École Doctorale n° 104 : Sciences de la Matière, du Rayonnement et de l'Environnement

Thèse de Doctorat

pour obtenir le grade de docteur délivré par

Université de Lille

Discipline "**Biologie de l'environnement, des organismes, des populations, ecologie**"

ECOLOGIE DES PLANTES ET DES POLLINISATEURS DANS LES PRAIRIES CALCAIRES LE LONG D'UN GRADIENT LATITUDINAL EN FRANCE : DIVERSITE DES ESPECES ET STRUCTURE DES RESEAUX D'INTERACTION PLANTES-POLLINISATEURS

Présentée et soutenue publiquement par

Natasha DE MANINCOR

le 25 Juin 2019

Jury

Isabelle DAJOZ, Université Sorbonne - iEES, Paris	Rapportrice
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Laboratoire Évolution, Écologie et Paléontologie (EEP)

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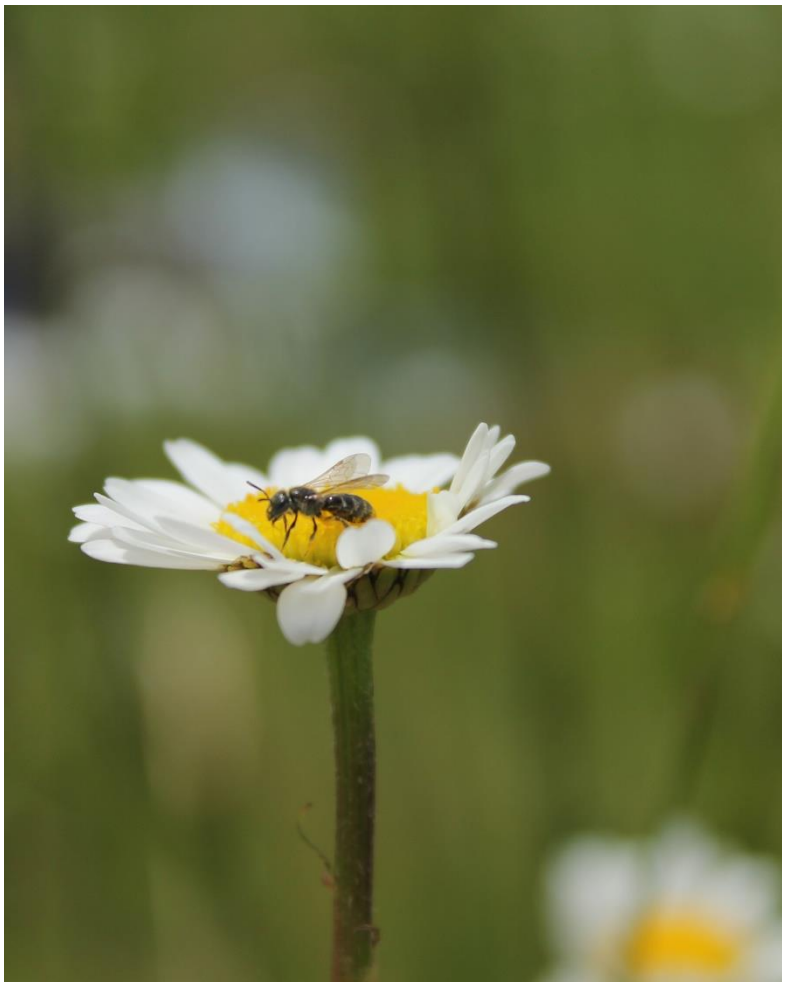
Ecology of plants and pollinators in calcareous grasslands along a latitudinal gradient in France: species diversity and the structure of plant-pollinator interaction networks

Natasha de Manincor



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"L'ape lavora sempre per il bene comune, e non può essere comprata o corrotta più di quanto non lo possa essere un fiore"

[C. Preston, "Le Api"]

Per Ida.

Contents

Introduction

Background: plant-pollinator interactions and the importance of pollination

Pollinators are a fundamental part of the global biodiversity and play a crucial role in the maintenance of flowering plants. Plant communities, in turn, are essential to the functioning of ecosystems and more generally of the planet and its atmosphere (Potts et al. 2010; Bilz et al. 2011). The pollination of flowering plants by animals represents an ecosystem service of great value for humanity, since pollinators are critical to the reproduction of most wild angiosperms and crops (Ollerton 2017). The global economic value of plant-pollinator interactions was estimated in 2005 at 153 billion euros (Gallai et al. 2009) and about 70% of agricultural production directly depends on pollinators (Klein et al. 2007). Without pollinators, many plants cannot reproduce and set seed; conversely, without plants to provide different rewards, many pollinating animal populations would decline, with consequent cascading effects for other species (Kearns et al. 1998). Thus, in this mutualistic interaction both partner species find a benefit: pollinators derive benefits in terms of food (pollen and nectar), mating and brood sites and the plants in terms of reproductive success.

Pollination of angiosperms by animals (vertebrate and invertebrate) has been estimated at 87.5%, with a mean of 78% in temperate-zone communities (40° to 64° latitude) to 94% in tropical communities (0° to 29° latitude) (Ollerton et al. 2011). Plants are sessile organisms, so they need to use “innovative” ways of dispersing gametes and propagules. Angiosperms invest in a suite of floral signals to attract diverse pollinator species that disperse their male gametes (pollen). The two most studied pollinator attractants are pollen and nectar, but flowers can vary in multiple features. Other types of floral signals have been developed to attract diverse and more efficient pollinator species, such as volatile compounds, floral oil, different colour phenotypes and other morphological traits such as floral size and floral display (Junker and Parachnowitsch 2015). Most pollinators are insects; bees in particular have co-evolved since the Cretaceous with the first flowering plants and nowadays constitute one of the largest and most diversified groups of obligate flower visitors (Kearns and Inouye 1997; Kearns et al. 1998; Ollerton 2017). Pollinator-mediated selection is considered as one of the major causes of floral trait variation (Schiestl and Johnson 2013; Gervasi and Schiestl 2017; Zu and Schiestl 2017). These traits include flower shape and size, colour, odour, floral rewards and phenology, which are sometimes associated with particular pollinator groups (Rosas-Guerrero et al. 2014). Unrelated plant species can display convergence in their floral traits when they are pollinated

by the same pollinators (Fenster et al. 2004). Nevertheless, it has also be demonstrated that differences in the identity of the most efficient pollinators associated to the different plant species of the same genus can lead to divergence in floral traits among species (Reynolds et al. 2009). Thus, pollinators are at least partly responsible for floral trait evolution, with some amount of coevolved specificity, and changes in pollinator communities could have a profound impact on plant-pollinator interactions.

Species diversity in Europe: angiosperms, bees and hoverflies

Species richness within most groups of organisms decreases with latitude, with higher species richness at low (tropical) latitudes than at high (poles) latitudes (Schemske et al. 2009). Different possible mechanisms may explain the biodiversity gradient (*i.e.* competition, mutualism, predation, number of habitats, evolutionary time, solar energy, local climatic variability etc.) but, nowadays, no consensus view on the cause of the pattern seems to be emerging (Gaston 2000; Gaston and Spicer 2004).

According to this latitudinal rule, plant and pollinator abundance and richness in European countries are not equally distributed and they are expected to vary along the latitudinal gradient (Ollerton 2017). Two recent reports of the European Red List of Species (Bilz et al. 2011; Nieto et al. 2014) have shown that Southern Europe and in particular the Mediterranean countries (Iberian peninsula, Southern France, Italy and Greece) display the highest species richness in Europe of both angiosperms and bees (Fig. 1). However, these countries also show the highest deficit of data due to the insufficient amount of surveys (Fig. 2), studies and taxonomical specialists. Moreover, for some groups of invertebrates, such as hoverflies, a European Red List is not yet available, while some national and regional red lists have already been published (Radenković et al. 2017). The data deficit of these groups could then compromise the possibility (i) to assess precisely the distribution of wild bees and hoverfly diversity in the European countries and (ii) to assign the correct threat status that will allow to mitigate more adapted measure of conservation.

In France species richness also progressively decreases with latitude for both angiosperms and bees. The total number of vascular plant species in France reaches 4,630 species (Walter and Gillett 1998); the total number of wild bees is estimated around 1,000 species (Patiny et al. 2009; in Europe 1965 species, Nieto et al. 2014) and the number of hoverfly species is 540

(around 94 genera in Europe and 931 listed species in the "Syrph the Net" database 2017, Larrieu et al. 2015; Speight et al. 2017). While bee species richness seems to decrease with latitude (Patiny et al. 2009), hoverfly species richness displays no such latitudinal trend (Keil et al. 2008).

