grassland, (ii) intensive grassland, (iii) leguminous, (iv) oilseed rape, (v) sunflower, (vi) maize, (vii) other intensive crops, and (viii) wood. For each one, at each UTM square, we calculated the mean field size in hectare and the number of fields per square, to access a proxy of the agricultural intensification. Indeed, a more complex landscape is generally characterised by smaller field size and higher connectivity between landscape elements. To reduce the number of landscape data, both for land use and agricultural practices, and to increase the pertinence of the analyses, we selected variables. First, we applied a logarithm transformation to the following land-use variables to ensure that the residuals normality was satisfied: crop, favourable grassland, resinous tree forest, road, built area. Then, we selected the five more pertinent variables using permutation tests in constrained ordination: Crop (*F* = 13.07; *p* = 0.005), deciduous tree forest (*F* = 12.34; *p* = 0.005), Road (*F* = 10.14; *p* = 0.005), Built area (*F* = 5.32; *p* = 0.005) and Favourable grasslands (*F* = 1.85; *p* = 0.07), explaining 16.54% of the variance of total bee data. These five land-use variables were used for all bee group analyses.

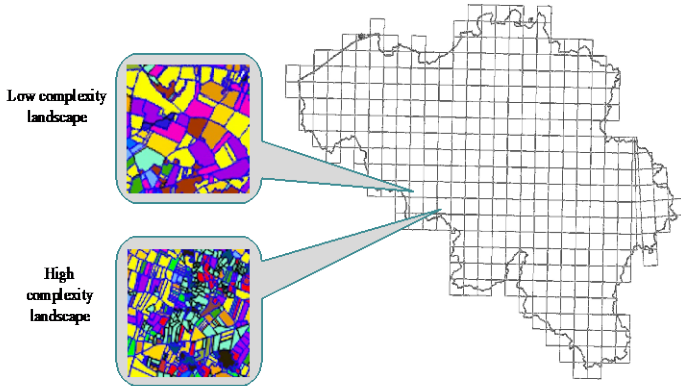


Figure 43. The Universal Transverse Mercator projection system (UTM) covering Belgium (squared of 10 km side) and schematic potential intensity landscapes.

Similarly, we selected the seven more pertinent variables for agricultural intensification: mean size of intensive crops (*F*=3.41; *p* = 0.015), number of intensive crop fields (*F* = 2.68; *p* = 0.045), number of oilseed rape fields (*F* = 3.06; *p* = 0.005), number of maize fields (*F* = 3.52; *p* = 0.010), number of intensive grasslands (*F* = 2.95; *p* = 0.015), number of favourable grasslands (*F*= 2.54; *p* = 0.040), number of leguminous fields (*F* = 1.99; *p* = 0.045). These seven variables have been used for all bee group analyses as agricultural intensification (Figure 43).

Comparative analysis of the explicative power of the environmental factors

To identify the specific or different driver combination roles, we used comprehensive comparative analyses, using the techniques of redundancy analysis (RDA). Analysis presented here focus on the more recent period (1990-2017) across Wallonia region (due to actual lack of some data for the Flanders region). Overall, these analyses allow to measure the proportion of variance explained by different factors and their interaction with covariance. Every analysis in the study was run at the regional scale and over four different bee groups. We first analysed the impact of environmental stressors on all the wild bee species recorded in the region (228 species recorded in 219 UTM squares) and secondly on three taxonomic sub-samples, well represented at the region scale and selected according to some of their ecological traits: *Bombus* species (24 species in 207 UTM squares), cuckoo bees (e.g. *Nomada* ssp and *Sphecodes* ssp; strongly depending on their host abundance and distribution in the landscape; 56 species in 190 UTM squares), and ground nesting bee species (e.g. *Andrena* ssp, *Halictus* ssp and *Lasioglossum* ssp; 93 species in 200 UTM squares).

Altitude as driver of environmental factors

Altitude is a good predictor of the climatic conditions at the Belgium scale and can replace a pool of complex variables (temperature, humidity…), as shown in previous studies (Dufrene & Legendre, 1991). Altitude has also a strong explanation power on the environmental factors, as observed in previous studies. Potential ecological factors, such as edaphic factors, show a strong continuous gradient correlated with the altitude and can partly explain agriculture organisation at the national scale studies (Dufrene & Legendre, 1991). Crops, built areas and unused areas are more associated with lower elevation areas (i.e. Loam ecoregion at the north and western with higher population density). At the revers, resinous trees, clearcutting areas and grasslands are associated with higher elevation areas, like the Ardennes ecoregion in the southeast. Indeed, the Ardennes ecoregion mostly consists in grassland and forested landscapes with lower population density. Consequently, the strong explanation power of altitude could be interpreted as a proxy of the landscape composition and intensification practices on wild bee population trends. As shown by the Venn diagrams (Figure 45), altitude strongly correlated with other environmental factors, and the percentage of the total variance explained exclusively by altitude (without multi-factor interactions) is very low (from 0.1% to 0.4% of the total explained variance in bee group communities).

Explanation power of environmental factors according to bee groups

Altitude is a good predictor of both environmental factors and bee group trends. But surprisingly, variance explained by altitude was twice higher for bumblebees (13.8%) than for others bee groups (6.5% for cuckoo bees, and 7.6% for ground nesting bees). Indeed, there is a strong correlation between altitude and climate, and bumblebees which are hairy bees with large body size (from 9 to 22 mm long) have a very efficient thermoregulation and strong plasticity to temperature variations. Bumblebees often can fly and visit flowers under cold conditions (less than 10°C). For example, *Bombus terrestris, B. hortorum* and *B. pascuorum* began to forage earlier in the morning and later in the afternoon than the honey bees. But this explanation power of the altitude in the variance of bumblebee community was strongly correlated to land use and in a second step to agriculture intensification, environmental factors which are higher for bumblebees (20.3% and 18.9%, respectively) (Figure 44) than for cuckoo bees and ground nesting bees. Thus, bumblebee's community trends seem to be more sensitive to the composition and intensification of the landscape than the other bee groups.

Floral resources explained a high part of the variability (including their interactions with the other factors) in bumblebee and ground nesting bee communities (19.1% and 15.9%, respectively) but are less important for cuckoo bees (11.6%; Figure 44). Bees use floral resources for both nectar and pollen. The second one is essential for larval development and is stocked in the cells of the nest. According to the species, strong floral preferences could occur for nectar or pollen or both, depending of some morphological characteristic (e.g. tongue length) or phenological overlap. Cuckoo bees are, for the great majority, cleptoparasite species, using host species nests for the development of their eggs and larvae. They generally lay an egg in a cell of the nest of a host. Then the parasite larva feeds on the food that had been provided for a host larva. In consequence, they don't need to collect a huge quantity of pollen for the development of their larva and focus more their foraging research on flower providing nectar resources. They can be considered as half less dependent on floral resources than other bee species. This specific behaviour may explain the lower explanatory power of floral resources in the landscape on the composition of the cuckoo bee community at a large scale.

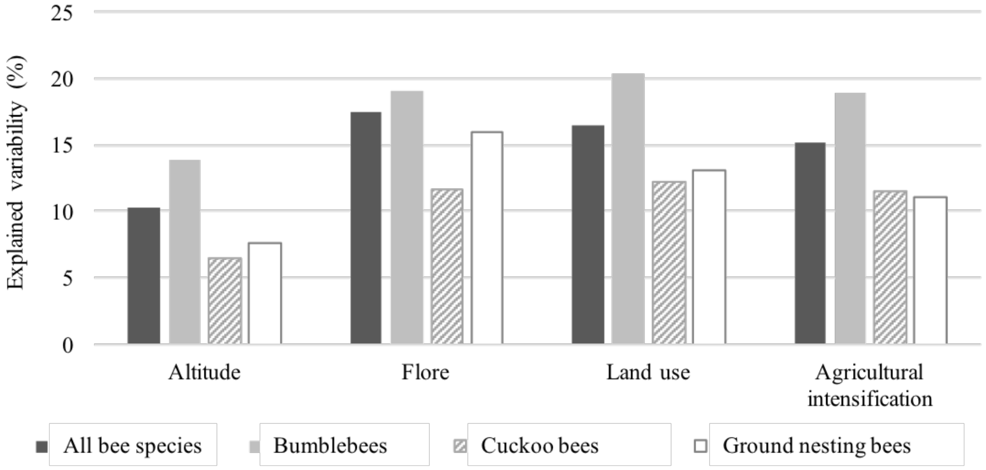


Figure 44. Variability of bee communities at regional scale explained (redundancy analysis) by the altitude, floral resources, land use and agricultural intensification for four bee groups: all the wild bee species recorded in the region, bumblebees, cuckoo bees and ground nesting bees.

Necessary but "not enough"

Our analyses have shown that there is a significant effect of environmental variables on bee community changes at the regional scale in Belgium, but with high residual variability values (from 73.2% for bumblebees to 85.6% for cuckoo bees) (Figure 45). One reason of the low explanation power of our models could be due to the spatial and temporal units used. As sampling effort and amount of data differed greatly between years and place, with low and heterogeneous records in some years, we needed to aggregate the data into a larger recent time-period. According to previous studies performed using the same database (Vray et al., 2019; Vray et al. in preparation) and in order to keep the comparability with some previous studies (e.g. Carvalheiro et al., 2013; Rasmont et al., 1993), we pooled bee species occurrence data from 1990-2016. Moreover, this period corresponds to new environmental management strategies with 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 since 1992. However, working on a large time period with summing species records data, can shade spatial variability and increase observation homogenisation.

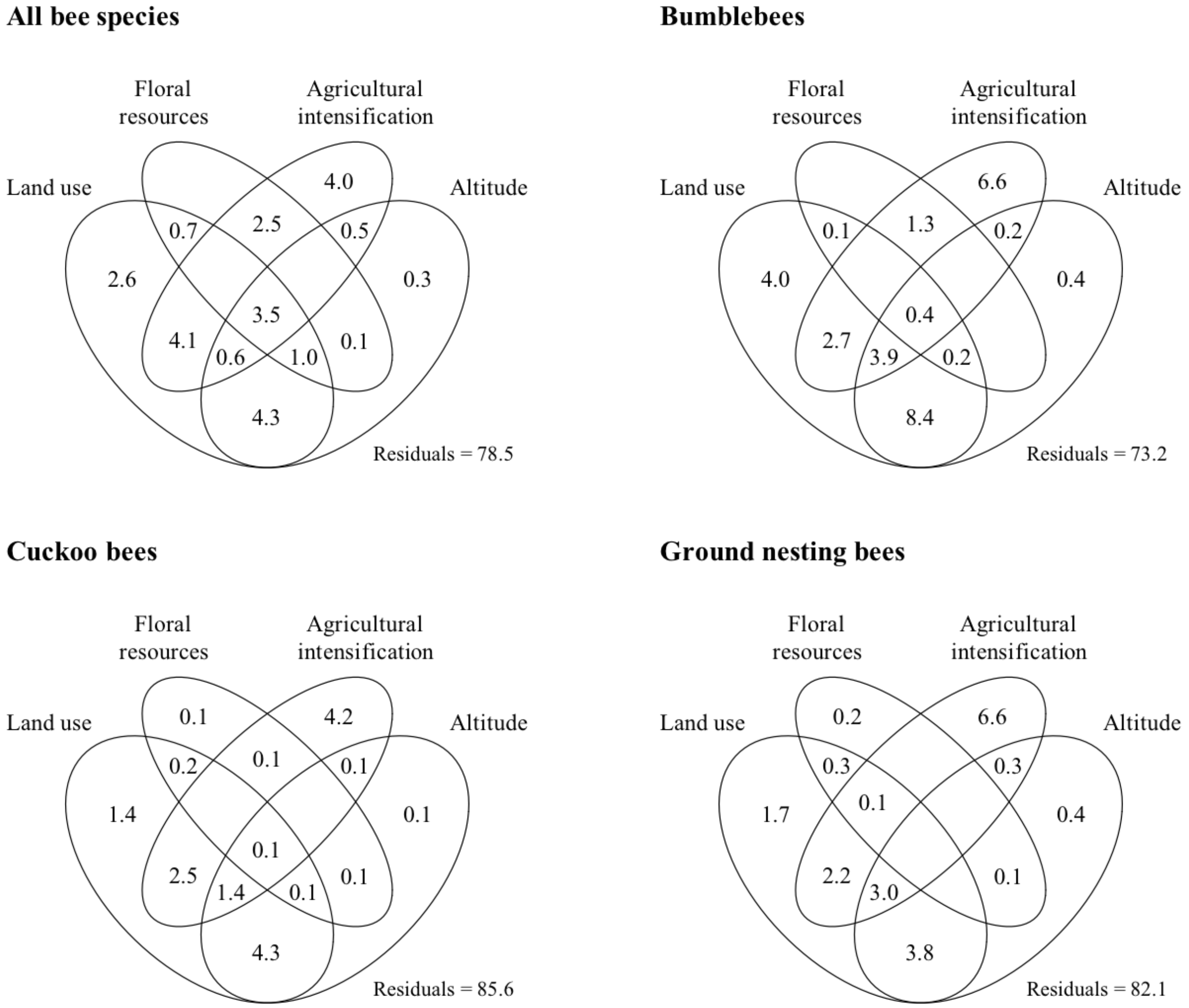


*Figure 45. Venn diagrams of the variance of bee community composition partitioning into land use, floral resources, agricultural intensification and altitude. Analyses were performed for four subsamples of bees: all bee species, bumblebees (*Bombus *species), cuckoo bees (species from Nosema and Sphecodes genera) and ground nesting bees (species from* Andrena, Halictus *and* Lasioglossum *genera). Figures are positively adjusted coefficients of determination (expressed in percentage) and represent the variability explained by each subspace being either a single variable (e.g. altitude) or shared effect between two or more variables. Values =0 are not shown.*

On another hand, we used the Universal Transverse Mercator projection system, with squares of 10 km side covering Belgium. Similarly to yearly sampling, there is a strong heterogeneity in bee records, including many areas with an insufficient sampling effort. In consequence, choosing small grid cells, such as UTM squares of 1 km or 5 km side, would increase data heterogeneity with a high proportion of gaps. Squares of 10 km side was the best compromise between the amount of data per grid cell and the spatial resolution. However, both small and large bees have fly capacities smaller than 10 km. Shift in their spatial organisation can occur between our UTM squares but also within one square, which can occult strong variations at local scale. In the same way, the mean values for environmental factors can increase artificial homogenisation of landscape components and practices.

Our results reveal that more factors drive shifts in bee communities, and probably local environmental conditions could be underestimated to predict bee species occurrence at large spatial scale. They are in agreement with the difficulty to study historical bee datasets due to the difficult or impossible assessment up to a high level of detail for datasets of landuse and floral resources in old periods, even if based on a large number of museum bee specimens.