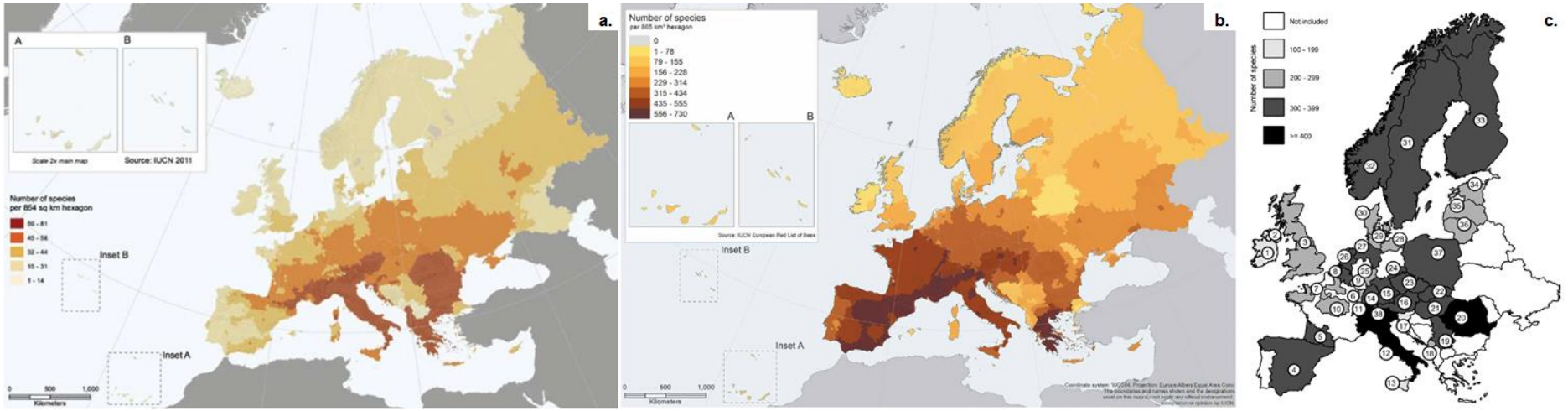

Figure 1. Maps of the species richness of European (a) vascular plants, (b) wild bees (in the European Red List of species of Bilz et al. 2011; Nieto et al. 2014) and (c) hoverfly species richness in 38 European regions used in the study of Keil et al. (2008).

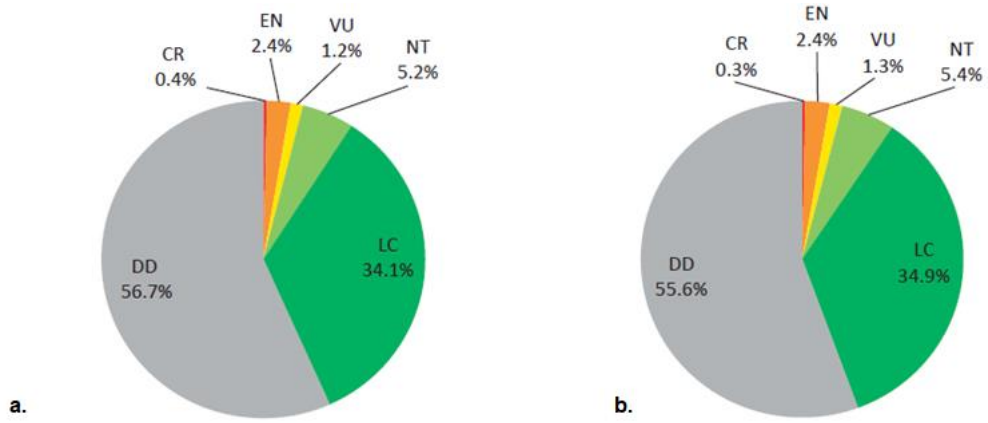

Figure 2. Red List status of bees in (a) Europe and in (b) EU27, showing the highest proportion of data deficient (DD) information (Nieto et al. 2014).

In this study we focus on two group of pollinators: (i) wild bees (Apoidea: Anthophila) and hoverflies (Diptera: Syrphidae). Wild bees and hoverflies are among the most efficient pollinators both for wild angiosperms and crops (Jauker and Wolters 2008; Albrecht et al. 2012; Garibaldi et al. 2013; Ollerton 2017; Klecka et al. 2018a). Bees have been recorded worldwide, on all continents and all habitats where flowering species are present, and hoverflies are often used as bioindicators of the habitat quality due to the broad variety of resources used during their life cycle, from larvae to adults (Keil et al. 2008; Larrieu et al. 2015).

Wild bee species are divided into six families belonging to two larger groups: (i) Apidae and Megachilidae form the group of long-tongued bees; (ii) Andrenidae, Colletidae, Halictidae and Melittidae comprise the group of short-tongued bees. Bees are known to collect different plant resources (pollen, nectar, oil, materials for nesting, etc.) with various foraging strategies. Then, they can visit a wide range of plant species; some groups are more specialised on one plant family or one genus. We can generally divide pollinator species in three groups based on their foraging behaviour: monolecty (one host plant species); oligolecty (one host plant family) and polylecty (more than one host-plant family) (Cane and Sipes 2006, Müller 1995; Müller and Kuhlmann 2008).

Hoverflies show differences in their resource preferences depending on their development status (Larrieu et al. 2015): while the larvae have a wide range of feeding habits (from herbivore to mycophages, but also deadwood decomposers and insectivores, sometimes used as biocontrol, Sommaggio 1999), adults of all the species are floricolous and pollinate plants. Indeed, adult hoverflies usually behave opportunistically, *i.e.* from being pollen generalists as well as pollen or nectar specialists, only limited by morphological constraints (Iler et al. 2013; Klecka et al. 2018a; Lucas et al. 2018). However, their generalisation could be the result of serially specialized diets, since pollen retrieved on hoverfly individuals usually comes from a single plant taxon (Lucas et al. 2018) and depends on flower availability and phenology (Cowgill et al. 1993; Colley and Luna 2000). Moreover, some hoverflies have preferences regarding plant colour, morphology and inflorescence height (Branquart and Hemptinne 2000; Colley and Luna 2000; Lunau 2014; Klecka et al. 2018b,a).

Plant-pollinator networks

Plant-pollinator networks are among the most studied types of mutualistic networks (Bascompte and Jordano 2007). In pollination networks, plant and insect species are represented as nodes and they are connected by pairwise interactions (links) with a bipartite structure, which means that interactions only exist between two distinct, non-overlapping sets of species (Fig. 3), but not within each set (*i.e.* insects interact with plants, but not insects with insects or plants with plants). Each node is characterised by its degree, *i.e.* the number of links it shares with other nodes, in other words the species generalisation level. The measure of the degree distributions corresponds to the frequency distribution of the number of links per species (Bascompte and Jordano 2013). Interactions between plants and pollinators range from being highly specialized (one or few partners) to generalized (high number of links), and the degree of specialization seems to depend on the latitude, even if previous studies have led to contrasting outcomes on this subject (Dalsgaard et al. 2011a; Schleuning et al. 2012; Pauw and Stanway 2015). However, plant species with generalist interactions, in which one species interact with multiple mutualistic partners, occur more often than specialised interactions at higher latitudes than in lower ones (Waser et al. 1996; Olesen and Jordano 2002).

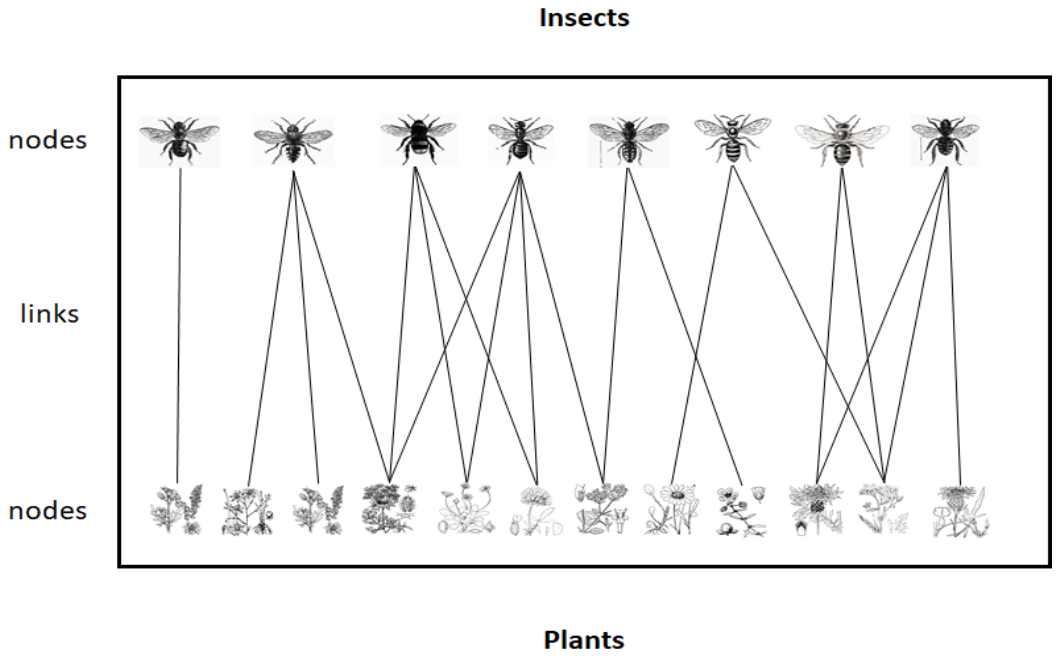

Figure 3. Example of plant-pollinator networks. Insect and plant species are represented as nodes and the interaction between the as a link.

Networks can generally be represented as an interaction matrix, whose dimensions are A × P, where A is the number of animal species and P of plant. The product A × P is the maximum number of possible interactions. The proportion of actually observed links to all possible links in the web is its "connectance" C = L/(AP), where L is the number of realized links. This index is a measure of specialization at the community level, considering only "binary interactions" (*i.e.* 0 or 1, presence or absence of the interaction, Blüthgen et al. 2006). When information on the intensity and frequency of interactions among nodes is included, such networks are called weighted or valued. In weighted networks, we can also measure a specialisation index at the species level (*d'*), which is a measure of "partner diversity" based on the Kullback-Leibler divergence between the realized and expected weights of interactions (see details in Blüthgen et al. 2006), and at the community level (H_2), which calculates the Shannon diversity of links (*i.e.* the interaction diversity).

Describe the network structure is important to understand the ecological and evolutionary dynamics of multispecies assemblages. Ecological networks are generally known to display two assembly patterns: (i) a nested architecture (nestedness) and (ii) a modular structure (modularity). Nested networks show a hierarchical structure where interactions of specialist species are included in those of generalist species. A nested structure reduce the competition increasing the number of coexisting species, thus influence the network robustness to random extinction (Bascompte et al. 2003; Tylianakis et al. 2010). Modular networks are characterized by the existence of densely subsets of species, called modules, in which species preferentially interact within modules than among them (Olesen et al. 2007). Finding modules which maximize some measure of link density within modules can help make sense of network structure (Olesen et al. 2007; Dupont and Olesen 2009, 2012). Modularity and modules can provide information about habitat heterogeneity, divergent selection regimes, phenological mismatch and phylogenetic clustering of closely related species, highlighting non-random patterns of interaction (Bascompte et al. 2003; Olesen et al. 2007). Individual modules can differ in size and link density because of variation in species number (sum of pollinator and plant species) and might be dominated by a few species, taxonomically or functionally delimited. These functional patterns provide information regarding the organization of interactions within ecological communities. Fortuna et al. (2010a) evidenced a significant correlation between nestedness and modularity in plant-pollination networks, but not in seed-disperser or host-parasite networks. They found that, in communities with low connectance, nestedness is positively correlated with modularity, and the opposite pattern is found in communities with high connectance (*i.e.* higher nestedness corresponds to lower modularity). Moreover, both modularity and nestedness influence network resilience in theoretical models of ecosystem stability (Thébault and Fontaine 2010).

Many other indices have been proposed to better describe the ecologically and evolutionary aspects of mutualistic networks (Dormann et al. 2009), but most of them (including nestedness and modularity) are influenced by network dimensions and could be mere reflections of sampling effort (Blüthgen et al. 2008; Staniczenko et al. 2013).

A fundamental challenge of ecological network analysis is to understand the functioning of communities and the variation of network dynamics in space and time. In this thesis, I will mostly refer to "space" as the latitudinal gradient and to "time" as seasons within a single year, based on the phenological differences between species. In plant-pollinator networks, species need to be present in the same site at the same moment along the season, thus the flowering of plants and the foraging of pollinators have to coincide in space and time for interactions to occur (Olesen et al. 2011). Plant and pollinator species could display different phenologies along the season: some species can occur early in the season, some later, and species can display longer or shorter phenologies. We refer to "phenology overlap" to the amount of time measuring when the phenophases of a given plant species and a given insect species coincide. Interannual differences in phenophase can have profound impact on network structure by modifying the duration of phenology overlap between mutualistic partners and, in turn, the probability for these partners to interact (CaraDonna et al. 2017). Longer phenology overlap between plant and insect species might increase their probability of interaction (Olesen et al. 2011).

Ecological interaction networks are properties of species communities in a given area and at a given time period and thus can vary stochastically or in response to environmental changes (Tylianakis and Morris 2017). Moreover, our perception of networks is influenced by sampling effort and it is difficult to know exactly how complete the data are (Dormann et al. 2017). Species interaction networks are generally based on temporally aggregated data (Fig. 4), despite the fact that networks are not static over time. Quantifying the within-season turnover, *i.e.* to which extent networks change over time, has important implications for our understanding of species interactions. Following this rationale, one hypothesis is that interactions occurring at one specific moment are at least partly determined by phenological overlap (CaraDonna et al. 2017). Even if some links are more predictable and easier to observe, some links between species can remain unobserved while some of them are truly forbidden, *e.g.* when there is a complete spatial or temporal mismatch between two potential partners, which

occurs when their phenophases and locations do not overlap at all (Olesen et al. 2011).

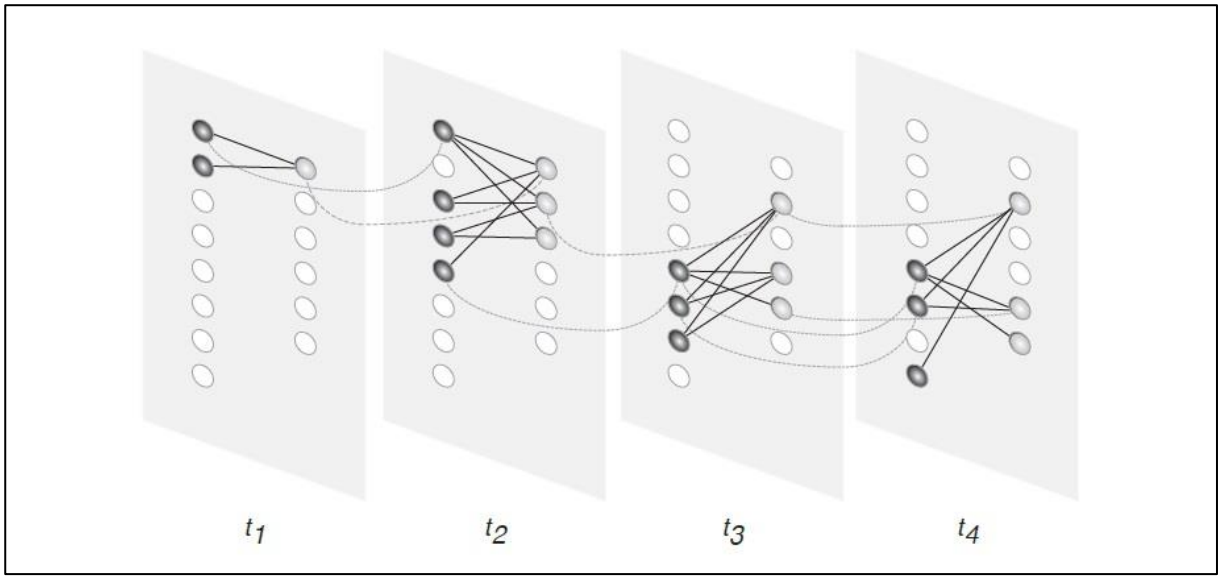

Figure 4. Example of a temporal sequence of interactions in a bipartite network. The complex network is an aggregate of distinct temporal slices. Different species that persist for variable time lengths (phenophases) are indicated as dashed lines joining nodes between successive time slices (Bascompte and Jordano 2013).

The seasonal dynamics of complex networks are the result of matching patterns of phenophases of the animals and the plants. The differences in plant and insect phenophases can influence the probability and the frequency of interaction between them, but we do not know to what extent this can affect network structure. The duration of the phenological overlap and the seasonality are crucial factors to influence network vulnerability and resilience to phenological changes. Even if the interaction is not related to a particular season (each partner is present at an appropriate phenophase year-round and so the interaction can occur any time), the cost and the benefits of the mutualistic interaction could change with shifts in partner phenologies (Rafferty et al. 2015). Further on the topic of temporal variability, there is still little information as to how differences between years (inter-annual variation) can affect the probability and the frequency of interactions, the stability of network structure and the species roles (Norfolk et al. 2015; Chacoff et al. 2017; Cirtwill et al. 2018). Interactions between plants and pollinators are also influenced by variation in species richness and the landscape context, and the number and the type of interactions in pollination webs are strongly affected by the composition of plant communities (Norfolk et al. 2015) and the type of habitat.

The architecture of mutualistic networks can have profound implications for network robustness, which is defined as network resistance to species loss (Bascompte and Jordano 2007). Past studies have shown that different types of networks respond to species loss in different ways. Mutualistic networks, such as pollination networks, are highly dependent on the degree distribution. If species

extinction starts from the most generalist plant species, network collapse can occur quite fast. Conversely, if species extinctions start from the most specialized plant species, the same network will display very few changes. Moreover, the robustness of plant-pollinator networks depends on which plant traits or assemblage of traits influence the probability of extinction of the plant species (Astegiano et al. 2015a,b).

Due to their complexity and variation among years (Chacoff et al. 2017), most studies of mutualistic networks have focused on predicting and comparing classic network metrics (nestedness, connectance, modularity, etc.). However, these metrics are all influenced by network size, *i.e.* the number of plant and insect species, which compromise or limit the temporal and spatial comparison of networks with different sizes (Blüthgen et al. 2008; Fortuna et al. 2010b; Staniczenko et al. 2013; Poisot and Gravel 2014; Astegiano et al. 2015b). Moreover, few studies have compared interaction networks along environmental gradients (Devoto et al. 2005; Schleuning et al. 2012; Sebastián-González et al. 2015; Pellissier et al. 2017). Thus, new methodological approaches are needed to better explore the ecological processes influencing the network structure and determining species interactions (Bartomeus et al. 2016). In order to compare networks of different sizes, a better alternative is to switch from network-derived metrics to the comparison of output of regression models, which can consider multiple factors and latent variables and assume that the sampled data are just part of a larger unobserved dataset (Grace et al. 2010). Thus, new modelling approaches such as Structural Equation Models (see Chapter II for further details) or Redundancy analyses on the result of random-dot product graph decomposition (RDPG-RDA approach based on Dalla Riva and Stouffer 2015 and formalized by Joffard et al. 2019, see Appendix 2 Adrien Berquer internship) and Exponential Random-Graph Model (ERGM, e.g. Labeyrie et al. 2016), could be new useful tools to further explore network structures and to make comparisons between them while avoiding circularity.