4.4.2. WP4. Task 2. Modelling

4.4.2.1. WP4. Task 2. Subtask 4.2.1. Prospective modelling of the relationship between bumblebees, land use and climate, at the Belgian scale

Most models of future biodiversity change utilize only climate change variables and ignore land use land cover (LULC) variables or use only LULC variables based on current conditions (static) (Bellard *et al.* 2012; Titeux *et al.* 2016; see Figure 46) The aim of this study was to 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 used three land use change scenarios (BAMBU, GRAS, SEDG; Box 1) representing alternative socioeconomic futures, which have been specifically developed to evaluate the impacts of environmental changes on biodiversity, (ALARM (Assessing LArge-scale environmental Risks with tested Methods) Scenarios; (Spangenberg *et al.* 2012)). These were previously downscaled at a finer spatial resolutions (250 m) to aid in their role as drivers of future distributional changes (Dendoncker *et al.,* 2006). We hypothesized that we would observe clear differences in the projected distributions produced by climate only models versus models which include LULC. Furthermore, we hypothesized that the differences between static and dynamic LULC models will be species-specific and less pronounced, and will most likely depend on the spatial scale and resolution (Luoto *et al.,* 2007; Martin *et al.,* 2013). Overall, the goal was to illustrate the inherent bias associated with using climate change only scenarios when modelling bumblebees that will clearly be affected by land use change.

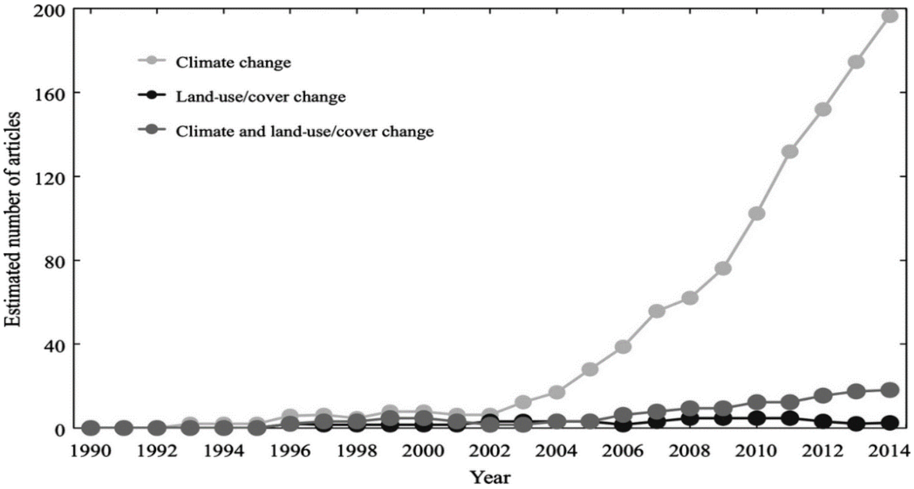


Figure 46. Inclusion of Land-use/land cover data in studies of future biodiversity change scenarios; reproduced from Titeux, N., Henle, K., Mihoub, J.-B., Regos, A., Geijzendorffer, I.R., Cramer, W. et al. (2016). Biodiversity scenarios neglect future land-use changes. Global Change Biology.

- **BAMBU** ('Business as Might Be Usual')

  IPCC A2 scenario - Mean projected temperature rise in Europe at 2100 is 4.7˚c – An intermediate change scenario based on extrapolated current socioeconomic and policy decisions.
- **GRAS (**'Growth Applied Strategy')

  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.
- **SEDG** ('Sustainable European Development Goal')

  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.

Box 1 Description of the land-use change scenarios used in the analysis.

Species and Spatial Data

The study focussed on the genus *Bombus*, which has declined significantly in the last one hundred years (Rasmont *et al.,* 2005; Biesmeijer *et al.,* 2006; Carvalheiro *et al.,* 2013; Kerr *et al.,* 2015). Forty-eight European bumblebee species were included in the analysis. 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). The collection records were obtained for 22 European countries and from multiple sources including professional and amateur scientists (Figure 47). Data were collated as part of the EU FP7 project STEP (Potts *et al.,* 2011) and is aggregated and available on the Atlas Hymenoptera webpage (Rasmont & Iserbyt, 2010-2013). Records from 1970 until 2000 were used. The total number of available records was 462,636. The spatial extent was limited to the extent of the ALARM future projections of European land use, which in turn limited the species collection records available to be used (Figure 47). At the scale of BENELUX (Belgium, Netherlands and Luxembourg) three resolutions were used; 5 × 5 km, 10 × 10 km, 20 × 20 km. At the European scale a 50 × 50 km European grid was used.

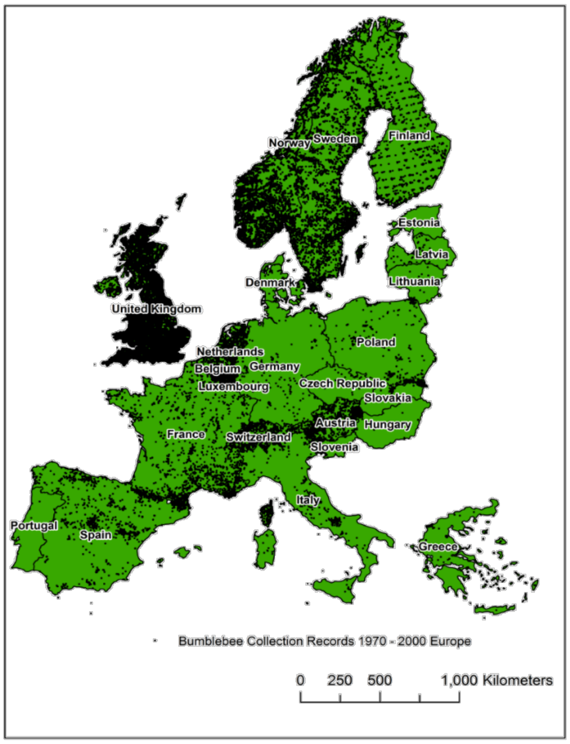


Figure 47. Extent of study area and bumblebee collections records from 1970-2000.

Land-use and Climate Data

Current climatic conditions were produced from monthly-interpolated rainfall and temperature data from 1971-2000, at a 10′ resolution (Mitchell *et al.,* 2004; Fronzek *et al.,* 2012). Future climate scenarios were derived from a coupled Atmosphere-Ocean General Circulation Model (HadCM3; (New *et al.,* 1999)). Five climate variables (TABLE XVII) were selected for the current period as well as for each of three future scenarios of climate change (BAMBU, GRAS, SEDG) in 2050 and 2100 for the four grid resolutions outline above. To avoid collinearities, only variables with Pearson correlation coefficients less than 0.7 were selected (Dormann *et al.,* 2013).

The role of the covariates was 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; and 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.

TABLE XVII. List of climate and land-use variables used in the analysis.

Climate Variables	**Land-Use Variables**
Average precipitation of the wettest month	Percentage Cover Arable Land
Total annual number of growing degree-days above 5 degrees Celsius	Percentage Cover Forest
Mean diurnal range (Mean of monthly difference between daily maximum and minimum temperatures)	Percentage Cover Grassland
Annual Temperature Range (Max Temperature of Warmest Month - Min Temperature of Coldest Month)	Percentage Cover Permanent Crops
Water Balance - annual water balance (Mean monthly precipitation minus the monthly potential evapotranspiration; (Gerten *et al.* 2004)).	Percentage Cover Urban

Model Construction and Performance

We used a Species distribution modelling approach with an ensemble of different model algorithms. Ensemble modelling aims to limit the many uncertainties of forecast modelling and has become increasingly used in studies of future biodiversity change (Thuiller, 2014). We chose three algorithms to include in the ensemble model: (1) a generalized linear model, GLM with linear and quadratic effects, and stepwise selection based on the Akaike Information Criteria (AIC); (2) a generalized boosted model, GBM, with 3,000 trees and five cross validation folds; and (3) maximum entropy, MAXENT with linear and quadratic features. 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 modelled 48 species for which we had at least 50 unique records. As real absences were not available we generated randomly distributed pseudo-absences for GBM and GLM and selected a background sample for MAXENT (Phillips *et al.,* 2009; Elith *et al.,* 2011).

We used target-group sampling to select our background points (Mateo *et al.,* 2010). For each of the 48 species we produced ten runs of each algorithm, for each of the three model hypotheses, and for each of the four grid resolutions (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). 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.,* 2013).

The mean AUC values for all species were 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 < 0.001, Figure 48).

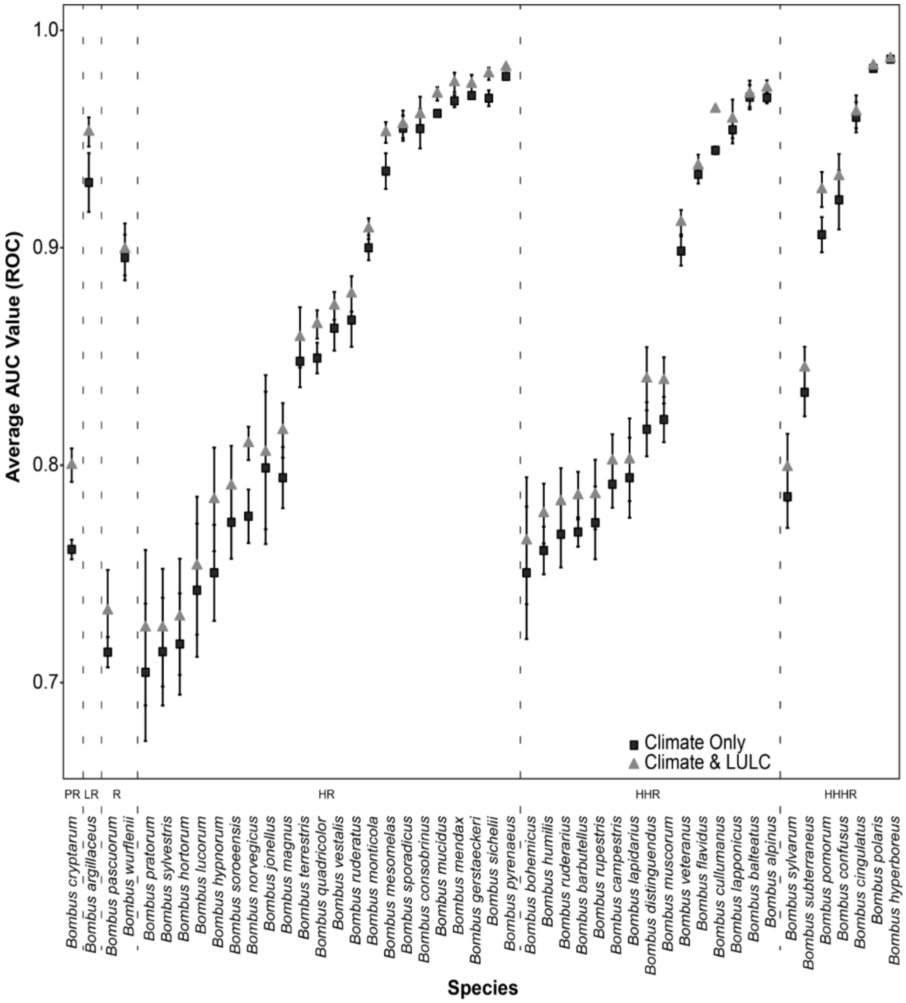


Figure 48. Area under the curve (AUC) statistics for median-ensemble-model performance visualized per species. Black squares represent models with only climate covariates and grey triangles models with land use land cover (LULC) covariates and climate covariates. Groupings represent Climatic risk as 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).

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 highly important for a number of species. Overall LULC variables contribute to 15% of total variable importance. Variable importance is highly variable across the 48 species however, LULC covariates have significant influence on training models when included (Figure 49).

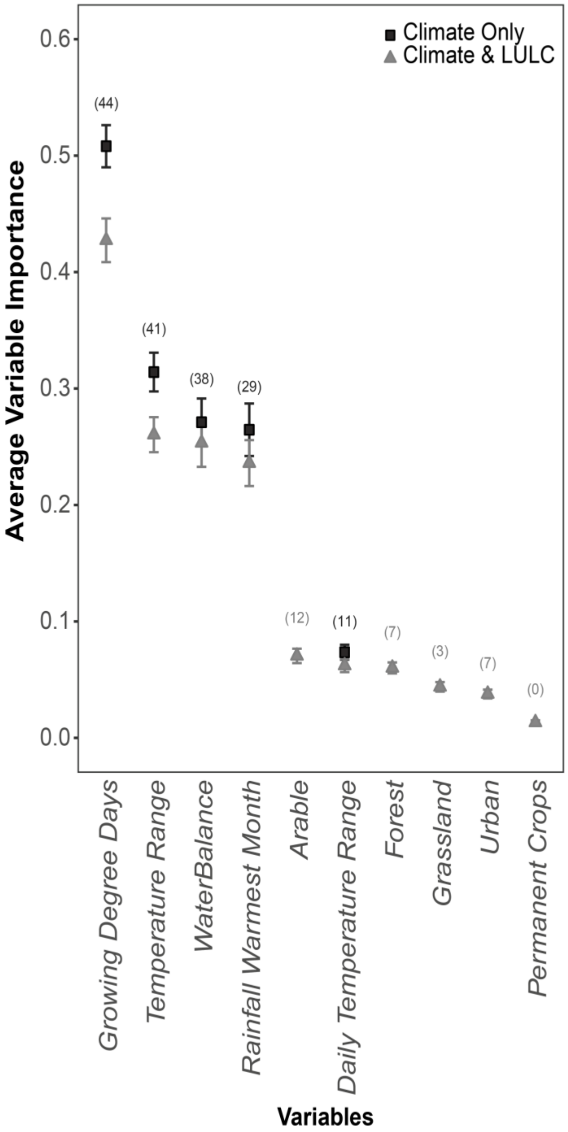


Figure 49. Average variable importance values and standard errors of all covariates included in the training models. Black squares represent models with only climate covariates and grey triangles models with land use land cover (LULC) covariates and climate covariates. The numbers in the brackets represent the number of species for which this variable was one of the four most important variables.

Comparison between climate change only model and dynamic and static land-use change models

For each species we produced a median representation across all model runs. We chose the median value as it is less sensitive to extreme values than the mean. These selected ensemble models were then projected to 5 × 5 km, 10 × 10 km, 20 × 20 km, onto BENELUX. We also projected the data trained at 50 × 50 km onto the entire European study area. For each species we produced habitat suitability (continuous values between 0 and 1) and binary maps (either 0 or 1). One map per species 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.,* 2013).

To compare the projected distributions of the three model hypotheses we measured the (1) loss in range, by looking at areas of occupancy (AOO) decreases between the current and future periods; (2) gain in range, by looking at areas of occupancy (AOO) increases between the current and future periods; and (3) spatial shifts, by looking at centroid of the species range from the present 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 were 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.

Projected range loss showed the greatest difference between model types (Figure 50). There is considerable variability between species and between scenarios but model type has a significant effect on whether species will lose range and how much range loss will occur (TABLE XVIII). At the BENELUX scale overall species are more likely to lose range under DLMs than both COMs and SLMs ($p < 0.001$ and 0.002; TABLE XIX). However, when 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%; $p < 0.001$; TABLE XIX). Under COMs greater mean range loss across scenarios and resolutions occurs for 11 species, however five species show greater range loss under DLMs (Figure 50 a). The relationship between projected range loss of SLMs and DLMs, while not significant at the BENELUX scale (TABLE XVIII) also appears to be species specific, with a number of species below the equal projection line, indicating greater range loss under DLMs (Figure 50 b). At the European scale SLMs project significantly less range loss than both COMs and DLMs (2.9% and 1.7%; $p = <0.001$ and 0.02, TABLE XIX). 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 50 c, d). Eighteen species are projected to lose greater range under COMs whilst fourteen species are projected to lose greater range under DLMs (Figure 50 c). 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 (Figure 50 d, TABLE XVIII).

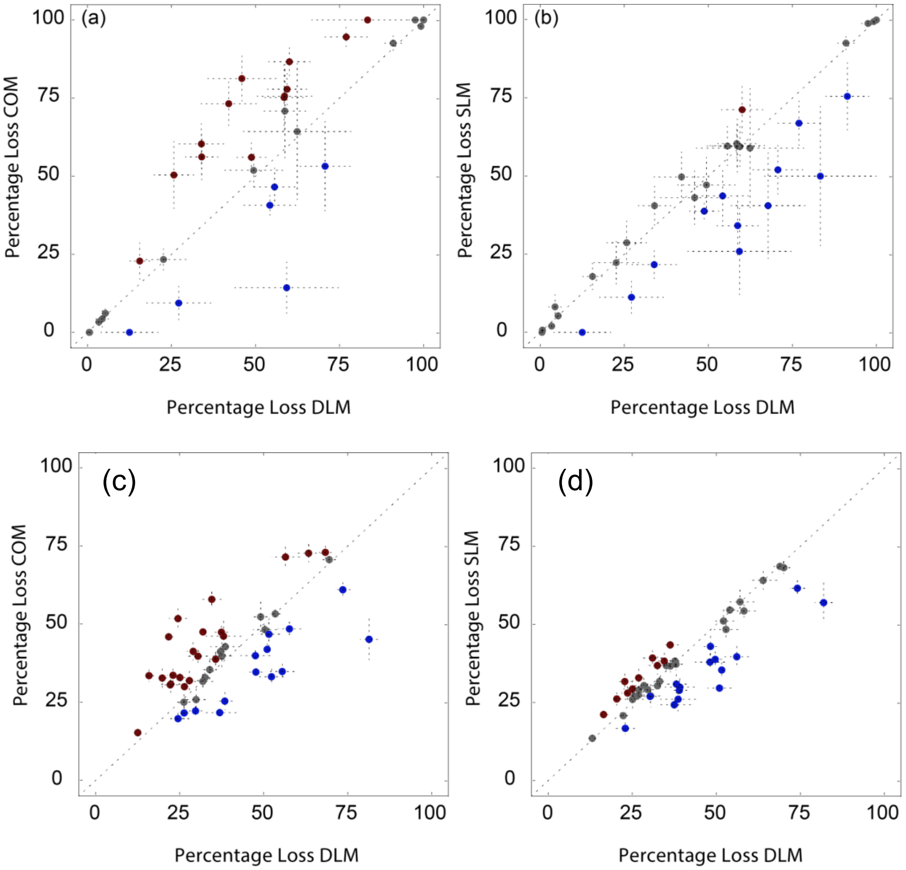


Figure 50. Comparison of percentage loss projections between model types for BENELUX and Europe 2000-2050. (a) BENELUX: Climate Only Models (COM) and Dynamic Land Use Models (DLM). Results are averaged across resolution (5 × 5, 10 × 10, 20 × 20 km) ;(b) BENELUX: Static Land Use Models (SLM) and DLM. Results are averaged across resolution (5 × 5, 10 × 10, 20 × 20 km); (c) Europe: Climate Only Models (COM) and Dynamic Land Use Models (DLM), 50 × 50 km resolution. and (d) Europe: Static Land Use Models (SLM) and DLM, 50 × 50 km resolution. (a) N=36, (b) N=38, (c) N=48, (d) N=48. All results are average over scenario (BAMBU, SEDG, GRAS) and 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.

These results suggest that including LULC covariates creates a wider bioclimatic envelope and in turn projects longer persistence within the landscape. We did not observe the same results across all 48 bumblebee species, and there are species which respond differently to climate and LULC. This inconsistent relationship indicates that dynamic LULC model predictions are not simply a level up or down from climate only models. However, the results can only be interpreted in so much detail due to the low number of land use classes. In the case of bumblebees, we know that to model wild bee species adequately we need ecologically relevant LULC covariates that represent local management (Marshall *et al.,* 2015; Aguirre-Gutiérrez *et al.,* 2016). Therefore, new scenarios should emphasize relevance to biodiversity and land use management, for example, separating between natural-grassland and agricultural-grassland, and intensive and less intensive farming systems.

TABLE XVIII. Effects of SDM variability on the Distributional Change of Bumblebees. 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 Supplementary.

Explanatory variables	**Europe** *(50 × 50 km)*			**BENELUX** *(20 × 20, 10 × 10, 5 × 5 km)*				
	Percentage Loss	*Percentage Gain*	*Centroid Shift*	*Probability of Loss*	*Percentage Loss*	*Probability of Gain*	*Percentage Gain*	*Centroid Shift*
Single Terms								
Range Size Present Europe	***	***	***	-	**	-	-	-
Model Type *(COM, DLM, SLM)*	***	***	***	***	***	***	**	-
Period *(2000-50, 2050-80)*	***	***	***	-	***	***	***	***
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	853	856	847	1706	1511	1617	726	1361

P-values: 0.01 ≤ p ≤ 0.05 = *, 0.001 ≤ p ≤ 0.01 =* * and <0.001 = ***

TABLE XIX. Pairwise comparisons between model types. Showing the fixed effect and the significance of the best models as chosen by Bayesian information criteria BIC. Null hypothesis tested: the difference between contrasts is equal to 0. Values are averaged over other explanatory variables included in the model (see Table S1.)

Contrasts	**Europe** *(50 × 50 km)*			**BENELUX** *(20 × 20, 10 × 10, 5 × 5 km)*				
	Percentage Loss	*Percentage Gain*	*Centroid Shift (km)*	*Probability of Loss (Odds Ratio)*	*Percentage Loss*	*Probability of Gain (Odds Ratio)*	*Percentage Gain*	*Centroid Shift (km)*
COM – DLM	*1.17*	*1.62****	*51.7****	*0.13****	*1.32****	*0.30****	*1.17*	*NA*
COM – SLM	*2.91****	*1.97****	*48.2****	*0.34****	*1.32****	*0.58**	*1.45***	*NA*
DLM – SLM	*1.74**	*1.21***	*-3.5*	*2.57***	*1*	*1.93****	*1.24**	*NA*
P-values: 0.01 ≤ p ≤ 0.05 = *, 0.001 ≤ p ≤ 0.01 =* * and <0.001 = ***								

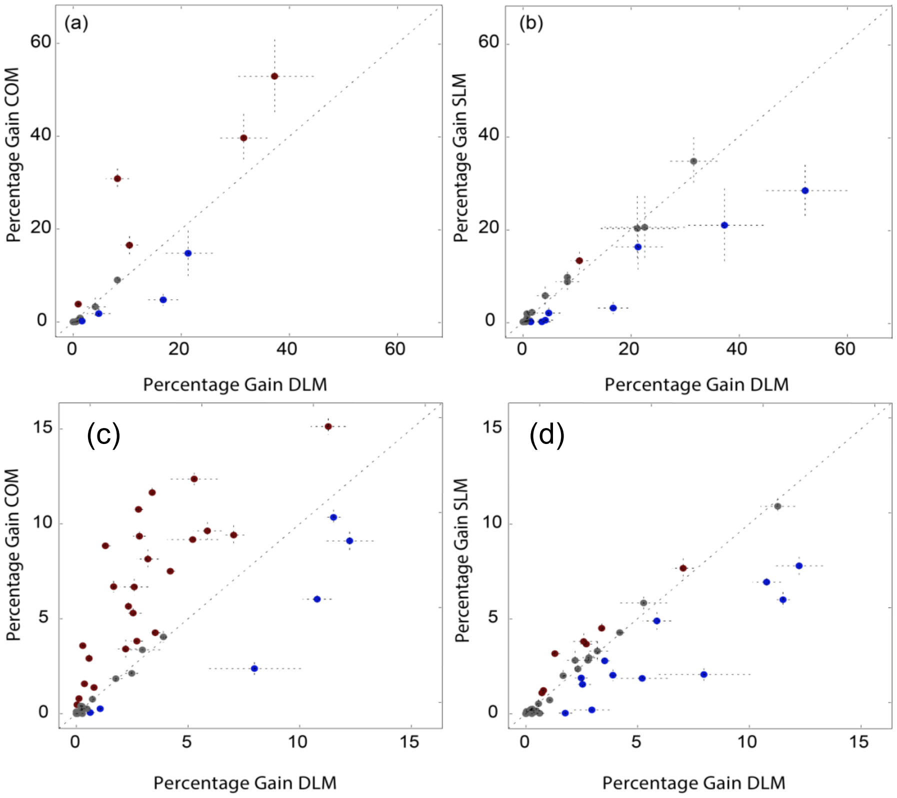


Figure 51. Comparison of percentage gain projections between model types for BENELUX and Europe 2000-2050. (a) Climate Only Models (COM) and Dynamic Land Use Models (DLM). Results are averaged across resolution (5 × 5, 10 × 10, 20 × 20 km); (b) Static Land Use Models (SLM) and DLM. (a) N=25, (b) N=35 bumblebee species in BENELUX for 2000-2050. Results are averaged across resolution (5 × 5, 10 × 10, 20 × 20 km); (c) Europe: Climate Only Models (COM) and Dynamic Land Use Models (DLM), 50 × 50 km resolution and (d) Europe: Static Land Use Models (SLM) and DLM, 50 × 50 km resolution. (a) N=36, (b) N=38, (c) N=48, (d) N=48. All results are average over scenario (BAMBU, SEDG, GRAS) and 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.

Only 50% of species were projected to gain any range at all within BENELUX by 2100 (Fig 50 a, b). The odds of range gain are significantly higher for DLM projections than for COM and SLM ($p < 0.0001$; TABLE XIX). 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%, $p < 0.0001$ & 0.03; TABLE XIX). This can be visualized in figures 51 a, where variation between species is evenly distributed and clustered at zero and 51 b, where seven species have a considerably greater range gain under DLMs. At the European scale overall greater range gain is projected by COMs than SLMs and DLMs (2.3% and 1.8%; $p < 0.001$; TABLE XIX). DLMs project greater suitable habitat gain than SLMs (1.3%, $p = 0.01$, TABLE XIX). This relationship is clearly visible in figure 51 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 becomes more apparent when range gain is high (Figure 51 c, d).

Case Study of one atypical and one representative species

On top of presenting results for *Bombus* as a genus we chose two species, *B. argillaceus* (Scopoli, 1763) (increasing range) and *B. veteranus* (Fabricius, 1793) (decreasing range), which demonstrate different expected changes under climate change (Rasmont *et al.,* 2015). We chose these two species as they are at opposite ends of the spectrum of climate risk, both had high model performance values, both have 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. The current distribution of *B. argillaceus* is Southern and South Eastern Europe as well as Western Asia (Rasmont & Iserbyt, 2010-2013). 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* in the subgenus *Thoracobombus* exhibits an already patchy distribution in the plains of Northern Europe and has already experienced decline in Belgium, shifting from an abundant species to one which is barely present (Rasmont & Iserbyt, 2010-2013). Under future climate only projections *B. veteranus* is expected to decrease in range considerably (Rasmont *et al.,* 2015).

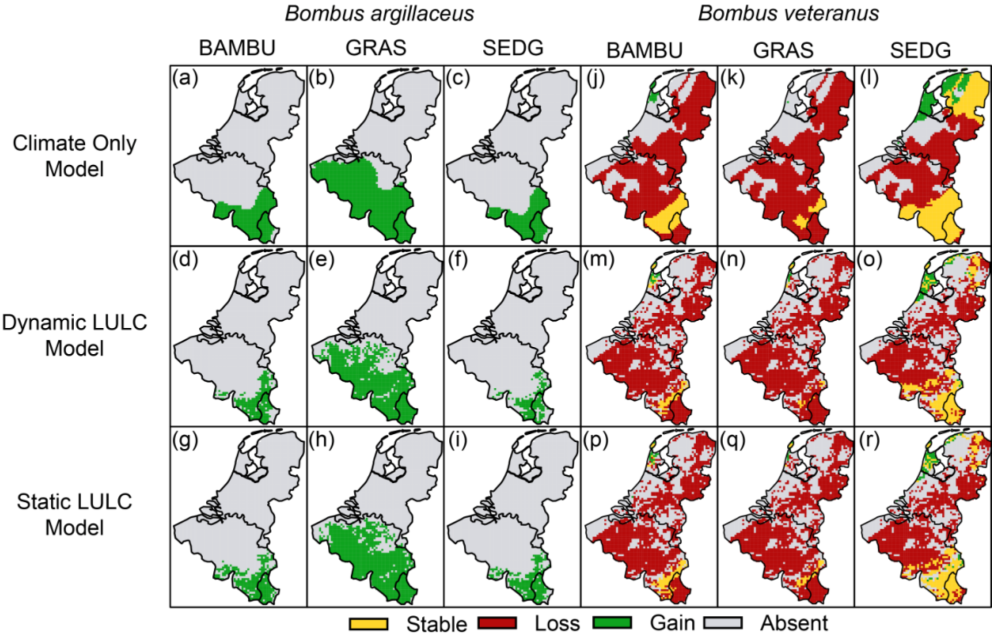


Figure 52. BENELUX maps showing 5 × 5 km resolution of change in habitat suitability between 2000 and 2100 for two species, Bombus argillaceus *(a-i; atypical) and* Bombus veteranus *(j-r; representative of many species). Habitat suitability change is shown for three future change scenarios (BAMBU, GRAS (, 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.*

We observed that 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 52 a-c) than DLMs (9%, 34%, 7%; Figure 52 d-f) or SLMs (10%, 36%, 10%; Figure 52 g-i). 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 of Europe (Figure 52).

Bombus veteranus under BAMBU and GRAS is expected to lose almost its entire suitable habitat in the 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 52 k, n, q). Significant gain is only projected under SEDG for COMs (25%; Figure 52 l). At the European scale *B. veteranus* loses more range under COMs (54%, 67%, 38%; Figure 53 j-l) than SLMs (32%, 50%, 19%; Figure 53 p-r) and DLMs (40%, 55%, 26%; Figure 53 m-o). *Bombus veteranus* is projected to expand into Northern Europe, more under COMs than SLMs and DLMs. Overall SLMs project more persistence in the landscape but less Northern shift. Furthermore 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, Fig. 53 m-r). These maps are available for all 48 species modelled.