Effects of global changes on plant-pollinator interactions and pollinator decline

Because of the functional importance of biotic interactions for biodiversity and ecosystem functioning, recent evidence of pollinator decline due to global changes, especially for insects such as hoverflies, wild bees and bumblebees (Marshall et al. 2018; Powney et al. 2019), is a pressing concern worldwide. Indeed, recent biodiversity decline is a globally reported phenomenon and could lead to catastrophic disruptions of plant-pollinator interactions and associated ecosystem services (Biesmeijer et al. 2006; Potts et al. 2010; Burkle et al. 2013; Powney et al. 2019). A small community

of pollinators is often not enough to provide the pollination service (Winfree et al. 2018), and different studies showed that pollination carried out only by honeybees greatly reduces the reproductive success of cultivated plants compared to pollination by wild bee communities (Albrecht et al. 2012; Garibaldi et al. 2013). Moreover, in natural habitats with richer plant and pollinator communities such as calcareous grasslands, honeybees exploit only one third of all the flowering plants visited by wild bees which also have larger foraging distances (Steffan-Dewenter and Tscharntke 2000). Global warming can affect mutualistic interactions in different ways among years: (i) altering the timing of life-history events, displacing species distributions or increasing species extinction rates (Parmesan 2006; CaraDonna et al. 2014; Rafferty et al. 2015; Rafferty 2017), (ii) disrupting the matching of functional traits between partners, *e.g.* the match between flowers with deep corolla tube and longue-tongue pollinators; (Miller-Struttmann et al. 2015), and (iii) leading to fitness losses in pollinators and plants (*e.g.* due to the desynchronization between solitary bees and host plants, Schenk et al. 2018).

One of the most visible effects of climate change on species features is phenological shift, *i.e.* changes in the active season of pollinators, and budding, flowering and fruiting periods in plants (Rafferty 2017). Phenological shifts are expected to have large effects on the functioning of interaction networks, such as mutualistic networks and food webs in seasonal terrestrial systems, and in particular plant-pollinator networks, because they rely on the precise timing of species activity (Rafferty et al. 2015). More generally, climate change can disrupt the structure of interaction networks through interaction mismatches, since the absence of one partner species at the location and/or time at which the interaction should have taken place will prevent it from occurring (Willmer 2012; Miller-Struttmann et al. 2015). Phenological mismatch can have different effects depending on the type of community (alpine, Mediterranean, tropical, etc.; Petanidou et al. 2008; CaraDonna et al. 2014), the lifespan and the life cycle of organisms, as well as their degree of specialization (Joffard et al. 2019). For example, in subalpine plant-pollinator networks, a temporal mismatch can have a large impact on the pollination service and thus might reshape the plant community, with modifications of species abundances (CaraDonna et al. 2014) or population dynamics (Kudo 2014). Indeed, synchrony between insect emergence and the flowering period of their host plant is crucial to ensure pollination, especially for "specialist plants" (Hutchings et al. 2018). Moreover, in multi-layered ecological networks, which consider more than one functional group (*e.g.* plant-pollinator-herbivore communities, Pilosof et al. 2017), phenological asynchrony has the potential to cause cascading effects throughout the layers of the network (Koh et al. 2004) and the consequences might be different if the phenological mismatch mainly affects one group or the other (Fabina et al. 2010; Pocock et al. 2012).

A variety of approaches, including observational studies (Hutchings et al. 2018), experimental manipulations (Bartomeus et al. 2013; Petanidou et al. 2014; Rafferty et al. 2015; Schenk et al. 2018) and theoretical approaches (Memmott et al. 2007a; Fabina et al. 2010; Bartomeus et al. 2011), have been developed to explore the impact of climate change on species phenologies and the indirect consequences of phenological shifts on species interactions in plant-pollinator networks. Different studies have pointed out that plant and insect species are not all equally affected by changes in environmental cues, in turn translated into species-specific phenological shift. In general, species which occur earlier in the season are more affected by changes in temperature (Fitter and Fitter 2002; Bartomeus et al. 2011; CaraDonna et al. 2014; Petanidou et al. 2014; Rafferty 2017) and plant species tend to modify their flowering period in response to such changes (Kudo 2014). These shifts in plant phenology sometimes occur at faster (Iler et al. 2013) or slower rates than their pollinators' in response to the same abiotic changes, with a risk of disruption of this interaction in both cases (Balfour et al. 2018; Hutchings et al. 2018). Pollinators have an important pressure to adapt to this shift either through interaction with other plant species or through changes of their own phenology. Some studies have shown that pollinators are capable of phenotypic plasticity in the face of thermal changes during their development (such as extending the prepupal diapause in the genus *Osmia*, Sgolastra et al. 2012). However, rapid changes can differentially affect species with different phenologies (Schenk et al. 2018). Phenological shifts can also drastically alter pollinator fitness through modified temporal overlap between pollinators and their floral resources which can, in extreme cases, lead to local extinctions (Memmott et al. 2007b). Nevertheless, high pollinator richness might ensure plant pollination over time due to the phenological complementarity among pollinator species and thus help maintain synchrony (Bartomeus et al. 2013), except in case of very specific interactions (Hutchings et al. 2018). In any case, these responses depend on the type of community (CaraDonna et al. 2014; Petanidou et al. 2014), on the level of network connection (Encinas-Viso et al. 2012) and on the level of specialization (Koh et al. 2004; Schleuning et al. 2016).

Another factor that has been demonstrated to cause global biodiversity decline is land-use changes (Murphy and Romanuk 2014). Several studies have shown that land degradation, habitat loss and fragmentation have a negative effect on the richness, abundance, biomass, diversity, composition and population size of plants and insects and, thus, on their interaction networks (Evans et al. 2013; Spiesman and Inouye 2013; Astegiano et al. 2015a; Hallmann et al. 2017; Kaiser-Bunbury et al. 2017). Theoretical studies suggest that network asymmetry and redundancy might ensure network persistence in the face of habitat loss (Fortuna and Bascompte 2006; Bascompte and Jordano 2007), but empirical studies have also shown that habitat loss directly leads to species loss and the reorganization of plant-pollinator interactions (Spiesman and Inouye 2013). However, some semi-

natural or restored habitats (through the removal of exotic species or the reintroduction of native ones) might ensure network resilience and its integrity, ensure plant-pollinator interactions through shifts in interactions (Evans et al. 2013; Kaiser-Bunbury et al. 2017).

Finally, habitat loss and temporal (*i.e.* phenological) mismatches, do not occur independently and, combined with other factors (invasive alien species, pesticides and parasites), could act synergistically and drive pollination decline (Hegland et al. 2009; Potts et al. 2010; Goulson et al. 2015). Because foraging pollinators are highly sensitive to the composition and the abundance of flowering communities, and likewise plants are highly dependent on pollinator richness, the response of plants to environmental changes can cause cascading effects leading to the disruption of mutualisms, and specialist interactions are likely to be the most affected (Biesmeijer et al. 2006). Thus, it is important to consider both temporal and spatial variation in plant-pollinator interaction networks to better understand how these networks can respond to increasing global changes and still ensure much needed ecosystem services. In particular, studies of pollination networks along different natural gradients, considering both abiotic and biotic factors, may provide critical insights as to how climate changes affect plant-pollinator communities in order to ensure adequate conservation measures.

Objectives

The general aim of this project is to understand the consequences of spatial heterogeneity, eco-evolutionary processes and dispersal on species interactions, using an integrative approach which combines fieldwork observations and sampling, greenhouse experiments and the development of sophisticated statistical analyses. With this PhD project, I aim to understand, and to improve the ability to predict, the effects of environmental changes on ecological communities by studying and characterizing plant-pollinator associations along an environmental gradient in France, which corresponds to different natural variations in biodiversity and complexity of the interaction networks.

My objective with this work is to answer the following questions:

How do plant-pollinator communities vary in space and time and can this variation be linked to environmental gradients?
How do environmental changes affect the structure of plant-pollinator networks?
How biased is our perception of plant-pollinator networks through direct field observation and can we improve on such techniques?
Is there variation among plant traits associated with variation in pollinator communities and, if so, is this variation adaptive?

In order to answer these questions, this thesis is composed of four chapters dealing successively with (i) the characterization and diversity of plant-pollinator communities in calcareous grasslands, (ii) the analysis and comparison of interaction networks along a latitudinal gradient, (iii) the comparison of networks obtained by direct observation and via pollen analysis, and finally (iv) the spatial variability of floral scents in four focal species. Here is a slightly more detailed summary of what each and every thesis chapter contains:

My first objective was to study and compare the taxonomical diversity variation at various spatial and temporal scales, along environmental latitudinal gradient, between and within regions, and along the flowering season (**Chapter I**). I provide and analyse a new database made of geo-localized data characterizing plant-pollinator associations at the species level, spatial variation in community structure and trait assemblage, focusing on the case of plant-pollinator networks in six different calcareous grasslands in France.
My second objective was to analyse and compare plant-pollinator networks to study the consequences of environmental gradients on plant-pollinator interaction probability. To avoid the problem of comparing networks with different sizes using classic network metrics which are sensitive to differences in network dimensions, I used a new methodological approach based on Bayesian

Structural Equation Models (SEM, **Chapter II**). This methodology allows us to consider latent variables which are unknown *a priori* but can be estimated *a posteriori*, which can help avoid circularity (*e.g.* regressing probability of interactions on species degrees). I used the information of insect and plant abundances and phenologies to assess whether the effect of phenology overlap on the probability and intensity of interactions was similar in all networks. I then compared 16 models to understand which effects were more likely to play a role in the structuring of the networks.

Plant-pollinator "visit-based networks", *i.e.* constructed using the information recorded in the field based on the observed interaction between a foraging insect and a flowering plant, are often a subsample of all possible interactions. Some potential links often remain unobserved in the field while some links are truly forbidden. I used the pollen found on insect bodies, which is a natural marker of the recent history of pollinator interactions, to build a more complete interaction network ("pollen-based network"). My third objective (**Chapter III**) was then to understand how distorted is our vision of plant-pollinator networks sampled following classic methods, so I compared the "visit-based networks" to the "pollen-based networks", focusing on changes in the network structure and insect roles within the network.