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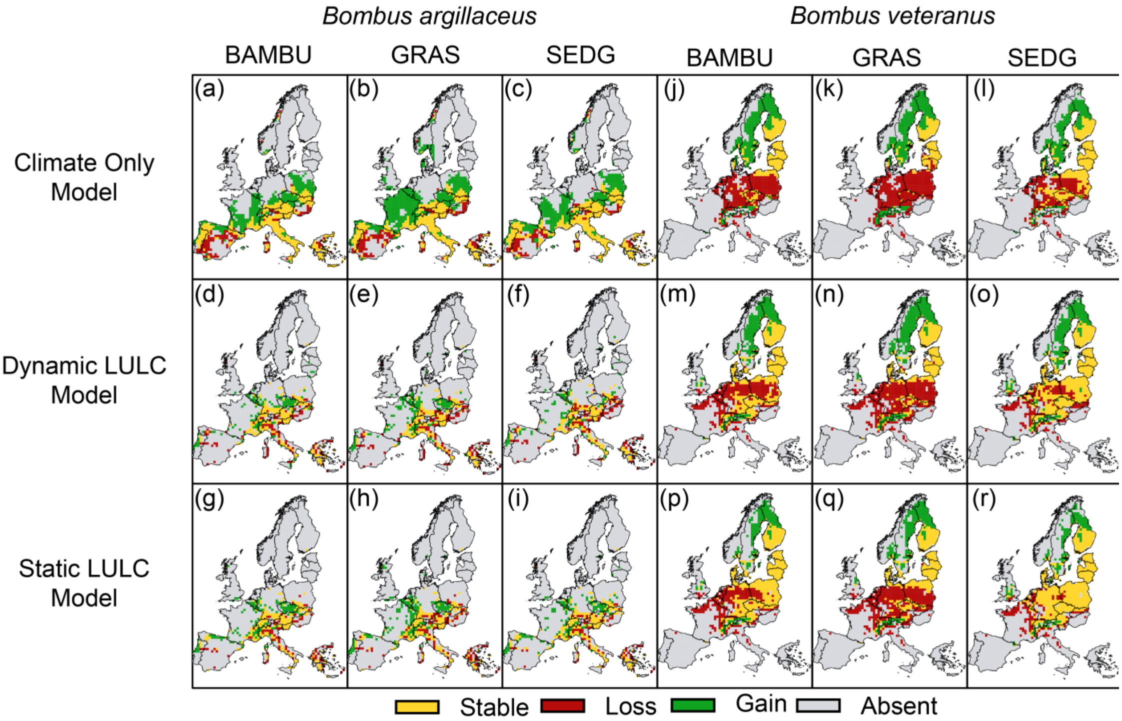

Figure 53. European maps showing 50 × 50 km resolution of change in habitat suitability between 2000 and 2100 for two species, Bombus argillaceus *(a-i; atypical) and Bombus veteranus (j-r; representative of many species). Habitat suitability change is shown for three future change scenarios (BAMBU, 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.*

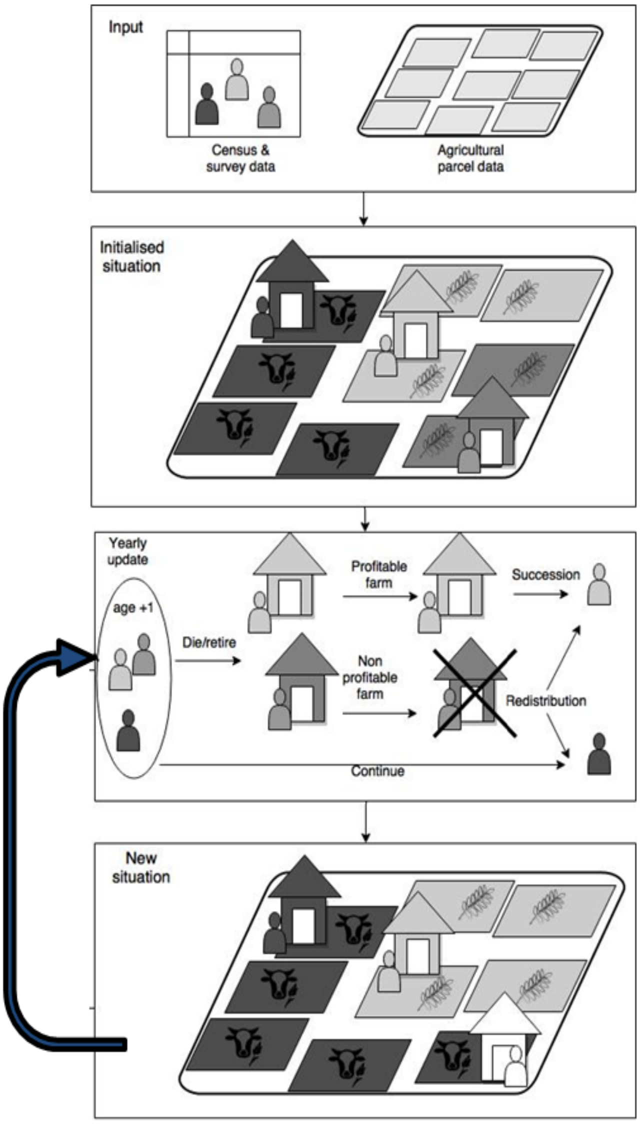

Figure 54. Overview of the methodology of the Agricultural Dynamics through Agent-based Modelling (ADAM), Beckers et al. 2018. In preparation.

4.4.2. WP4. Task 4. Subtask 4.2.2. Prospective modelling of the relationship between bumblebees, land use and climate, for selected municipalities.

Originally, this subtask focused on four Belgian municipalities for which land use change models already existed. UNamur sampled bumblebees between April and September 2015 within the municipalities for which we have higher future land-use thematic resolution (currently Beauvechain and Grez-Doiceau). However the spatial scale of these four municipalities was insufficient to test the hypothesis outlined in subtask 4.2.2 (Martin *et al.,* 2013), that higher thematic resolution is a necessity to better capture the effect of land use on species trends. Therefore this subtask was extended to include land use changes in the whole of Belgium.

The key goal of this subtask is to separate land use classes used in subtask 4.2.1 to provide increased thematic resolution. Namely, the separation between pollinator friendly crops and other arable crops, agricultural grassland versus semi-natural grassland, green urban versus grey urban and also to have an estimate of more specific natural habitats including heathland. This requires the incorporating a series of different models. Firstly the distribution of 48 European bumblebees will trained for 2010 using Corine European land cover data at the 1 × 1 km resolution.

The future projections of land use change will come from two sources; (1), ADAM (Agricultural Dynamics through Agent-based Modelling), which models agricultural changes as a result of farmer decision making (BELSPO MASC project, Figure 54) and (2), an Activity-based Cellular Automata (ACA) land-use model which uses socio-economic baselines and scenarios to estimate land use changes at the Belgian scale, produced by VITO as part of a BELSPO funded project.

We will produce land-use changes with 12 classes (TABLE XXI) for 2050 for three scenarios directly comparable to those used in subtask 4.4.1.

- Business as usual scenario: medium emission scenario (RCP 4.5-8.5)
- Global Economy (~A1F1): high emission scenario (RCP 8.5)
- Strong Europe (~B1): low emission scenario (RCP 4.5).

TABLE XXI. Increased Thematic Resolution Land-use Classes

1. Oil seed rape
2. Fruit trees
3. Cereals
4. Other Crops
5. Forest
6. Heathland
7. Dunes
8. Marshes
9. Semi-natural grassland
10. Agricultural Grassland
11. Green Urban
12. Grey Urban

Climate change variables for the different RCP scenarios will be obtained from the WorldClim database (http://www.worldclim.org/). For each species we will produce a distribution map for the present (for Europe) and for each of the three scenarios at 2050 (Belgium only). We will repeat this using 12 land use classes and aggregating the land use to the same five classes used in subtask 4.4.1. We will compare the output of the different models by examining the range and distributional shifts from the present until 2050. To determine the influence of greater thematic resolution when modelling the projected distribution of bumblebees. We will focus specifically on those species included on the Redlist of bumblebees for Belgium, to determine the projected change in their distributions with and without high thematic resolution land use data.

We will use a similar methodology as outline in WP 4.4.1. However, we will use only MAXENT SDMs as with previous experience and in other similar studies MAXENT provides sufficient high-quality models for collection data of this kind (Elith *et al.* 2011; Aguirre-Gutierrez *et al.* 2013; Marshall *et al.* 2018). We will create 10 model runs per species per scenario for models with high thematic resolution and models with low thematic resolution. We will measure; (1) loss in range, by looking at areas of occupancy (AOO) decreases between the current and future periods; (2) gain in range, by looking at areas of occupancy (AOO) increases between the current and future periods; and (3) spatial shifts, by looking at centroid of the species range from the present and the future, all at the time period of 2050. We expect to observe finer scale changes in distribution and record areas in Belgium of important habitat that may act as refuge for bumblebee species in the future.

4.5. WP5. Stakeholder and policy makers integration

Stakeholders and decision-makers integration into an interdisciplinary research project is arguably one of the key elements to enhance the project's legitimacy and uptake of the results. In BELBEES, stakeholders' integration was planned along four specific tasks of Work Package 5.

4.5.1. WP5. Task 1. Identification of stakeholders at municipal, regional and federal levels

Stakeholders were identified starting from previous knowledge of the project's scientific partners, to constitute a follow-up committee (FUC). Members of this were consulted in order to identify and select other stakeholders that could be interested by the project's results. In BELBEES, we defined stakeholders as anybody who has a stake in this project, hence all PI, members of the FUC, and other parties interested in the project. A list of about 50 stakeholders was established. All of them were invited to the first stakeholder meeting of the project.

4.5.2. WP5. Task 2. Organization of meetings with stakeholders to assess their needs and constraints with regards to pollinators

The first meeting allowed identifying the needs and constraints of the 27 stakeholders present (22 were excused). The needs came out from a world café and they were structured along the four main hypotheses of the project. For example, agricultural areas were identified as target land use of the project. There was also a need for more data and research on the impacts of climate change on wild bees (including bumblebees), but also more communication and awareness rising. There was also a need to further investigate requirements at the species level in terms of habitats and resources. Finally, the fact that the impact of pesticides could not be investigated further was identified as a big gap.

4.5.3. WP5. Task 3. Land use change scenario design at the municipal level

Due to time constraints, themselves mainly due to the fact that results from the retrospective modeling at the municipality level (identifying the precise correlation between wild bee decline and landscape and climate factors) could not be achieved before late in the project, stakeholders meetings at the municipal level to think about designing optimal landscapes for wild bees at this level was not an option. However, this remains a key task that should ideally be achieved as a next step to the research, to enhance wild bee conservation.

4.5.4. Organization of meetings with stakeholders to validate results

In addition to yearly meetings with the FUC, a final meeting was organized with stakeholders in order to present and validate the results produced by each partner during the project on 30th of May 2018 at RBINS. An overview of the scientific results obtained since the beginning of the BELBEES project was presented by each scientific partner. A presentation of the data provided by Natuurpunt (citizen science partner of the BELBEES project) has also been performed. In the meantime, these presentations included a “brainstorming” section which allowed a discussion between the scientists and the consultative committee (i.e. everyone interested in wild bee conservation).

The afternoon was devoted to the mitigation management. In order to establish recommendation on wild bee conservation, working tables were formed. In the first instance, a discussion related to the main factors of bee decline took place between the coordinator and the participants at the tables. The aim was to analyse the main threats and urgent needs on wild bee conversation in the current situation (future researches, issues, mitigation and policy making). At the end of this interactive session, the coordinator summarized the main points of this session. All the participants finally voted for the most relevant recommendations.

Stakeholders’ recommendations for policy

Biodiversity management concerns

During the meeting, the stakeholders made the next proposals:

- To develop corridors at the European and local level (increase the subsidies to motivate the actors);
- To determine why species have disappeared, and to study their potential reintroduction;
- To determine pollinator efficiencies in ecosystems services and how to feedback to policy;
- To define quality coefficients of pollinators integrating: abundance and richness, colony losses, botanical diversity and pesticides content;
- To determine single values of habitat quality;
- To develop practical measures against the drastic decline of wild bees, the decline being mentioned for many years but changes in such measures;
- To develop the conservation of ground-nesting bees;

- To study biodiversity from an intrinsic crop pollination point of view;
- To link policy making to habitats not to farmers or crops;
- To determine what species are missing and to propose to actors how to adapt to these missing species;
- To solve the problem of bee hostels which are growing too big;
- To replace honey bees with wild bee species;
- To improve the biodiversity by encouraging extensive management (increase of wild spaces) and awarding citizen to apply this kind of management on garden in the context of wild bee conversation, via education;
- To highlight the importance of natural reserves;
- To encourage bottom-up processes;
- To propose a European index of bees, with all Member States providing data on bee species;
- To develop more studies about the weeding of road sides;
- To develop new projects in order to improve our knowledge in the most successful mitigations;
- To develop a landscape approach (more than Natura 2000) in policy approaches;
- To fight the concept of “weeds”;
- To promote less specialised bee hostels;
- To improve the number of nest sides;
- To remove the law against thistles or make new regulation;
- To develop more specific habitats dispersed in municipalities;
- To install favorite plants in selected habitats: list of the best plants for pollinators adapted to local situation, development of specific seed mixtures for target bee species depending on the localization;
- To take into account that abundance richness of bee species is linked to sand; thus after an extracting sand from an, area, this shall not be covered by soil and trees;
- To link funding of greening to its impact on pollinators;
- To not use crop pollination as the only argument for biodiversity conservation.

Summary of stakeholders’ concerns:

Regarding to the biodiversity management concerns, stakeholders consider that furthers studies should be conducted in order to develop simple and operational pollinator indicators, to develop more researches about dispersal abilities of each bee species. Bee friendly landscape should be promoted by according prime for effort devoted to pollinator conservation, targeting seed mixtures adapted to the local conditions and the local bee species, promoting connection between favorable resources in landscape, promoting plant species abundance and diversity and finaly by providing further information on ground nesting bees in the context of wild bee conservation. The stakeholders also underlined that it is necessary to avoid an approach only founded on the economical role of pollinators.

In terms of legislation, several modifications should be taken in consideration. First, a simple and operational pollinator indicator should be determined and a bee friendly index should be established which could be applied to municipalities. Secondly, the landscape approach should be integrated in the applicability of policy and more specifically in the Plan Protection Products (PPP). Most of the wild bee flower resources are negatively seen by population as “weed” (“mauvaises herbes”, “onkruid”). This is particularly the case of thistles. This misconception should be fought by educative communication. As first, the regulation against thistles should be changed and restricted to agricultural fields only.

Monitoring concerns

During the meeting, the stakeholders made the next proposals:

- To develop more follow up projects;
- To give feedback to people concern by this kind of project;
- To share occurrence data at the European scale (more relevant for global change);
- To promote volunteering (only relevant at the parcel level);
- To develop monitoring studies on large farming areas as realistic labs;
- To define the evolution of wild bee species area (How do we keep a conservation idea while everything is changing?);
- To monitor abundance richness of species;
- To improve field studies to compensate the lack of information about bee health and monitoring of bees;
- To develop more researches to determine which bee species are indicators;
- To continue monitoring of bee for digitization and valorization;
- To increase the number of people capable of monitoring;
- To determine if the whole environment has to be monitored for bees: Inventory of habitats for bees;
- To establish permanent transects;
- To develop easily measurable monitoring parameters;
- To determine the foraging range in farming areas;
- To determine how to give monitoring feedback;
- To develop monitoring which allow applicable measures: ask to politicians how they can use information and develop new tools for them;
- To make a list of monitored generalist to specialist bees.

Summary of stakeholders’ concerns:

Regarding monitoring concerns, stakeholders consider that data gathered during the BELBEES project should be shared in order to perform actions and policy making. Nevertheless, more funding should be granted to researchers to allow further monitoring of wild bees. This would improve the development of measures and actions on the field like long term monitoring by using identification tools, indicator species and field guides of wild bees. The efficiency of wild bee hostels should be investigated.

Climate change concerns

During the meeting, the stakeholders made the next proposals:

- To study the interactions with natural resources to buffer the effects of climate change;
- To evaluate the dispersal capabilities of species (elevation and latitude movement including dispersion and genetic of separate populations);
- To study the phenology variations related to Climate change;
- To not overlook the climate change in the decline of wild bees;
- To increase the awareness-raising and communication about climate change;
- To develop measures related to the climate change like reducing the urbanization (smokers, greener urbanization);
- To encourage children to monitor pollinators (e.g. schools, scouts);

- To develop automatic pan trapping placement (identification of wild bees can be a problem because of the numbers of specimens);
- To monitor floral strips: initiatives related to plant diversity for farmers.

Summary of stakeholders' concerns:

Regarding Climate change concerns, stakeholders consider that further studies should be conducted to determine the buffering effects of natural elements able to mitigate their negative effects.

Pathogens concerns

During the meeting, the stakeholders made the next proposals:

- To determine the causes of changes of the pathogens;
- To link with other topics: pesticide, climate stress and modification of the landscape;
- To study the prevalence and pathogenicity of *Osmia* diseases in bee hostels;
- To restrict the importation of bees (ban *Osmia* importation and develop a sanatory certificate for all domesticate bees);
- To control pollen importation and establishment of a sanatory certificate for pollen as food resource.

Summary of stakeholders' concerns:

Regarding pathogens concerns, stakeholders consider that further studies should be conducted to determine the impact of wild bee pathogens. The importation of new domestic bees should be controlled by reducing the import of some bee species, establishing a health bee certificate. Stakeholders demanded the ban of *Osmia* importation.

Pesticides concerns

- To determine how to reduce the number and the concentration of herbicide;
- To determine how to avoid spillover of pesticide;
- To determine how to quantify the indirect effects of pesticide;
- To determine the toxicity of fungicides which are used on flowers in orchard and determine their synergetic effect with other pesticides;
- To determine how to quantity the use of pesticide in private gardening;
- To determine the buffering effects of landscape elements against pesticides;
- To change regulatory framework of pesticides, vet products and biocides (taking in consideration pollution rise context and landscape approach);
- To promote good phytopharmaceutical practices with control (DIN or ISO, AFSCA).

Summary of stakeholders' concerns:

Regarding the pesticides concerns, stakeholders consider that the regulation framework of pesticides should be changed. The toxicity of fungicides should be more explored in further studies.

Farming concerns

During the meeting, the stakeholders made the next proposals:

- To inform the farmers of the available subsidies;
- To promote management practices focused on livestock polyculture farmers;
- To share information with farmers on foraging plant and disperse seed;
- To encourage farmers to apply for correct subsidies;
- To provide pollinating primes for farmers who own area with pollinator friendly plants;
- To change agriculture system: increase heterogenous farming landscapes, promote organic farming, define target crop rotations, promote development of more different crops.

Summary of stakeholders' concerns:

Regarding the farming concerns, stakeholders consider that the current agricultural model should be modified. Actions should be conducted on a local scale with farmers and managers. Farmers should be awared to wild bee conservation by promoting a different agriculture model like organic farms or alternatives to pesticides. Pollination prime could also be granted to farmers. The abundance of floral resources available for wild bee should be improved by developing heterogenous crops, flower strips and leguminous fodder crops (which would reduce the use of chemical fertilizers). The abundance of nest site should be improved by increasing the presence of edges, wood edges and tuffs of grass in agricultural landscapes.

Awareness and education concerns

During the meeting, the stakeholders made the next proposals:

- To raise awareness to biodiversity and consumption;
- To communicate the results of Red List of Belgian wild bees;
- To communicate more information about honey bees and wild bees;
- To raise awareness population to the importance of wild bee-friendly seeding;
- To make cities greener with flowering roofs or walls.

Summary of stakeholders' concerns:

Regarding the awareness and education concerns, stakeholders consider that these two points should be more developed by granted more funding to promote educational programs about biodiversity, responsible consumption and nature protection. These programs could change the educational opinion on conservation.

Actions for wild bee conservation in the cities like plantation of tree species that provide good resources for wild bees (e.g. *Acer*) in parks and gardens could be performed. Stakeholders also suggested to promote late mowing on the road sides and to implante wild bee hostels in cities. Installation of green roofs and walls in cities could make cities greener.

Participation of citizen in actions for wild bee conservation could be improved by promoting actions in private gardens. The design of these gardens should promote a “nature” approach (e.g. wild garden with thistles and aromatic plants). Studies should be conducted to quantify the concentration of pesticides, herbicides and fungicide used in private gardens. Finaly, citizen should be awared to alternatives to chemical fertilizers by using leguminous plants.

Part II: RECOMMENDATIONS

The results of the BELBEES project show the vulnerability of the Belgian wild bees and the causes and mechanisms of their decline. Some research still needs to be done to have a deeper understanding of these phenomena but, clearly, we now have already a lot of knowledge and this information allows to start taking actions for wild bees' conservation.

The following recommendations result from the BELBEES researches and from the discussions that occurred during the BELBEES' scientific meetings, stakeholder's meetings and final meeting. These recommendations are made to set the future objectives of wild bees' conservation and give some leads (actions, legislation, etc…) on how we could achieve these aims in Belgium.

The overall project and its scientific studies show a real impact of food resource depletion, habitat loss, climate change and pesticides on wild bees and these causes clearly are a result of current human practices. Thus, our main objective should be to adapt the practices for the Belgian territory. This implies to encourage and develop new regulations, new management practices (in farming, public spaces, industries, green spaces, etc.) but also to promote good practices already existing. This first objective can only be achieved if the awareness raising and propagation of the good practices stimulate and enlighten the actions of all the Belgian actors. Finally, these changes in practices must be accompanied by associated scientific researches and wild bees monitoring in Belgium.

The following recommendation are very important for wild bees' preservation, as part of our main pollinators group. From our studies on the pollination service in Belgium (Jacquemin *et al.*, 2017) it is concluded that the productivity of main crops in Belgium only partially depends on pollinators. Nevertheless, this study evaluates the value of pollination service at 251 million euros per year in Belgium and some regions like Limburg and Flemish Brabant would be at considerable risk regarding their fruit production. Our further recommendations are thus essential in order to maintain food security and food quality on the long term in Belgium.

Adapt the management practices in Belgium to make them more “bee friendly”

To adapt the management practices, it is very important to take into account the scientific results of this project. Our recommendations will thus result from a close look at the impact of each cause and their consequences for wild bees.

Floral resources depletion

The studies conducted in this project showed a global floral resources depletion and a change in the plant-bee networks in Belgium during last century. These floral resource changes have an impact on bumblebees and other wild bees through nutrition (quality of the diet) that can impact their development and health. For instance, after a nutritive stress, bumblebees could be more vulnerable to diseases and infections and have to compensate by increasing their nectar and pollen consumption. It appears that generalist bee species may be able to compensate and shift their diet: this is the case of some of the more common Belgian bumblebees. In this case, the availability of diverse floral resources to reach this new diet balance is critical. Specialist species are more threatened by the disappearance of their main floral resources. They are more heavily affected than the generalist species; their foraging plants decreased more during last century and they appear to be less able to shift their diet.

As an answer to these results, we strongly advice to improve the floral resources availability and quality in the landscape in order to provide for the nutritional needs of wild bees and to improve their health and resilience to other decline factors.

Some more specific recommendations to achieve this goal would be the next:

- Promote a list of best resources plants for wild bees which would take into account their nutritional value, their importance for specialist and generalist bees and the local context (climate and soil). This action could be a huge leverage: for instance, it has been shown that some ornamental tree species can provide some good quality pollen and diet at different periods of the year whilst some can be toxic, such as *Tilia cordata*. Planting the recommended trees in cities (to replace toxic trees, trees that need to be replaced anyway, etc.) would already enhance the resource availability at urban scale. Encouraging the development of the adequate plants production (indigenous, non-treated and high-quality plants) would help the promotion of these bee-friendly plants.

- Enhance the availability of flowering plants for target bee specialist species that are rare, declining or ecologically important and of flowering plants that strongly decreased during last century. These plants could be implemented in the MAE schemes, in cities and green their spaces, in citizens' gardens, etc. This would enhance their presence at landscape scale. Regarding flower stripes a first study advices to adapt them for bumblebees by adapting the composition of flower mixes. These should take into account the various preferences of each bumblebee 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.

- Increase the areas that can provide floral resources in the landscape by increasing the floral resources in current flowery areas, but also by increasing the proportion of habitats and land uses that provide flowers for wild bees. For instance, in agricultural areas it would be important to maintain floral resources outside main crops both for helping wild bees and maintain pollination service. As a practical case, it has been shown that conserving abundant floral resources in Belgian orchards throughout the season allows conservation of bee fauna after the massive flowering of the orchad trees.

- Promote the good practices for flowering plants management in all types of areas. This is particularly important to allow managed and spontaneous flowering plants to express their whole potential for wild bees. These management practices should mainly aim to conserve the floral resource through time (e.g. maintaining the flowery meadows over the years; do not cut roadsides and meadow before September and the end of bumblebees colony cycle) and allow the maximum availability of floral resources for wild bees (e.g. avoid cutting plants before their flowering). This implies to follow a calendar, especially for trees and hedges pruning and grass mowing. One of our studies on Belgian heathland showed that some rare bumblebee species need the willow species (*Salix* spp.) to complement their diet and complete their life cycle. Thus, a specific management providing floral diversity and landscape heterogeneity is necessary, even in natural areas.

- Revise the law on thistle removal. Thistles are of great importance for wild bees and especially for bumblebee males. This law could negatively affect bumblebee populations that are already greatly threatened by global environmental changes. We recommend to abrogate the law, or at least to limit the law to *Cirsium arvense* within cultivated fields, and to move towards new measures that reconcile biodiversity conservation and agricultural need.

- Consider the potentially negative impact of invasive flowering plant removal. It has been shown that some exotic invasive plants (e.g. *Impatiens glandulifera* and *Buddleia davidii)* are foraged by generalist species such as bumblebees. They were shown to be important in their diet balance and their removal could have detrimental effects for bumblebees in a poor forage agro-environmental landscape. On the other side, the expansion of these invasive plants could also have a negative effect on specialist bee species. Thus, we suggest to assess the floral resource availability in the landscape before the mass removing of invasive flowering plants, and to adapt the further management in order to replace the floral resource they constituted by indigenous plants.

Habitat fragmentation and genetic pauperization

The studies conducted in this project showed that the Belgian landscape greatly changed during last century and that growing urbanization and agricultural intensification had a negative effect on bumblebees' assemblages in Belgium. Other wild bees, that are often smaller than bumblebees and with smaller dispersion abilities, are probably even more strongly affected by landscape fragmentation and habitat loss. At this stage, more studies including landscape and environmental (climate, etc.) factors are needed to better understand these phenomena. Surprisingly, it appeared that these great landscape and agricultural changes had no influence on the bumblebees' genetic diversity. Indeed, our studies showed that rarer bumblebees have a lower genetic diversity than the common bumblebees, but that it was already the case 100 years ago.

These first results bring us to strongly recommend to improve the wild bees' habitat availability and quality in the landscape. This will provide nesting resources (nesting sites, nesting material, etc.) and floral resources to bees, and allow bee communities to be established in the whole Belgian landscape. We also recommend to ensure wild bees' populations' connectivity to avoid any future genetic pauperization and to improve wild bees' resilience to other decline factors.

Some more specific recommendations to achieve this goal would be the next:

- Protect and restore wild bees' habitats. The protection, restoration and suitable management of existing high-quality wild bees' habitat would be the first step for their conservation and would allow them to find their basic requirements (nesting and flower resources) in safe areas (adapted management practices, no pesticides use, etc.). A list of the most important wild bees' habitats would be of great help to prioritise the areas of interest. Quality and connectivity of these preserved habitats should be included in their management plans. We also suggest to create natural reserves in Belgium for the sites hosting wild bees' communities of national or regional importance.

- Increase the hosting potential in agricultural and urban areas. Our studies showed that crops had a positive effect on bumblebees distribution a hundred years ago but now have a negative effect on them. This highlights the overall negative effect of agricultural intensification but also shows the potential of farming as a leverage for bumblebees and other wild bees conservation in Belgium. Improving farming practices by reducing the risks (pesticides, etc.) and improving floral and nesting resources in crops and on the farm, could have a massive influence on wild bees' preservation. To achieve such goal, it is necessary to promote and experiment pesticides alternatives (organic farming, agro-ecology, precision agriculture, etc.) and other bee-friendly farming practices (promote flowering crops and especially leguminous grops, crop rotations, flower margins, etc.). Agricultural areas represent the largest surface in Belgium and have the greatest effect on bumblebees' distribution, along with urban areas. In Belgium, the high density of settlements and crops in the north of the country were deserted by bumblebees, except for the few currently abundant species. Improving the welcoming potential and permeability of the cities to wild bees is thus necessary to mitigate the effect of massive urbanisation. This implies to lower risks for wild bees (pollution, habitat destruction) and to improve the availability nesting and floral resource in the cities and suburban areas. In the cities, urban planning could include bees' habitats in several support such as green roofs, floors, walls, etc. Bee-friendly actions can be taken by the citizens in private garden and balconies whilst public services and associations can improve green and public spaces. Promoting good practices for citizens and municipalities is thus a main goal in the urban areas

As a general rule, in highly anthropogenic areas, preserving undisturbed elements in the landscape such as hedgerows, micro-reliefs and embankments and respecting the spontaneity of wildlife will help providing natural nesting and flower resources.

- Move towards a “dynamic conservation” of wild bees. Indeed, wild bees are flying insects that seem to have good dispersion abilities and that could easily colonize new habitats that fit their nesting and floral requirements. In a context of global changes (landscape and climate), a dynamic conservation of wild bees through space (e.g. urban wildernesses moving around the city) and time (e.g. climatic sanctuaries) seems to be necessary for long term conservation in Belgium. This dynamic conservation implies to plan bees’ conservation and to identify the future protection areas. Indeed, the Climatic Risk Atlas for European Bumblebees shows that during next century suitable areas for bumblebees might greatly diminished and shift in Belgium and in whole Europe. Dynamic conservation also implies to better understand wild bees’ dispersion abilities in the landscape. It will contribute to habitats connectivity and thus allow gene flow between populations and reduces the risk of genetic isolation.

Disease emergence

Metagenomic surveys were conducted on wild bees (bumblebees’ and solitary bees) in Belgium to explore the spectrum of their potential pathogens. The surveys highlighted the fact that wild bees’ pathogens are very little known. However, they already revealed that wild bees carry plants and mosquitoes’ viruses, but also carry their own set of viruses. Most of these organisms were never described before and their function and pathogenicity are unknown. Other parasites such as microsporidia (*Tubulinosema* unprecedented detection in Europa), gregarines (cryptic parasite species in the genus *Apicystis*) and trypanosomes were also described and found to infect several wild bee generas. Researches are still needed to better describe the pathosphere of wild bees and especially to study the pathogenicity of the described potential pathogens.

These first results bring us to strongly recommend to apply the precaution principle regarding diseases in managed and wild bee species in order to limit diseases transmission and propagation.

Some more specific recommendations to achieve this goal would be the next:

- Study the pathogenicity of wild bee viruses and parasites in bumblebees, osmias, and other wild bees. In order to do so, more researches should be performed on these diseases.

- Evaluate the effect of some practices on diseases prevalence in wild bees’ populations. Indeed, we actually lack information on the effect of some measures on the prevalence of some diseases in the wild bees’ populations. We could imagine that measures that enhance the proximity of pollinators (such as insect hostels, isolated flower plots visited by all surrounding pollinators, proximity of hives, etc.) might increase the risk of infection and diseases transmission. Sanitary rules might come out of such evaluations and allow the improvement of wild bees’ management practices.