Beside pollen and nectar, other non-visual cues, such as volatile organic compounds (VOCs), play an important role in pollinator attraction at long distances. Thus, like other floral traits, the composition of floral scents is likely to be under pollinator-mediated selection. Geographical variation of floral scents and among-population differences are often interpreted as the result of local adaptation to pollinators. However, little is known about the mechanisms behind such variation and few studies have attempted to discriminate between genetic adaptation and phenotypic plasticity. My fourth objective was to study the mechanisms behind geographical variation of floral scents and among-population differences, and to verify if such variation is linked to variation in pollinator community (**Chapter IV**). I performed floral scent extraction in both natural (field) and controlled (greenhouse) environments to compare the floral bouquets, in term of quality (*i.e.* VOC identity) and quantity (often analysed as VOC proportions), in four annual species with generalist pollination, that were included in our surveyed plant communities, which were largely distributed along the studied environmental gradient.

General material and methods

Most studies of plant-pollinator interactions focus on a single species or a single community and often are limited in time, focusing on the richest months of the years to collect the highest amount of diversity (Hegland and Boeke 2006; Kantsa et al. 2018). Large datasets that account for the entire flowering season or multiple years are quite scarce (Basilio et al. 2006; Petanidou et al. 2008; Dupont and Olesen 2009; Cirtwill et al. 2018), and often studies that compare different pollination networks result from the compilation of multiple empirical studies performed in different countries following different protocols (Dalsgaard et al. 2011b; Bartomeus 2013; Carvalheiro et al. 2014; Schleuning et al. 2014). To cope with the lack of data, theoretical and statistical models have also been implemented to generate different possible scenarios to understand the effects of ecological and evolutionary dynamics on the current structure of interaction networks or to gauge how ecosystems can deal with global changes and pollinator loss (Memmott et al. 2007a; Stang et al. 2007; Bartomeus 2013; Poisot et al. 2015; Goldstein and Zych 2016; Schleuning et al. 2016; CaraDonna et al. 2017). One of the aims of this project was to provide a new geo-localized database characterizing plant-pollinator associations and the spatial variation in community structure and trait assemblage. This database contains the abundances of the studied plant species at the different locations and estimates of the abundances of the different pollinator species. The surveys took place in the same habitat but in different locations distributed along a latitudinal gradient of *ca*. 1000 km in France. This environmental gradient will be used to simulate the effects of global changes based on space-for-time substitution, and accounting for natural variation in species niche due to the network context and to their co-evolutionary history.

In the following sections, I present the material and methods used to generate the data presented in this thesis. I will present (1) the fieldwork protocol used to record plant-pollinator associations; (2) the palynological analyses; (3) the analysis of floral scents of four focal plant species (in the field and at the greenhouse). Furthers details on protocols and analyses are presented in the next chapters.

Fieldwork seasons (2016 and 2017)

Study sites

Our target sampling fields were chosen among calcareous grasslands. Calcareous grasslands are present in almost all European countries, and in France thy represent 15-20% of the total area covered by the European Natura 2000 network (Fig. 5). They are characterized by highly diverse plant communities with a high proportion of entomophilous species compared to other types of habitats (WallisDeVries et al. 2002; Butaye et al. 2005). Calcareous grasslands also yield the greatest amount of plant nectar per unit area (Baude et al. 2016) and are richer in VOCs (Cornu *et al.* 2015).

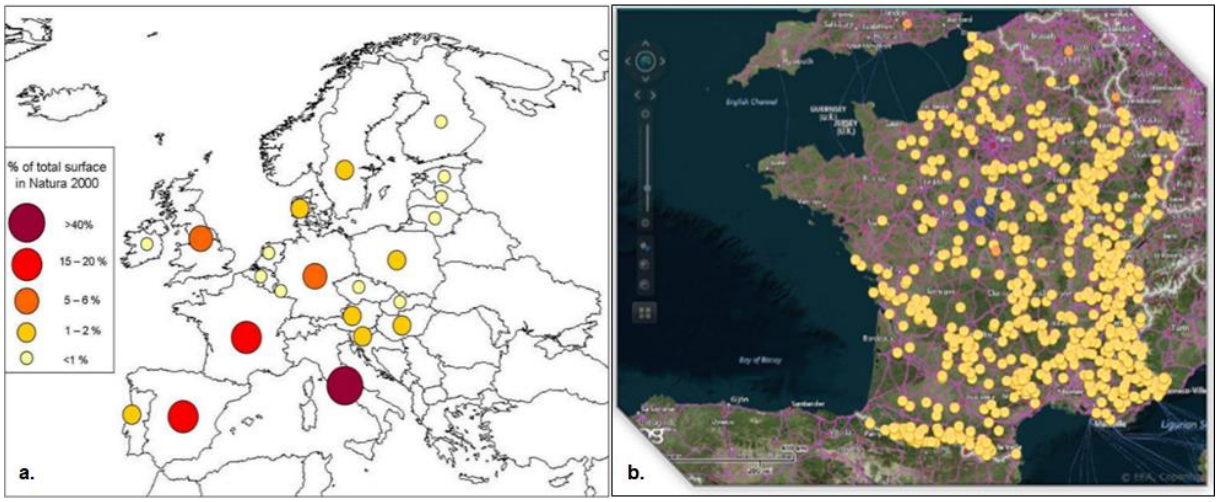

Figure 5. (a) Percentage of the total surface of dry grasslands in Natura 2000 in European countries and (b) distribution of dry grasslands in France (Natura 2000 viewer).

We conducted two years of fieldwork in six locations in three different French regions (Fig. 6), where we focused on an area of 1 hectare each: two sites in Hauts-de-France (Les Larris de Grouches-Luchuel, thereafter noted LAR, 50°11'22.5"N 2°22'02.9"E and Regional natural reserve Riez de Noeux les Auxi, noted R, 50°14’51.85”N 2°12’05.56”E, in départements Pas-de-Calais and Somme), two sites in Normandie (Château Gaillard – le Bois Dumont, noted CG, 49°14'7.782"N 1°24'16.445"E and les Falaises d’Orival, noted FAL, 49°04'40.08"N 1°33'07.254"E, départements: Eure and Seine Maritime) and two sites in Occitanie (Fourches, noted F, 43°56'07.00"N 3°30'46.1"E and Bois de Fontaret, noted BF, 43°55'17.71"N 3°30'06.06"E, department: Gard). The six sites are included in the European NATURA 2000 network; the four sites in Hauts-de-France and Normandie are managed by the Conservatoire d’espaces naturels of Normandie, Picardie and Nord – Pas-de-Calais and the

sites in Occitanie by the CPIE Causses méridionaux. Examples of three calcareous grasslands used in this study are shown in Fig. 7.

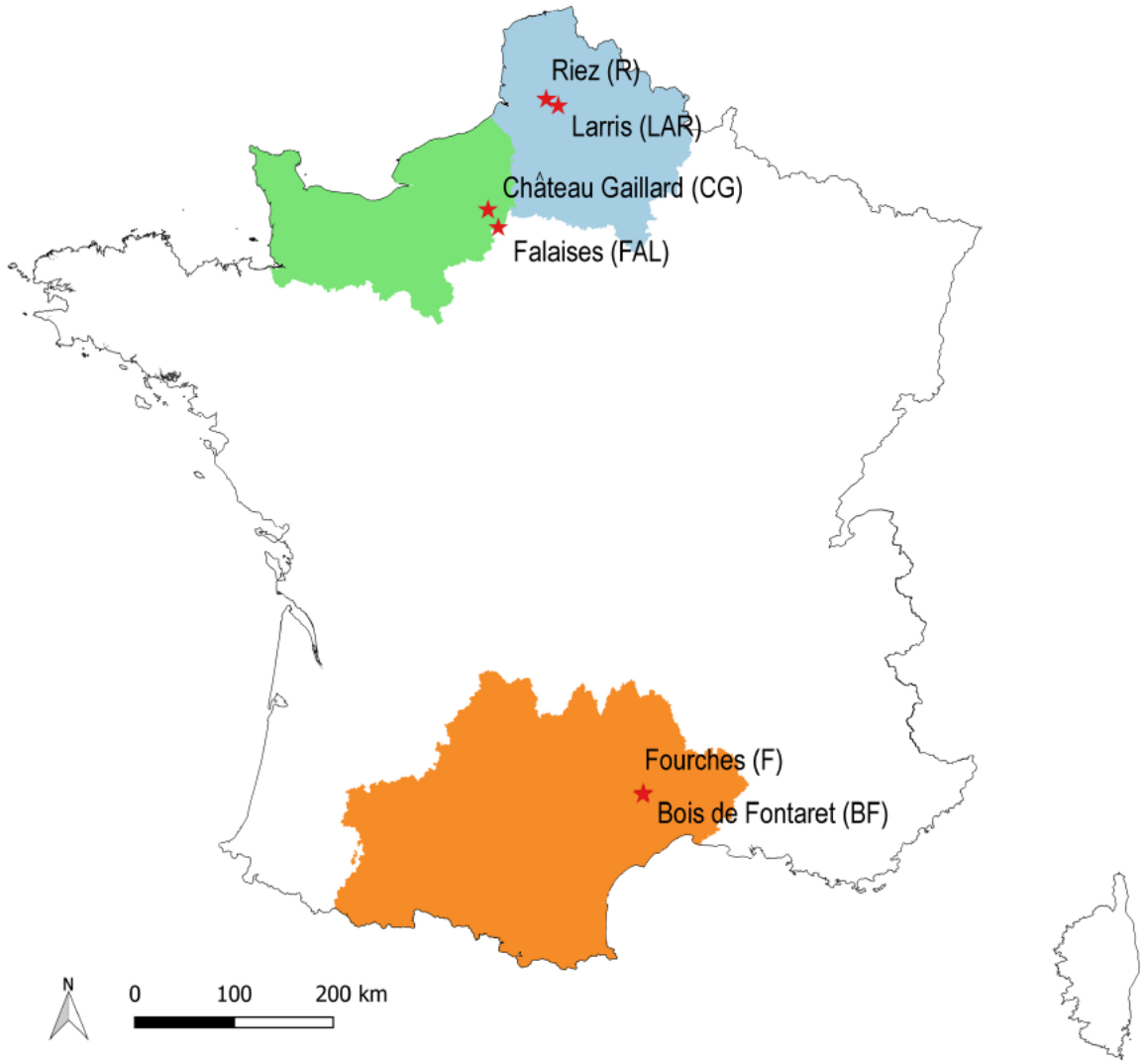

Figure 6. Site location and regions in France: in blue the French region Hauts-de-France (Les Larris de Grouches Luchuel, LAR and Regional natural reserve Riez de Noeux les Auxi, noted R, in départements Pas-de-Calais and Somme), in green the Normandie region (Château Gaillard – le Bois Dumont, CG and les Falaises d'Orival, FAL, départements Eure and Seine Maritime), in orange the Occitanie region (Fourches, F, and Bois de Fontaret, BF, department Gard). The six sites correspond to the red stars.