- Monitor the trade of pollinators in Belgium. The trade of managed pollinators such as honey bees (*Apis mellifera*) and new managed pollinators such as bumblebees (*Bombus terrestris*, *Bombus sp.*), Osmias (*Osmia cornuta*, *O. bicornis*, *Megachile rotundata*…) is growing in the world and in Europe. The movement of these managed pollinators bred in other countries will inevitably bring the genetic contamination of local populations and new diseases in Belgium. Since we are currently not able to screen for the diseases of wild bee species, we recommend to apply the precaution principle. We recommend to regulate the importation of pollinators in Belgium - or at least to keep records of the trades and organize traceability of the bees - and to elaborate pest controls in parallel. Regarding honey bees for which diseases are well known, we recommend to demand sanitary certificates when importating colonies and honey bees pollen in Belgium.

Pesticide management

The effect of pesticides commonly used in Belgium is often tested on honey bees but there still is a lack of knowledge on their effect on bumblebees and other wild bees as well as on their sublethal and chronic effects. Plus, we often have no idea of real pesticide doses spread and of the actual wild bees' exposure in the environment. In this context, the development of biomarkers (e.g. gene expression or protein levels) for pesticide intoxication in bees and wild bees could ultimately allow to asses' pesticides toxicity and presence in the environment. Exploratory studies showed that such biomarkers can be developed for honey bees and used to monitor imidacloprid intoxications, although results will depend on the context. However, these results cannot be easily expanded to bumblebees since the efficiency of their detoxification mechanisms is different and because it appears that their detoxification-induced gene expression is caste dependent. At the same time, studies also showed that pesticides formulations affect the bumblebee's micro-colonies survival at much lower doses that at the Maximum Field Recommended Concentrations, highlighting the high toxicity of pesticides mixes for bumblebees. More research is still needed on this new biomarker technique, especially for the study of protein level biomarkers and for some solitary bees' model species.

These preliminary results bring us to strongly recommend to limit the wild bee species exposure to pesticides in order to avoid sublethal and lethal negative effects and to enhance the health and resilience of wild bees to other decline causes.

Some more specific recommendations to achieve this goal would be the next:

- Further study and develop biomarkers for pesticides intoxications for bees. The development of a precise biomarker in honey bees would allow the monitoring of pesticide exposure risks in the Belgian landscape. In parallel, studies on pesticides toxicity and on biomarkers development are essential to better understand the effect of pesticide molecules, and pesticides mixes, on bumblebees and solitary bees.

- Adapt the regulatory framework and promote good sanitary practices for pesticides use. At present stage some first measures could already reduce the risks for pollinators by controlling the use of pesticides. This would imply to change the regulatory framework of currently used pesticides, as well as for veterinary products and biocides that are suspected to be very toxic for wild bees too and to take into account the pollinators in the condition of use, especially regarding the moment of pesticides spreading (date, daytime, bee activity in the field, weather, etc.), the doses used (promote a landscape approach to regulate global use of pesticides, lower the maximum field recommended concentration, etc.) and the treatment calendar (avoid some pesticides mixes, minimum time between treatments, etc.). These measures would have more chances of success if accompanied by adapted technologies (e.g. precision farming) and of independent controls of pesticides use.

- Promote alternatives to pesticides for farmers and all land managers. New agricultural and green spaces management practices are needed in order to prevent, or limit the pesticides use in Belgium. Agricultural areas probably represent the largest area and the greatest use of pesticides at the country scale. Thus, we strongly recommend to accompany farmers in a transition toward new practices (e.g. crop diversification, agroforestry, agroecology, organic farming, etc.). In the non-agricultural areas (e.g. road edges, public green spaces, citizens garden, etc.) alternative and adapted practices also need to be shared to field actors (organic treatments, differentiated management, etc.). On the long term, these alternative practices need to be monitored to evaluate their positive impact on wild bees. For instance, flower stripes in agriculture can act as an ecologic trap in some circumstances. In the same way, mulching to avoid using weed-killer products might have a negative effect on soil nesting bees. These very complex ecological questions need to be addressed through partnership between field and research actors.

Climate change

The studies conducted on climate change focused either on the global warming and temperature increase, or on the punctual extreme events such as heat waves. A third aspect of climate change is phenological shifts of wild bees and/or plants, which still requires to be studied in Belgium. The Climatic Risk Atlas of European Bumblebees is the most comprehensive work allowing us to have a modelling of future projections of bumblebee ranges in Europe. Results are very alarming since, in 2100, we expect to have 36% of European bumblebees at high climatic risk, whilst only 3 oriental species would benefit from climate change and only 3 bumblebee species would remain in Belgium. Some more precise models run for Benelux show some range loss for bumblebee species by 2100 and this range is more variable (positively or negatively, depending on the species and projections) if the landscape variables are integrated to the models. As a summary, climate effects are predicted to be strong but predictions are complexified by other intricated global changes such as landscape change. Another study showed that the size of some bumblebee queens increased during last century in Belgium, but in this case climate effect is hard to tell apart from other effects such as habitats fragmentation. Even if climate change has to be studied in a dynamic way (integrating other environmental factors) some functional consequences have been shown and generalist species, or species with a narrow climatic niche are more at risk. The effects of climate change can thus be amplified by extinction cascades from plant-pollinator networks. For Belgian bumblebee males, it has been shown that resistance to heat waves varies among species, with the boreo-alpine species such as *B. jonellus* that are more at risk. The resource quality and colony size were shown to influence the bumblebees' reaction to heat stress. The synergies between climatic effect and other decline causes should not be underestimated.

These results bring us to strongly recommend to support programs for climate change mitigation and to take actions to reduce climatic risks for wild bees.

Some more specific recommendations to achieve this goal would be the next:

- Further study the effect and mechanisms of climate change for wild bees. A good understanding of the climate change effects, mechanisms and synergies with other decline factors will allow us to find out mitigation measures for wild bees. Further researches are needed on wild bees and host plants phenological shifts. A better understanding of the interactions between climate change, landscape change and other environmental factors is needed to better understand the leverage we have (through these other factors) for wild bees' conservation. Furthermore, studying and modelling the future climatic sanctuaries for wild bees would allow us to plan wild bee conservation.

- Focus on reducing all the other decline causes of wild bee. Climate change is a global issue for which no answer can be found at Belgian scale. In this context, one should focus on all the other decline causes (floral resources depletion, habitat regression, pesticides, diseases, etc.) that can be managed at the country scale. For more information on how to achieve this goal, see the previous paragraphs.

Awareness raising and promotion of good practices

The previous paragraphs present different measures or actions to achieve the goal of wild bees' preservation in Belgium. These measures need to be implemented at several scales, with many different actors, and need to evolve as our knowledge about wild bees' conservation grows. To do so, the planning of actions is necessary, for instance through national or regional action plans. In parallel, naturalist, scientific and technical information need to spread in order to allow people willing to take actions to do in an adequate way. This is why we consider that the dissemination of information and valorization of scientific results is critical.

Some more specific recommendations to achieve this goal would be the next:

- Support scientific results popularization. In the scientific world, popularization is not always a competence of the researchers or researchers don't have time to do sobecause of the pressure in this competitive sector. Financial or technical support (partnerships between research and awareness raising structures) would improve the spreading of scientific results toward the civil society.

- Raise awareness among the broad public. Raising awareness amongst citizens is necessary for a large-scale understanding of the pollinators' decline issue and to launch actions. This can be done through various medias such as conferences, films, events, citizen sciences programs, etc. The content of the citizen science message should evolve in consideration with the circumstances and the public that is aimed for.

- Promote biodiversity and wild bees' contents in schools' programs. Schools would probably be the most efficient way to raise awareness amongst Belgian citizens. The main topics to approach would be the importance of plants and pollinators for food and ecosystems, the diversity of wild pollinators and their decline, the actions that can be taken at global and invidual scale for wild bees' conservation. Fighting the concept of "weed" and of "stinging and dangerous" insects would also be important. Also, basic courses on pollinators should be mandatory in farming, landscape gardener courses as well as in all environmental and biological courses in order to prepare the future professionals to cope and adapt their practices in favour of wild bees.

- Support the elaboration and promotion of good practice guidelines. In order to bring people and professionals to take actions for pollinators, good supports are needed. These documents should include general information, a few statistic and scientific information, but also technical support to enable people to implement measures that are adapted to their context (profession, administrative and bioclimatic region, etc…). These good practices guidelines should be upgradable and could be accompanied by some exemplary pilot projects (e.g. experimental farms, ideal garden for wild bees, etc.).

- Support the development and implementation of a pollinator indicator. A simple and comprehensive pollinator indicator would be of great help to take wild bees and other pollinators into account in the management of Belgian managed areas (agricultural and natural areas, etc.) and for the planning of bees' conservation at the administrative scale (municipalities, provinces, regions, etc.) and for the communication toward local stakeholders and decision makers.

Scientific monitoring and wild bees monitoring

All the previous recommendations need to be included in a general context of scientific monitoring and wild bees monitoring. The scientific monitoring would imply to keep going with the fundamental researches on wild bees, but also to keep investigating their causes of decline in Belgium and the mechanisms and synergies involved. Indeed, a better understanding of wild bees and of the problems they face will help to find the better answers. In the meantime, first actions need to be taken and evaluated, for instance through pilot projects, in order to estimate the effect of these measures on wild bees and biodiversity in general. As a finality, the cost-benefit ratio would be important to prioritize the measures for wild bees' conservation.

This scientific monitoring can only be done if a long-term and standardized monitoring of Belgian wild bees is organized in Belgium, and if wild bee fauna databases are well managed and made available. The standardized monitoring of wild bees will allow a precise follow-up of populations health and decline whilst the good management of wild bees' data will allow the sustainability of wild bees monitoring and of researches.

Some more specific recommendations to achieve this goal would be the next:

- To organize the wild bee data collection, digitization and distribution in concertation at the country scale. In order to implement and large scale and standardized monitoring at regional of national scale, concertation is necessary to succeed and have exploitable data in the end. Some subjects such as required data fields, protocols and data management need to be discussed and common methods shared for the success of the operation. The monitoring that would result from this concertation should cover the whole country and also focus on some areas considered at risk (e.g. dunes, heathlands, and higher altitude areas like the Hautes-Fagnes). In parallel to avoid the negative effects of genetic pauperization and landscape fragmentation, the monitoring of genetic diversity of both declining and common bumblebees is needed.

- Ensure the durability of wild bees' databases in Belgium. Currently wild bees' databases are managed by different structures and people with different means and goals. Data sharing happens in the frame of some specific projects and is not perennial. The same happens for financial support. A country scaled data management and subvention would guaranty a more consistent and lasting wild bee database.

- Engage a transition toward open data for a better data valorization. On the long term, the sharing of data on wild bees could increase the opportunities for wild bees' conservations. For instance, it could allow a greater publication of scientific works in relation with Belgian wild bees, or bring more local actors to take local wild bees fauna into account in their management practices or construction projects.

5. DISSEMINATION AND VALORISATION

5.1. WP6. Valorisation

In the frame of the BELBEES project, several articles were published in peer-reviewed journals (e.g. Nature Communications, Global Change Biology) addressing the major factors of bee decline like climate change, landscape modifications and pathogen virulence. Simultaneously, the results of the project were regularly displayed in national and international seminars through posters and oral presentations. Several master and PhD theses have fully addressed the topics of BELBEES. The project also led to the diffusion of the findings in different medias (e.g. social networks, radio and television interviews). Moreover, several educational works and illustrated identification tools were published by RBINS. Below is an exhaustive list about the valorisation of the project.

The international Eurbee8 congress (18-20/09/2018, University of Ghent, Ghent, Belgium) was the opportunity for scientific partners of BELBEES to expose their results to individuals and scientific community working on bees. This international congress gathered scientists from all over the world. A consortium entitled " Where have all the wild bees gone, long time passing? " focused on BELBEES. It was presented by the scientific partners, including seven oral communications as well as posters that were displayed during the congress.

Web Pages

Atlas Hymenoptera, Thematic page of 'Belgium'. Mons, Gembloux. Web. 19 octobre 2018. http://www.atlashymenoptera.net/liste_them.asp?them=Belgium

Belbees, Web, 19 octobre 2018. http://www.belbees.be/

National and International Seminars

Brasero N., Lecocq T., Brigitte F. & Rasmon P. “Comparative study of long-term stability cuticular hydrocarbons in queens, workers and males of *Bombus terrestris* and related species (Hymenoptera, Apidae)”. ZOOLOGY 2014 21st Benelux Congress of Zoology, Liège (Belgique).

Folschweiller M., Lecocq T., Dufrêne M. & Rasmont P. "Adaptation to a changing world: How wild bees cope with climate change" in "21st Benelux Congress of Zoology", Liège, Belgium (2014).

Lecocq T., Michez D. & Rasmont P. “Methods for species delimitation in bumblebees: towards an integrative approach”. ZOOLOGY 2014 21st Benelux Congress of Zoology, Liège (Belgique) Oral Presentation.

Vray S., Denconcker N. & Rasmont P. “May land use and climate changes threaten bumblebee populations (*Bombus* spp.) in Belgium”. ZOOLOGY 2014 21st Benelux Congress of Zoology, Liège (Belgique).

Vray S., Dendoncker N. & Rasmont P. "May land use and climate changes threaten bumblebee populations (Bombus spp.) in Belgium?" in "BEES Christmas market", ULg campus Agro-Bio Tech Gembloux, Belgium (2014).

Maebe K., Eurbee 6, Murcia (Spain), 9-11 September 2014.

Marshall L. 6th Belgium Ecosystem Services Christmas Market, Gembloux (Belgium), 17 December 2014.

Rasmont P., "Atlas des risques climatiques des bourdons d'Europe" in "Apoidea Gallica", Avignon, France (2015).

Schoonvaere K. & de Graaf D.C. “Towards characterization of the pathosphere of four wild bee species in Belgium”. Belgian Society for Parasitology and Protistology conference on November 26, 2015.

Dufrêne M. & Jacquemin F. Participation to the BELBEES meeting of 15/10/2015 (poster and presentation).

Jacquemin F. Participation to the BELBEES day (12/12/2015) (poster).

Marshall L. 6th Belgian Geography Days, Brussels (Belgium), 13-14 November 2015.

Jacquemin F., Folschweiller M., Drossart M., Rasmont P., Violle C. & Dufrêne M. "Naturalist historical databases help us to better understand plant-bee interactions and their dynamics across space and time" in "Symposium «Entomology in Belgium 2016», Organized by the Royal Belgian Entomological Society", Brussels, Belgium (2016).

Rasmont P., Martinet B., Franzen M., Harpke A., Kerr J. K., Lecocq T., Michez D., Potts S.G., Roberts Stuart P.M. & Schweiger O. "How does climate change affect bumblebee diversity?" in "Eurbee7 ", 1,76, Cluj-Napoca, Roumanie (2016).

Rasmont P. "The high climatic risk of European wild bees and bumblebees" in "EUROPEAN WEEK OF BEES AND POLLINATION - 5TH EDITION", European Parliament, Brussels, Belgium (2016).

Rasmont P. "The high climatic risk of European bumblebees" in "BDIV Seminar", Louvain-la-Neuve, Belgique (2016).

Roger N., Moerman R., Carvalheiro L., Aguirre-Guitiérrez J., Jacquemart A.-L., Kleijn David, Lognay G., Quinet M., Rasmont P., Richel A., Vanderplanck M. & Michez D., "Impact of pollen resources drift on common bumble bees in NW Europe" in "Euro IUSSI ", Helsinki, Finlande (2016)

Vray S., Dendoncker N. & Rasmont P. "Trends of bumblebee (*Bombus*) populations in Belgium: a 100-year record" in "Symposium «Entomology in Belgium 2016», Organized by the Royal Belgian Entomological Society", Brussels, Belgium (2016).

Marshall L., Biesmeijer J. C., Rasmont P., Vereckeen N. J. & Dendoncker N. “The interplay of climate and dynamic land use land cover changes affects the distribution of EU Bumblebees”, International Society for Ecological Modelling Conference, Towson (USA), 08 May 2016.

Jacquemin F., Rasmont P., Mahy G., Gérard M., Violle C. & Dufrêne M. “Influence des dynamiques des ressources florales sur les abeilles sauvages en Belgique”. Thesis committee, Montpellier (Lauret, France), 13/05/2016.

Jacquemin F., Violle C., Munoz F., Taudiere A., Rasmont P. & Dufrêne M. 2016. “Plant diversity loss forces shift in ecological strategies for wild bees: insights from historical time series at a country-wide scale” –Montpellier (France), EcoSummit 2016 - Ecological Sustainability: Engineering Change, Poster 2.260, 31/08 & 01/09/2016.

Pecheur E., Laura M. & Jacquemin F. “Ecosystem services in Wallonia...What’s up?” Antwerp (Belgium), European Ecosystem Services Conference – Helping nature to help us, Stand (posters & games), 20/09/2016.

Jacquemin F., Violle C., Munoz F., Taudiere A., Rasmont P. & Dufrêne M. “Plant diversity loss forces shift in ecological strategies for wild bees: insights from historical time series at a country-wide scale” –Marseille (France), SFécologie 2016 – International Conference of Ecological Sciences, oral presentation (Ecological networks session), 27/10/2016.

Jacquemin F., Folschweiller M., Drossart M., Rasmont P., Violle C. & Dufrêne M. “Naturalist historical databases help us to better understand plant-bee interactions and their dynamics across space and time” – Bruxelles (Belgium), Entomology in Belgium 2016, 02/12/2016.

Pecheur E., Laura M. & Jacquemin F. “Ecosystem services in Wallonia...What’s up?” Gent (Belgium), BEES Christmas Market, Stand (posters & games), 13/12/2016.

Jacquemin F., Violle C. & Dufrêne M. (2017) “Decline of pollinators: mapping the dependency of crops in Belgium –Sofia (Bulgarie), MetEcoSMap Conference 2007”–Mapping and assessment of ES –Science in action. Poster

Michez D. Conference “Qui fait quoi pour les abeilles ?» Presentation to communicate the UMONS results for the Food depletion task of the BELBEES project, scientific section « Etat des lieux et tendance des abeilles sauvages en Belgique », Bruxelles (Belgium), 15 May 2017. Oral Presentation

Rasmont P. Apoidea Gallica in Tours (France) from 20/01/2017 to 22/01/2017 Rasmont P. Congress CARI in Namur, 29 January 2017. Oral Presentation

Rasmont P. IPB meeting - Workshop on understanding the knowledge of pollinator and pollination in EU, Bruxelles (Belgium), 23 October 2017. Oral Presentation

Marshall L. SCAPE Conference in Oslo (Norway), 28 October 2017. Oral Presentation

Folschweiller M., Lecoq T., Dufrêne M. & Rasmont P. (2018). Adaptation to a changing world: How wild bees cope with climate change. Eurbee 8th, Ghent, “Where have all these wild bees gone, long time passing?”. Poster

Michez D. Conference at Toulouse University 3 (France), 12 December 2017. Oral Presentation

Pauly A., Sonet G., Noël G. & Boevé J.-L. Barcoding halictine bee species from Europe and Africa. Poster iBOL Conference 2017, South Africa. Oral Presentation

Rasmont P., Boevé J-L., Francis F., Dendoncker N., Dufrêne M., Smagghe G., Barbier Y., Brasero N., D’Haeseler J., Dekoninck W., Desmet L., Foschweiller M., Jacquemin F., Maebe K., Marshall L., Martinet B., Meeus I., Michez D., Moerman R., Pauly A., Roger N., Schoonvaere K., Vanderplanck M., Van Ormelingen P., Vray S. & de Graaf D.C. (2018) Were have all the wild bees gone, long time passing ? Oral Presentation

Michez D., Roger N., Moerman R. & Vanderplanck M. (2018) Drift in distribution and quality of host-plant resources in common bumblebees. Oral Presentation

Jacquemin F., Violle C., Munoz F., Mahy G., Rasmont P., Michez D., Vereecken N.J., Roberts S.P.M, Vray S. & Dufrêne M. (2018). The role of bees in interaction networks with plants as a conservation argument. Oral Presentation

Vray S., Rollin O., Michez D., Dendoncker N. & Rasmont P. (2018). Extreme shift in abundance and distribution of bumblebee composition in Belgium. Eurbee 8th, Ghent, “Where have all these wild bees gone, long time passing?”.

Maebe K., Meeus I., Vray S., Dekoninck W., Boevé J-L., Rasmont P. & Smagghe G. (2018) A century of temporal stability of genetic diversity in wild bumblebees. Eurbee 8th, Ghent, “Where have all these wild bees gone, long time passing?”. Oral Presentation

Schoonvaere K., Francis F. & de Graaf D.C. (2018). Viruses in wild bees : to be included in future monitoring programs. Eurbee 8th, Ghent, “Where have all these wild bees gone, long time passing?”. Oral Presentation

Folschweiller M., Jacquemin F., Drossart M., Dufrêne M., Michez D. & Rasmont P. (2018) Status and trends of wild pollinators in Belgium and north of France. Eurbee 8th, Ghent, “Where have all these wild bees gone, long time passing?”. Poster

Jacquemin, F., Violle, C., Coppée, T., Folschweiller, M., Drossart, M., Rasmont P. & Dufrêne, M. (2018). Spatio-temporal floral resource shifts in Belgium. Eurbee 8th, Ghent, “Where have all these wild bees gone, long time passing?”. Poster

Maebe, K., D’Haeseleer J., Teepe A., Ronse A., Smeets P., Meeus I. & Smagghe G. (2018) Genetic analysis on recently found *B. veteranus* specimens in Belgium, does the supposed extinct species returned or just never left? Eurbee 8th, Ghent, “Where have all these wild bees gone, long time passing?”. Poster

Marshall L., Biesmeijer J.C., Rasmont P., Vereecken N.J., Dvorak L., Fitzpatrick U., Francis F., Neumayer J., Ødegaard F., Paukkunen J.P.T., Reemer M., Roberts S.P.M, Straka J., Vray S. & Dendoncker N. (2018). Declining distributions for BENELUX Bumblebees: climate and land use change interactions. Oral Presentation

Martinet B., Vanderplanck M., Rasmont P., Barraud A., Renaudeau C., Carvalheiro L.G. & Michez D. (2018). Ensuring access to high quality flower resources can reduce impacts of climate change on bumblebee colony development. Eurbee 8th, Ghent, “Where have all these wild bees gone, long time passing?”. Poster

Pauly A. & Boevé J-L. (2018). An assessment of the Belgian Halictids species, with an overview of the endangered species in other countries in Europe. Poster

Rollin O., Michez D., Jacquemin F., Marshall L., Rasmont P., Dufrêne M., “A multi-stressor analysis of spatio-temporal shifts in Belgian bee community” Eurbee 8th, Ghent. Poster

Vray S., Lecocq T., Roberts S.P.M., Rollin O. & Rasmont P. (2018). May regulations against thistles threaten bumblebees? Eurbee 8th, Ghent, “Where have all these wild bees gone, long time passing?”. Poster

Zambra E., Martinet B., Michez D. & Rasmont P. (2018). Hyperthermic stress resistance of bumblebees: what about of sub-boreal Belgian species? Eurbee 8th, Ghent, “Where have all these wild bees gone, long time passing?”. Poster

Media interview

Rasmont P. & Quin E. "L'invité _ Pierre Rasmont - 28 minutes", 28 Minutes, ARTE France (2014)

De Muelenaere M. & Rasmont P. "L'avenir est incertain pour les abeilles sauvages", La Libre (2015)

Du Brulle C., Lecocq T., Rasmont P. & Michez D. "Les bourdons ont le bourdon en Belgique" (2015)

Michez D., Rasmont P. "Déclin des abeilles en Europe", RTL - Journal parlé de 19H, Mons (2015)

Rasmont P., Dehon M., Gerard M., Martinet B., Roger N., Vray S. & Zerck P-L., "Mons - Ce labo qui étudie les abeilles est une référence dans le Monde !", Télé Mons-Borinage, les Reportages, Mons (2015)

Wauthy D. & Rasmont P., "Des bourdons sur la Liste Rouge", L'Avenir du Luxembourg (2015)

Lenaerts C. & Rasmont P., "Le tilleul argenté tueur d'insectes" RTBF info (2016)

Rasmont P., Interview for the Belgian radio RTBF “Les bourdons et les abeilles sauvages” given by Fabienne Vande Meerssche (20/05/2017).

Rasmont P., Dussart F. & Legrand C., « Hiver bien froid ? moins d'insectes en été ! », RTBF.BE, Mons (2017).

Vray S., « Pollinisation : les populations de bourdon se raréfient en Belgique », RTBF (26/03/2018). https://www.rtbf.be/info/societe/detail_pollinisation-les-populations-de-bourdons-se-rarefient-en-belgique?id=9877098

Vray S., "Le déclin des bourdons" RTBF, TV news 13h and 19h30 (31/03/2018).

Vray S., " Le bourdon en voie de disparition ", Télé Mons-Borinage, TV news, Mons (31/03/2018).

Vray S., Bel RTL, Matinale (29/03/2018).

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Vray S., "Pollinisation : les populations de bourdons se raréfient en Belgique" , rtbf.be (26/03/2018).

Vray S., "Le grand silence des bourdons", Le Soir (newspaper 27/03/2018).

Vray S., "Les populations de bourdons ont fortement décliné en Belgique ces 100 dernières années", SillonBelge.be (26/03/2018).

Vray S., "Hommelpopulatie gedecimeerd; wetenschappers eisen dat België prioriteit maakt van biodiversiteit", DeMorgen (26/03/2018).

Vray S., "Aantal hommels gedecimeerd: “Er is onmiddellijke actie nodig!”, HLN (26/03/2018).

Vray S., "Ca bourdonne à l'UMONS), Daily Science (05/06/2018) http://dailyscience.be/05/06/2018/ca-bourdonne-alumons/?fbclid=IwAR1xzxrIvDJrbYZmW8gX4Z9bvkJyp5DpzFGDk2xfDRipZldTVATWCeHplUY

Vray S., « Faut-il encore arracher les chardons ? », l’Avenir (14/09/2018).

Vray S., « Faut-il encore arracher les chardons ? », l’Avenir (14/09/2018).

Vray S., « Pourquoi les bourdons disparaissent depuis un siècle en Belgique ? », L’UMONS élément n°29 (June-Auguste 2018).

Meetings

First annual meeting of BELBEES’ consultative committee, 12/06/2014.

Second annual meeting of BELBEES’ consultative committee: “BELBEES Halfway Stakeholders Meeting”, 21/10/2015.

BELBEES day, 12/12/2015.

Third annual meeting of BELBEES’ consultative committee: “Transposing sciences results to wild bee conservation”, 30/05/2018.

5.1.1. WP6. Task 1. Publication of vade-mecum for wild bee managing

Pauly A. « Abeilles de Belgique et des Régions limitrophes, Partie 1: Famille Halictidae. (502 pp.) ». In prep.

Pauly A. « Abeilles de Belgique et des Régions limitrophes, Partie 2: Famille Megachilidae ». In prep.

5.1.2. WP6. Task 2. Publication of a vulgarisation booklet for large public

Wild bees identification tools and data gathered during the BELBEES project allowed to disseminate information about wild bees to citizen during activities and public events.

5.1.3. WP6. Task 3. Field guide to wild bees of Belgium

Gosselin M., Moerman R., Terzo M., Vereecken N. & Rasmont P. (2019) « Abeilles sauvages, bourdons et autres insectes pollinisateurs. » Collection Agrinature n°9. Service public de Wallonie, Direction générale de l'Agriculture, des Ressources naturelles et de l'Environnement. SPW Editions.

Pauly, A. « Abeilles de Belgique et des Régions limitrophes, Partie 1: Famille Halictidae. (502 pp.). » In prep.

Pauly, A. « Abeilles de Belgique et des Régions limitrophes, Partie 2: Famille Megachilidae. » In prep.

Pauly, A & Coppée, I. (2017) « Abeilles et chardons. » Bulletin de la Société royale belge d'Entomologie/Bulletin van de Koninklijke Belgische Vereniging voor Entomologie, 153 (2017): 155-159.

6. PUBLICATIONS

6.1. WP6. Task 4. Publication in peer reviewed international journals

Publications in peer review

Lecocq T., Brasero N., Martinet B., Valterova I. & Rasmont P. (2015) Highly polytypic taxon complex: interspecific and intraspecific integrative taxonomic assessment of the widespread pollinator Bombus *pascuorum* Scopoli 1763 (Hymenoptera: Apidae). *Systematic Entomology*, 40, 881–890.

Lecocq T., Rasmont P., Harpke A. & Schweiger O. (2015) Improving international traderegulation by considering intraspecific variation for invasion risk assessment of commercially traded species. *Conservation Letters*, DOI: 10.1111/conl.122.

Maebe K., Meeus I., Ganne M., De Meulemeester T., Biesmeijer K. & Smagghe G. (2015) Microsatellite analysis of museum specimens reveals historical differences in genetic diversity between declining versus stable *Bombus* species. *PLoS ONE*, 10, e0127870; 10.1371/journal.pone.0127870.

Martinet B., Lecocq T., Smet J. & Rasmont P. (2015) A Protocol to Assess Insect Resistance to Heat Waves, Applied to Bumblebees (*Bombus* Latreille, 1802). *PLOS ONE*, 10.

Pauly A., Devalez J., Sonet G. Nagy Z.T. & Boevé J.-L. (2015) DNA barcoding and male genital morphology reveal six cryptic species in the West Palearctic bee complex *Seladonia smaragdula* (Vachal, 1895) (Hymenoptera: Apoidea: Halictidae). *Zootaxa*, 4034, 257-290.

Lecocq T., Coppée A., Michez D., Brasero N., Rasplus J.-Y., Valterová I. & Rasmont P. (2016) The alien's identity: consequences of taxonomic statuses for the trade of domesticated bumblebees. *Biological Conservation*,195, 169-176.

Schleuning M, Fründ J, Schweiger O, Welk E, Albrecht J, Albrecht M, Beil M, Benadi G, Blüthgen N, Bruelheide H, Böhning-Gaese K, Dehling DM, Dormann CF, Exeler N, Farwig N, Harpke A, Hickler T, Kratochwil A, Kuhlmann MP, Kühn I, Michez D, Mudri-Stojnic S, Plein M, Rasmont P, Schwabe A, Settele J, Vujic A, Weiner CN, Wiemers M & Hof C. (2016) Ecological networks are more sensitive to plant than to animal extinction under climate change. *Nature Communications*, 7:13965, DOI: 10.1038/ncomms13965.