Figure 7. Examples of a calcareous grasslands: (upper panel) Réserve Naturelle de Le Riez, Nord-Pas-de-Calais; (middle panel) les Falaises d'Orival, Eure; (lower panel) the high diversity of plants found in the Bois de Fontaret site, Causses du Blandas

Study model: plant-pollinator associations

We sampled plant and pollinator species and we recorded their interactions from April to October to cover the entire flowering period, the pollinators' life cycle (to include seasonal species), and to follow the fluctuation and variation of community structure. We visited each site once a month in 2016 and 2017. To collect information at the community level, in each site and at each session, we realized: (i) a botanic inventory of the flowering species and (ii) a sampling of pollinators. In order to evaluate the impact of the environmental conditions on plant-pollinator associations, we measured at each field survey at different time of the day the ambient temperature and the relative humidity using Tinytag recorders.

(i) The botanic inventory at each field survey consists in listing the plant species flowering in the area and recording information on their abundance-dominance. Flowering plants were identified at the species level in the field. To record the abundances of all flowering species, at first, we estimated the total percentage of surface covered by all flowering species in the selected area. We then estimated the relative abundance of each flowering species. We used Braun-Blanquet coefficients of abundance-dominance (Poor 1955) to rank flowering species: coefficient ***5*** = 75-100% of flowering area, coeff ***4*** = 50-75%, coeff ***3***=25-50%, coeff ***2*** = 10-25%, coeff ***1*** = 1-10%, coeff **+** = fewer individuals than

< 1% coverage, coeff ***i*** = 1 individual. All inventories were realized by the same surveyors to avoid potential biases.

(ii) The pollinator sampling for the two groups Anthophila and Syrphidae (Fig. 8). To sample the pollinator in the area we used two methodologies: (a) active sampling to record the plant-pollinator associations and (b) passive traps to widely sample the insect community.

For the active sampling, we used a **hand net** along a variable transect for a fixed time period. Pollinator observations were performed by the same team of 3-5 persons each day. The surveyors walked slowly around any potential attractive resource patch included in the selected 1-hectare area for 4h each day. We split the sampling period into 2 hours in the morning (about 10-12h) and 2 hours in the afternoon (about 14-16h) to cover the daily variability of both pollinator (hoverflies are more active in the morning than in the afternoon; D'Amen *et al.* 2013) and flower communities. Sampling took place when we had suitable weather conditions for pollinators (following Westphal *et al.* 2008). We sampled all flower-visiting insects and for each insect we recorded the plant species that it was visiting (*i.e.* observed interactions). All sampled insects were immediately and individually put in a killing vial with ethyl acetate (Fig. 9) and we recorded the study site, the data and hour of capture, the collector and the plant in interaction to assess to each insect an individual ID.

For the passive sampling we used two clusters of 3 **pan traps** each. Each cluster was composed of UV-bright blue, yellow and white pans to obtain an exhaustive representation of the pollinator presence in the area. The pan traps were placed in all sites at the beginning of the field journey and left active until the end of the field work (for at least 6 hours of activity). The three coloured pan traps were placed at vegetation height and at the border of the study area (Fig. 10), in order not to disturb the other activities, such as the transect walks (Westphal et al. 2008).

All the insects sampled in the field were prepared and pinned in the laboratory to be identified at the species level by expert taxonomists (for wild bees: David Genoud, Matthieu Aubert, Alain Pauly, Eric Dufrene, Michael Terzo, Denis Michez; for hoverflies Cédric Vanappelghem and Martin C.D. Speight).

Figure 8. Examples of hoverflies (red rectangle), Sphaerophoria sp. and Syrphus sp.; and wild bees (black rectangle), Eucera sp., Bombus lapidarius and Bombus hortorum (Family Apidae), Hylaeus sp. (Family Colletidae), Lasioglossum sp. (Family Halictidae) and Panurgus sp. (Family Andrenidae) sampled during the fieldwork in the site of Bois de Fontaret and Fourches (Occitanie), Falaises (Normandie) and Larris (Hauts-de-France).

Figure 9. Example of (a) insect sampling with the hand net in the site of Château Gaillard and (b) insect collection (Andrena sp.) in the killing vials.

Figure 10. Examples of pan trap cluster disposition (a) and the three-cluster bowls with UV-bright blue, yellow and white colours (b).

Palynological analysis

We divided the palynological analysis in three main tasks: (i) the Pollen Atlas preparation (performed by Eric Schmitt), (ii) the preparation of pollen slides from pollen collected on the insect bodies (performed by Marie Zélazny) and (iii) the identification of pollen for the network analysis (as part of P. Moreau's internship).

Pollen Atlas

The aim of the pollen atlas is to provide information on the pollen grains present in the area that we can possibly compare with the pollen grains found on the bodies of sampled insects.

During each field session, from April to October, in 2016 and 2017, we sampled plant anthers of all flowering species presented in the 1-hectare area in each site. We put the anthers in individual Eppendorf tubes filled with 70% ethanol to preserve them. From this collection, we prepared a pollen atlas representative of the pollen diversity in the three areas. In the laboratory, we extracted and transferred the pollen released by anthers in the Eppendorf tube on a microscopic slide mounted with a cube of glycerine jelly (Kaiser's Glycerol Gelatine for microscopy) to maintain the natural colour of the pollen grains, and we sealed the cover slip with nail varnish. For each slide, we recorded the plant species and the site and date of collection, and we took a photograph of the pollen grain as reference. In total, we prepared 533 pollen slides which correspond to 44 families and 245 plant species sampled during the fieldwork campaign in 2016.

Pollen preparation and identification

I investigated pollen found on insect bodies to add missing links between plants and pollinators which have not been observed in the field. To do so, I identified the pollen grains found on insect bodies by comparing them with pollen grains inventoried in the pollen atlas, from the same site on the same day.

Due to the large number of sampled insects, we only used the insects collected during the month of July 2016, when we recorded the highest diversity of both plants and pollinators, and only in three sites (one for each region) to compare pollinator preferences among sites along the gradient. We focused on wild bees (superfamily: Apoidea, clade Anthophila) because they are very efficient pollinators with specialized structures for pollen collection. The pollen was collected, when possible, by separating the pollen scattered on their body (PS) from the pollen actively collected in specialized structures (*i.e.* curbiculae and scopae, PC, Fig. 11a, b). The type of pollen (collected vs. scattered) is

a valuable piece of information about pollinator preferences. PC is usually collected for the nest, so it is the pollen that the insects collect on purpose. By contrast, PS represents the pollen collected accidentally, when adult bees visit a flower for nectar or other activities. Before removing pollen grains from insect bodies, we assigned a "pollen score" to each insect body regions (head, wings, thorax, abdomen, first-second-third pairs of legs). The "pollen score" is an estimation of the insect body surface cover by pollen grains, and we assigned five scores following Kearns and Inouye (1993):

0, No pollen on region; **1**, Very sparse pollen, fewer than 10 grains; **2**, Patchy pollen cover, 10-40 grains; **3**, General pollen cover, much of the area covered and **4**, Heavy pollen cover, grains may be several layers deep.

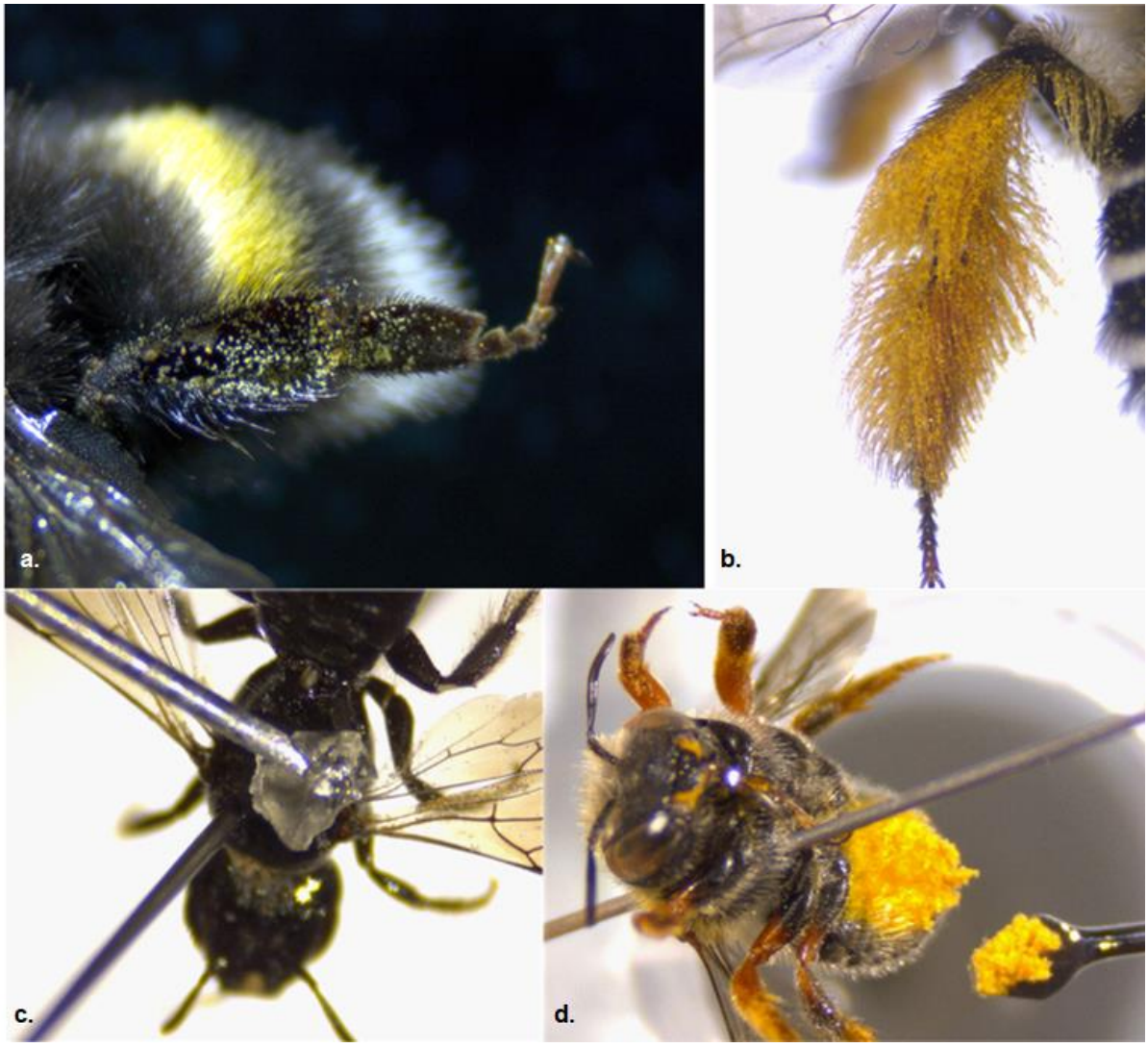

Figure 11. Examples of pollen structures: (a) curbiculae in Bombus sp. (Apidae family) and (b) scopae in Dasypoda hirtipes (Melittidae family). Example of pollen removal techniques with (c) small cube of glycerine jelly and (d) by brushing the Anthidium (Megachilidae family) scopae with a small spoon.

We collected PS from insect bodies using a small cube of glycerine jelly (volume 2 mm^3, Fig. 11c) following (Kearns and Inouye 1993). PC was removed by brushing the specialised structures with a small needle or a small spoon (Fig. 11d) and put in an Eppendorf tube filled with 70% ethanol for conservation. Only a fraction of the PC (10 µl) was used to prepare pollen slides. Pollen identification was performed at the lowest taxonomic level (mostly at species level) by an expert (K. Bieri at the *Biologishes Institut für Pollen analyses*, Kehrsatz, Switzerland), or in the laboratory, using a combination of diagnostic keys and comparison with the Pollen Atlas. When it was not possible to discriminate between two closely related species, we aggregated them in higher categories (family, genus or morpho-type). Microscope slides were observed at 400x magnification by random transects until we counted 100 pollen grains, then the rest of the slide was searched for undetected pollen types. However, for statistical analyses we did not keep plant species for which we detected ≤ 5 pollen grains, which we considered as infrequent or accidentally collected (Bosch et al. 2009; Fisogni et al. 2018).

In total, we collected pollen from 580 insects and 993 slides were prepared, 573 PS and 420 PC. In network analyses, we only considered pollen from female bees that were observed in interaction with a plant and for which we prepared the two different microscope slides (PC and PS). Thus, we used 782 pollen slides from 391 female individuals: 346 slides for the site of Fourches (Occitanie), 256 slides for the site of Chateau Gaillard (Normandie) and 180 slides for the site of Riez (Hauts-de-France).

The missing links found with the pollen analysis were integrated in pollen-based networks and compared with the visit-based networks. For more details see chapter III.

Floral scent production and composition

Floral scents are considered as a major non-visual trait acting as long distance attractant for many pollinator species (Schiestl 2010; Parachnowitsch and Manson 2015). Thus, like other floral traits, their composition is likely to be under pollinator-mediated selection (Dötterl and Vereecken 2010; Schiestl and Johnson 2013).

In this part of the project, we compared the floral bouquets (compound identity and their proportion) emitted in natural and controlled conditions in four annual species (Fig. 12): *Anthyllis vulneraria* (AV, Family: Fabaceae), *Globularia vulgaris* (GV, Family: Plantaginaceae), *Pilosella officinarum* (PO, family: Asteraceae) and *Ranunculus bulbosus* (RB, Family: Ranunculaceae). Even if the four

species display different morphologies and phenologies, all of them are entomophilous and considered attractive for the local pollinators. We chose plant species which are widely spread along the environmental gradient and which were present in at least some of the study sites (Fig. 12): GV populations were present in both sites in Occitanie (BF and F) and both sites in Normandie (CG and FAL), RB and AV populations were found in both sites in Occitanie and Hauts-de-France (LAR and R), PO populations were found only in the southern site of Fourches (Occitanie) and in both sites in the other two regions (CG and FAL in Normandie and LAR and R in Hauts-de-France. The populations of AV displayed different colours along the gradient (yellow in the region Hauts-de-France and pink in Occitanie; Fig. 12).

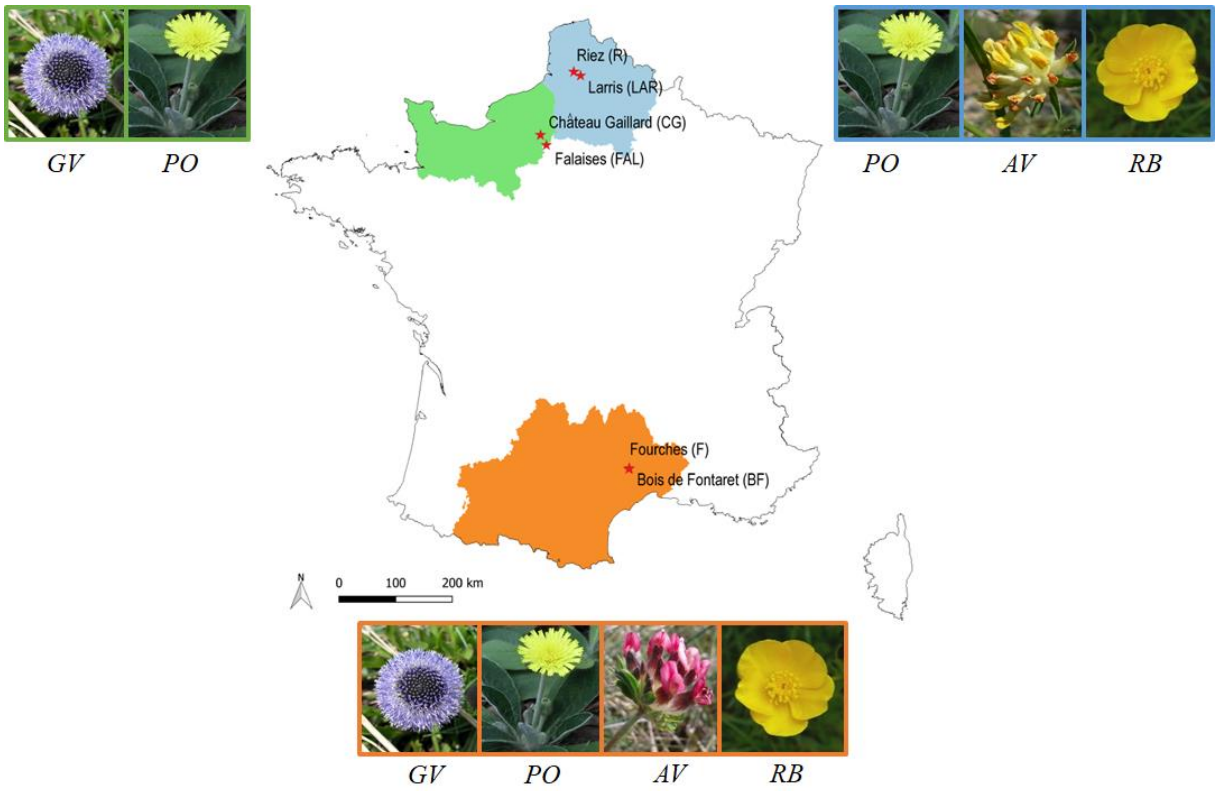

Figure 12. Population locations among the regions (Hauts-de-France in blue, Normandie in green and Occitanie in orange). The six sites correspond to the red stars. Globularia vulgaris (GV) is present in the Occitanie region and in the Normandie region; Ranunculus bulbosus (RB) is present in the Occitanie region and in the Hauts-de-France region; Anthyllis vulneraria (AV) is present in the Occitanie region (with the pink colour) and in the Hauts-de-France region (with the yellow colour) and Pilosella officinarum (PO) is present in the three regions.