Schoonvaere K, De Smet L, Smagghe G, Vierstraete A, Braeckman BP & de Graaf DC (2016) Unbiased RNA Shotgun Metagenomics in Social and Solitary Wild Bees Detects Associations with Eukaryote Parasites and New Viruses. *PLoS One* 11, e0168456.

De Smet L., Hatjina F., Ioannidis P., Hamamtzoglou A., Schoonvaere K., Francis F., Meeus I., Smagghe G. & de Graaf D. (2017) Stress indicator gene expression profiles, colony dynamics and tissue development of honey bees exposed to sub-lethal doses of imidacloprid in laboratory and field experiments. *PLoS One* 12, e0171529.

Jacquemin F, Violle C, Rasmont P & Dufrêne M (2017) Mapping the dependency of crops on pollinators
in Belgium. *One Ecosystem* 2: e13738. https://doi.org/10.3897/oneeco.2.e13738

Lecocq T., Gérard M., Maebe K., Brasero N., Dehon L., Smagghe G., Valterova I., De Meulemeester T., Rasmont P. & Michez D. (2017) Chemical reproductive traits of diploid *Bombus terrestris* males : consequences on bumblebee conservation. *Journal of Insect Science*, 24, 623-630.

Lecocq T, Gérard M, Michez D & Dellicour S (2017) Conservation genetics of European bees: new insights from the continental scale. *Conservation Genetics*, 18, 585-596.

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Marshall L, Biesmeijer J.C, Rasmont P, Vereecken N.J, Dvorak L, Fitzpatrick U, Francis F, Neumayer J, Ødegaard F, Paukkunen J.P.T, Pawlikowski T, Reemer M, Roberts S.P.M, Straka J & Dendoncker N. (2017) The interplay of climate and dynamic land use land cover changes affects the distribution of EU Bumblebees. *Global Change Biology* 24, 101-116.

Moerman R, Vanderplanck M, Fournier D, Jacquemart AL & Michez D (2017) Pollen nutrients better explain bumblebee colony development than pollen diversity. *Insect Conservation and Diversity*, 10, 171-179.

Pauly A. & Coppée I. (2017) Abeilles et chardons. *Bulletin de la Société royale belge d'Entomologie*, 153 : 155-159.

Rasmont P, Devalez J, Pauly A, Michez D & Radchenko VG (2017) Addition to the checklist of IUCN European wild bees (Hymenoptera : Apoidea), *Annales de la Société entomologique de France (N.S.)*, 53, 17-32.

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

Pauly A. (2018) Les Abeilles sauvages de la lande de Streupas (Hymenoptera : Apoidea). *Belgian Journal of Entomology*, 60 : 1-36.

Pauly A. & Vereecken N. (2018) Les abeilles sauvages des pelouses calcaires de Han-sur-Lesse (Hymenoptera : Apoidea). *Belgian Journal of Entomology*, 61: 1-39.

Schoonvaere K, Smagghe G, Francis F & de Graaf DC (2018) Study of the Metatranscriptome of Eight Social and Solitary Wild Bee Species Reveals Novel Viruses and Bee Parasites. *Frontiers in Microbiology*, 9, 177. doi: 10.3389/fmicb.2018.00177

Vray S., Rollin O., Rasmont P., Dufrêne M., Michez D., & Dendoncker N. (2019) A Century of Local Changes in Bumblebee Communities and Landscape Composition in Belgium. *Journal of Insect Conservation*. Doi: 10.1007/s10841-019-00139-9

Publications of book

Rasmont P., Franzen M., Lecocq T., Harpke A., Roberts S., Biesmeijer K., Castro L., Cederberg B., Dvorak L., Fitzpatrick U., Gonseth Y., Haubruge E., Mahe G., Manino A., Michez D., Neumayer J., Ødegaard F., Paukkunen J., Pawlikowski T., Potts S., Reemer M., Settele J., Straka J. & Schweiger O. (2015) Climatic Risk and Distribution Atlas of European Bumblebees. *BioRisk*, 10, 1–236.

Publications submitted

Pauly A., Noël G., Sonet G. & Boevé J.-L. *Lasioglossum medinai* (Vachal, 1895), a pseudocryptic species allied to *Lasioglossum villosulum* (Kirby, 1802) (Hymenoptera: Apoidea: Halictidae). *European Journal of Taxonomy*. Submitted.

Vanderplanck M, Martinet B (co-first), Carvalheiro LG, Rasmont P, Barraud A, Renaudeau C & Michez D. Ensuring access to high-quality resources reduces impacts of climate change on bees. Submitted.

Maebe K, Karise R, Meeus I Mänd M & Smagghe G (2018) Patterns of population structure between Belgian and Estonian bumblebees. *Scientific Reports.* Under review.

Jacquemin F, Violle C, Munoz F, Mahy G, Rasmont P, Michez D, Vereecken NJ, Roberts SPM, Vray S, Dufrêne M. Shift in bee-plant interactions: decrease of specialization in present vs. past networks. Submitted.

Vray S, Rollin O., Dendoncker N, Michez D, Dufrêne M, Roberts SPM & Rasmont P. Trends of bumblebee (*Bombus*) populations in Belgium along the last century: drastic shift in community composition correlated to species ecological traits. Submitted.

Publications in prep.

Beckers., V, Marshall. L., Rasmont, P. Van Rompaey, A & Denconcker, N. Increased Thematic Resolution of Land Use Change Models for Biodiversity Scenarios: Case study Belgium Bumblebees. In prep.

Drossart M., Vanormelingen P., D'Haeseleer J., Zambra E., Dufrêne M., Pauly A., Vereecken N. J., Vray S., Rasmont P., Michez D. Belgian Red List of bees. Mons : Presse universitaire de l'université de Mons.

Maebe K, Karise R, Vray S, Rasmont P, Mänd M, Meeus I & Smagghe G. Level of genetic diversity in European bumblebees is not determined by species abundance. *Frontiers in Genetics.* In prep.

Rasmont P, Ghisbain G, Terzo M. Les bourdons d'Europe et des contrées limitrophes, éd. NAP, Paris. In prep.

Rollin O., Jacquemin F., Marshall L., Michez D., Rasmont P. & Dufrêne M. Spatio-temporal shift in Belgian bee community : A multi-stressor analysis on one century monitoring. In prep.

Schoonvaere K, Brunain M, Baeke F, De Bruyne M, De Rycke R, de Graaf DC Comparison between Apicystis cryptica sp. n. and Apicystis bombi (Arthrogregarida, Apicomplexa): gregarine parasites that cause fat body hypertrophism in bees. In prep.

Schoonvaere K, Serteyn L, Sterk G, De Smet L, Rasmont P, Francis F, de Graaf DC. Dual-omics study investigating the effects of field relevant chronic exposure to agrochemicals in bumble bees. In prep.

Vray S, Marshall L, Michez D, Rasmont P & Dendoncker N Bumblebee species distribution modelling based on land use and climate in Belgium along the last century. In prep.

PhD thesis

Vray S. (2018). Cent ans de déclin des bourdons en Belgique : influence du climat et de l'occupation du sol. Thèse de doctorat. UMONS and UNamur, Mons and Namur, 490pp. ISBN : 978-2-9602170-0-1.

Marshall L. (2018). Wild bee biodiversity patterns across time and space: the role of land use and climate drivers. UNamur, Namur.

Jacquemin F. Influence des dynamiques des ressources florales sur les abeilles sauvages (Hymenoptera, Apoidea) en Belgique. ULg (Gembloux Agro-Bio Tech – Axe Biodiversité et Paysages) and Center of Functional and Evolutive Ecology (CNRS) in Montpellier (France). This PhD thesis is founded by Belspo and Interreg.

Schoonvaere K. Pathogen and pesticide exposure in the decline of wild bees in Belgium. Joint PhD at UGent (Ghent) and ULiège (Gembloux). In prep.

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Marlière, F. (2016) Etude spatio-temporelle d'une communauté d'apoïde en région ardennaise Le site Natura 2000 de la Fagne de Malchamps. UMONS.

Buchet, H. (2017) Etude des communautés d'apoïdes de la Montagne-Saint-Pierre, Liège, Belgique. UMONS.

Zambra, E. (2017) Le Syndrôme de stress hyperthermique chez les bourdons (Hymenoptera : Apidae) sub-boréaux de Belgique. UMONS.

7. ACKNOWLEDGEMENTS

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Philippe Thiry (GAWI), Didier Van Den Spiegel (Musée Royal d'Afrique centrale), Etienne Vandeghinste (SPW), Wouter Vanlanduyt (INBO), Pieter Verdonckt (INAGRO), Nicolas Vereecken (ULB), Felix Wäckers (BioBest NV), Marjoleine Walewijns (LNE), Sabine Wallens (SPF), Tom Wenseleers (KUL) and Filip Wouters (KONVIB).

Appendix – List of Stakeholders

Sector	Institution	Name	First name	Function/topic	e-mail
International					
Beekeeping association	Bee-life	Simon	Noa	Représentante	
Scientists	GBIF	Heughebaert	André	Biodiversity platform manager	a.heughebaert@biodiversity.be
National					
Public	BELSPO	Jamart	Georges		Georges.JAMART@belspo.be
	Museum RBINS	Peeters	Marc	Leader of national Bee WG;CBD national focal point	Marc.Peeters@natuurwetenschappen.be
	RBINS	Boevé	Jean-Luc	Scientific researcher / insect chemical-ecology	Jean-Luc.Boeve@sciencesnaturelles.be
	Musée Royal d'Afrique Centrale	Van Den Spiegel	Didier		didier.van.den.spiegel@africamuseum.be
	SPF Santé publiq,chaîne alim,environment	Kempenaer	Salima	National bee plan	Salima.Kempenaer@environnement.belgique.be
	SPF Santé publiq,chaîne alim,environment	Wallens	Sabine	Scientist senior biodiversity	Sabine.Wallens@environnement.belgique.be
Association	Société Royale Belge d'Entomologie	Coppée	Isabelle	Coordinator	isabelle.coppee@sciencesnaturelles.be
Farmers'union	UNAB	Depas	Gisèle	Representative	gisele.depas@gmail.com
Regional - Flanders					
Public	LNE	Walewijns	Marjoleine	Research on bee related stakeholders in Flanders	marjoleine.walewijns@lne.vlaanderen.be
	ILVO	Reubens	Bert		bert.vangils@ilvo.vlaanderen.be
	LV	Lamont	Jean-Luc		jean-luc.lamont@lv.vlaanderen.be
	INBO	De Bruyn	Luc		luc.debruyn@inbo.be
	INBO	Vanlanduyt	Wouter		wouter.vanlanduyt@inbo.be
	INBO	Brosens	Dimitri		dimitri.brosens@inbo.be
	INBO	Pollet	Marc		marc.pollet@inbo.be
	INBO	Maes	Dirk		dirk.maes@inbo.be
	FOD VVVL	Kollmorgen	Nadine		nadine.kollmorgen@health.fgov.be
Beekeeping association	KONVIB	Wouters	Filip	Lector&researcher High School Vives:ani.nutrition&entomo.	filip.wouters@vives.be
Agri.& horti. association	INAGRO	Verdonckt	Pieter		pieter.verdonckt@inagro.be
Farmers'union	Boerenbond	Cools	Karolien	In charge of bees	
	Boerenbond	Penninckx	Iris		
	VLM	Michiel	Karolien		karolien.michiel@vlm.be
Environmental association	Natuurpunt	Lambrechts	Jorg	Head of studies dpmt	jorg.lambrechts@natuurpunt.be
	Natuurpunt	D'Haeseleer	Jens	In charge of wild bees projects	
	Natuurpunt>Aculea (bijen groep)	Derycke	Samuel		sdry9@hotmail.com

	Velt	Rottiers	Lies		lies.rottiers@velt.be
University	UGent	de Graaf	Dirk	Prof. physiology dpmt	Dirk.deGraaf@UGent.be
	UGent	Smagghe	Guy	Prof. crops protection dpmt	guy.smagghe@UGent.be
	KUL	Wenseleers	Tom	Ecology, Evolution and Biodiversity Conservation	tom.wenseleers@bio.kuleuven.be
Private/economic	BioBest NV	De Jonghe	Roland	ex PDG	roland.de.jonghe@telenet.be
	BioBest NV	Wäckers	Felix	Manager R&D; prof. Lancaster Environment Center	felix.wackers@biobest.be
	IPM Impact	Sterk	Guido	Manager	guido.sterk@skynet.be
		Regional - Wallonia			
Public	SPW>DGARNE>DEMNA	Barbier	Yvan	DFF, biodiversity	yvan.barbier@spw.wallonie.be
	SPW>DGARNE>RCE	Mulders	Christian	Agriculture-environment	christian.mulders@spw.wallonie.be
	SPW>DGARNE>DPE	Vandeghinste	Etienne	National bee WG	etienne.vandeghinste@spw.wallonie.be
	Plan Maya	Stas	Arnaud	Head of dpmt	arnaud.stas@spw.wallonie.be
	Plan Maya	Saad	Layla	Plan Maya	Layla.SAAD@spw.wallonie.be
	CRAW	Hautier	Louis	Ecotoxicology, beekeeping, crops protection, agroecology	l.hautier@cra.wallonie.be
Beekeeping association	CARI	Bruneau	Etienne	Managing director;chairman of COPA COGECA honey WG;chairman of Apimondia technology&quality commission	bruneau@cari.be
Farmers'union	FWA	Marot	Jean	Studies dpmt > phyto	jean.marot@fwa.be
	FWA	Decock	Bernard		
	FUGEA	Gosselin	Matthias	CAP, entomologist	mg@fugea.be
Environmental association	Natagora	Paquet	Jean-Yves	Studies dpmt manager	
	IEW	Delvaux	Lionel	Coordinator: rurality, biodiversity	l.delvaux@iew.be
	Nature et Progrès	Fischer	Marc	General secretary	Marc.Fichers@natpro.be
Horticultural assocation	GAWI	Thiry	Philippe	Technician: fruit cultivation (FWH)	gawi.thiry@asblgawi.com
	CIM	Maréchal	Jean	Manager	jean.marechal@legumeswallons.be
University	ULg (Gblx agro-bio tech)	Dufrêne	Marc	Prof. biodiv and landscapes unit	
	UMONS	Rasmont	Pierre	Prof. and manager of zoology labo	
	UNamur	Dendoncker	Nicolas	Prof. geography dpmt	
	UCL	Jacquemart	Anne-Laure	Prof. Earth and Life institute	anne-laure.jacquemart@uclouvain.be
		Regional - Bruxelles			
Public	IBGE-BIM	Beck	Olivier	Biodiversity-monitoring	
	IBGE-BIM	Durieux	Jérôme	Beekeeping strategy	jdurieux@environnement.irisnet.be
University	ULB	Vereecken	Nicolas	Wild bees specialist	nvereeck@ulb.ac.be
Private/economic	ChemCom	Patiny	Sébastien	Senior scientist	patiny.s@gmail.com

ANNEXES

Publications relevant to the BELBEES topics conducted by the BELBEES partners but not founded by the BELBEES project

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