Floral scents in natural condition

Floral scent extractions were performed in the field (Fig. 13) between April and June 2017, during the species flowering peak and at the moment of the day with the maximum of ambient temperature (which ranged from 10°C in April to 29°C in June, recorded with the Tinytag) and minimum of humidity, due to their high dependence on the environmental conditions. Floral scents were collected when in the field the selected plant species had reached receptivity, *i.e.* when they attract their pollinators and always performed under natural light. We selected 5-15 individuals per site and species, according to flowering plant availability. Scent collections were performed between 12:00 to 16:00 hr, corresponding to the period of insect maximum activity during our field season.

Figure 13. Floral scent extraction in the site of Bois de Fontaret (Occitanie). The target individuals are identified with a yellow label and the control (empty bag) is placed in the vegetation around them (red circle).

Floral scents in controlled condition (Greenhouse)

At the end of the field season, we collected plant seeds from all different species from all the sites, when possible, to repeat the same methodology in controlled conditions. Our aim was to test whether the differences observed in the field are due to some genetic differences or to phenotypic plasticity link to these traits. Indeed, variation of the floral bouquet observed in the field can also be explained by phenotypic plasticity related to variation in the environmental conditions, such as light, temperature, type of soil or any environmental factor that varies among study sites (Majetic et al. 2009). However, few studies have investigated the role of abiotic factors and tested the differences on the production of floral scents' VOCs between wild plants and plants reared in common garden (Majetic et al. 2009; Chapurlat et al. 2018).

Floral scents extractions were performed in the greenhouse (Fig. 14) between April and May 2018 and we selected from 5 to 8 plant individuals per site and species, all belonging to different maternal families. All surveyed plants in greenhouse were put in a separated and closed chamber cell, then excluded from pollination events. In the greenhouse, floral scents were collected under a mix of natural and artificial lights and we settled the chamber temperature around 20°C.

Figure 14. Floral scent extraction of Anthyllis vulneraria in the controlled chamber cell in the greenhouse.

Floral scent extraction and analysis protocol

The same extraction protocol was used for all species in the two tested conditions. For further details see Chapter VI.

We covered each plant individual (the entire plant, including the leaves, Fig. 15) for one hour with a plastic bag made by polyethylene terephthalate (Nalophane®, Kalle Nalo GmbH, Wursthüllen, Germany) that were shut tighly with cotton string, to allow the accumulation of the VOCs. Scents were extracted using the dynamic Headspace technique (Grison-Pigé et al. 2002; Proffit et al. 2008; Soler et al. 2011; Dormont 2012). At each extraction session, one control (empty bag, Fig. 13) was sampled to further exclude, from the statistical analyses, compounds occurring in the ambient atmosphere. The VOCs are aspired and captured in adsorbent traps, ChromatoProbe® quartz microvials of Varian Inc. (length: 15 mm; inner diameter: 2 mm), previously cut closed-end , which are small filters filled with 3 mg of a 1:1 mix of Tenax-TA and Carbotrap® (60–80 and 20–40 mesh, respectively; Sigma Aldrich, Munich, Germany). One microliter of a solution of internal standards (n-Nonane and n-Dodecane, 108 ng/µl and 114 ng/µl of each) was added to each trap before scent

extraction. Traps were attached to silicone tubing within the collection bags and connected on the other end to flowmeters and a standard 12-V air pump. The 200 ml/min air flow was drawn out of the bag and over the trap for 20 minutes (Fig. 16). All samples were kept in clean glass vials and stored in the dark, in a portable cooler, until transport to a −20°C freezer where samples remained until analysis by Gas Chromatography-Mass Spectrometry (GC-MS) in the laboratory.

All samples collected under natural and controlled conditions were analysed at the "Platform for Chemical Analyses in Ecology" (PACE), technical facilities of the LabEx CeMEB (Centre Méditerranéen pour l'Environnement et la Biodiversité, Montpellier, France), using gas chromatograph coupled to mass spectrometer (GC, Trace™ 1310, and ISQ™ QD Single Quadrupole, Thermo Scientific™ Milan, Italy). For further details of the GC-MS analysis see Souto-Vilarós et al. (2018). Xcalibur™ software (Thermo Scientific™, Milan, Italy) was used to identify the chromatogram peak. We converted the retention times of each compound into a retention index (IR) using the retention times of the n-alkanes (Alkanes standard solution, 04070, Sigma Aldrich®). To identify the compounds, we matched the mass spectra with existing databases (NIST 2007 MS library, Wiley 9th edition), and with retention indices reported in the literature (Adams, 2007), or by comparison with reference compounds. We subtracted any potential contaminant compounds (treatment minus control) from the samples that we used for statistical analysis, by comparing their spectra to the controls collected on the same days of collection and under the same conditions.

Figure 15. Example of floral scent extraction in the field (on the left) and in the greenhouse (on the right) of the species Ranunculus bulbosus.

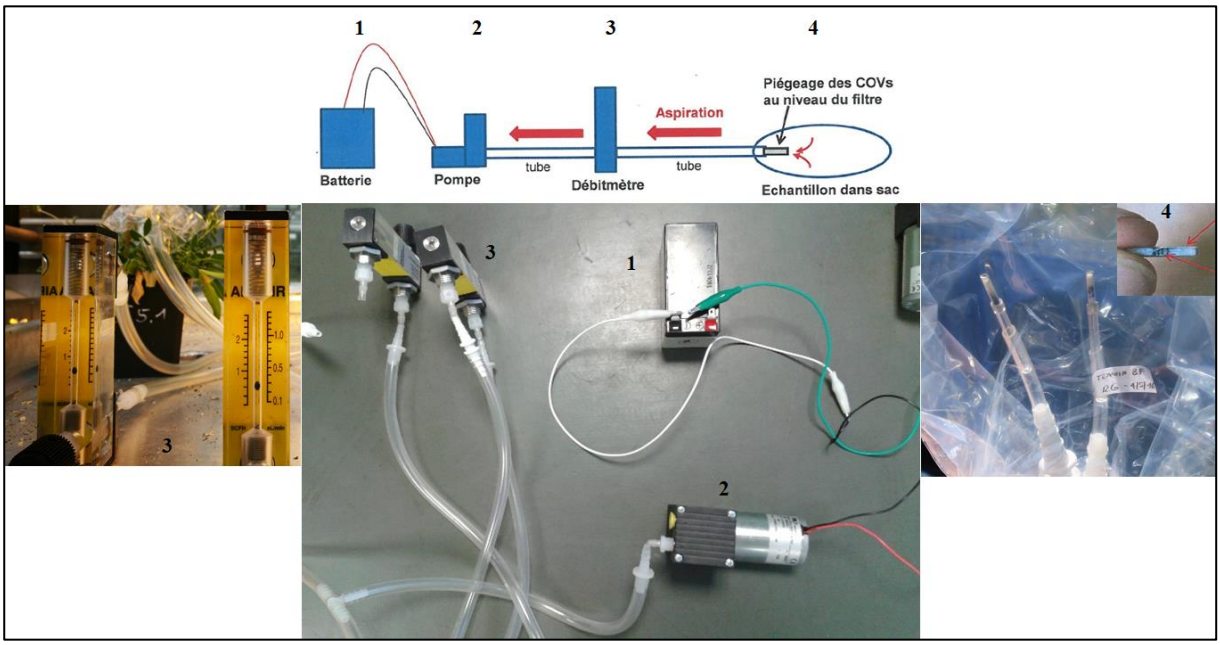

Figure 16. General scheme of the dynamic headspace to sample floral scents (Dormont 2012).

Additional floral traits

Floral reflectance

Because *Anthyllis vulneraria* displays colour variation between the two regions in which the species was surveyed (Fig. 17), with yellow colour usually in moister conditions and red in dryer habitats (Puidet et al. 2005), we quantitatively analysed floral reflectance from 300 nm to 700 nm (visible spectrum) on the same 15 individuals grown in greenhouse that were surveyed for scent analysis. We wanted to test whether the colour difference could have an impact on VOCs production, given that the volatile compounds and the colour production generally follow the same chemical pathways in the flower development, even if any clear pattern has been found (Majetic et al. 2008; Junker and Parachnowitsch 2015; Delle-Vedove et al. 2017). This specific combination of traits could also be the result of selective pressures acting concomitantly on flower colour and floral scent; colour polymorphisms may reflect different selection pressures driven by different pollinators, but also by herbivore protection or different local abiotic conditions (Dormont et al. 2014).

Two flowers were measured per individual and we used their average for statistical analysis. We used a spectrophotometer equipped with a Xenon light source (AvaLight-XE, Avantes, Eerbeek, The Netherlands). The spectrophotometer was calibrated with a white standard (made with Barium Sulfate) prior to the measurements.

Figure 17. Example of Anthyllis vulneraria dimorphism between natural (on the right) and reared individuals (on the left) in northern and southern populations. In drier habitat (southern populations), A. vulneraria displays white and purple flowers; in moister habitats (northern populations), a yellow phenotype.

Morphological measurement

In order to compare the effect of the environmental gradient on the assemblage of plant traits and to investigate the possibility of adaptation of interaction traits in response to the variation in the pollinator fauna, we also measured some morphological traits, such as: (i) number of inflorescences per plant, (ii) number of flowers per inflorescence or plant, (iii) flower and inflorescence size and (iv) plant surface. We performed plant trait measurements on the same focal plant species use for the

VOCs extraction (Fig. 18). A preliminary analysis was performed as part of Hineiti Lou Chao's internship. We found some differences for the morphological traits (using the Wilcoxon-Mann-Whitney test), such as the number of flowers per plant and the flower size in *G. vulgaris* and *R.bulbosus* plant species. However, these results will not be showed in Chapter IV, but it will be used in the discussion and perspectives (Chapter V and Annex 3, Hineiti Lou Chao Master's Dissertation).

Figure 18. Example of measurement of plant surface on one of the individual of Anthyllis vulneraria used for the floral scent extraction.

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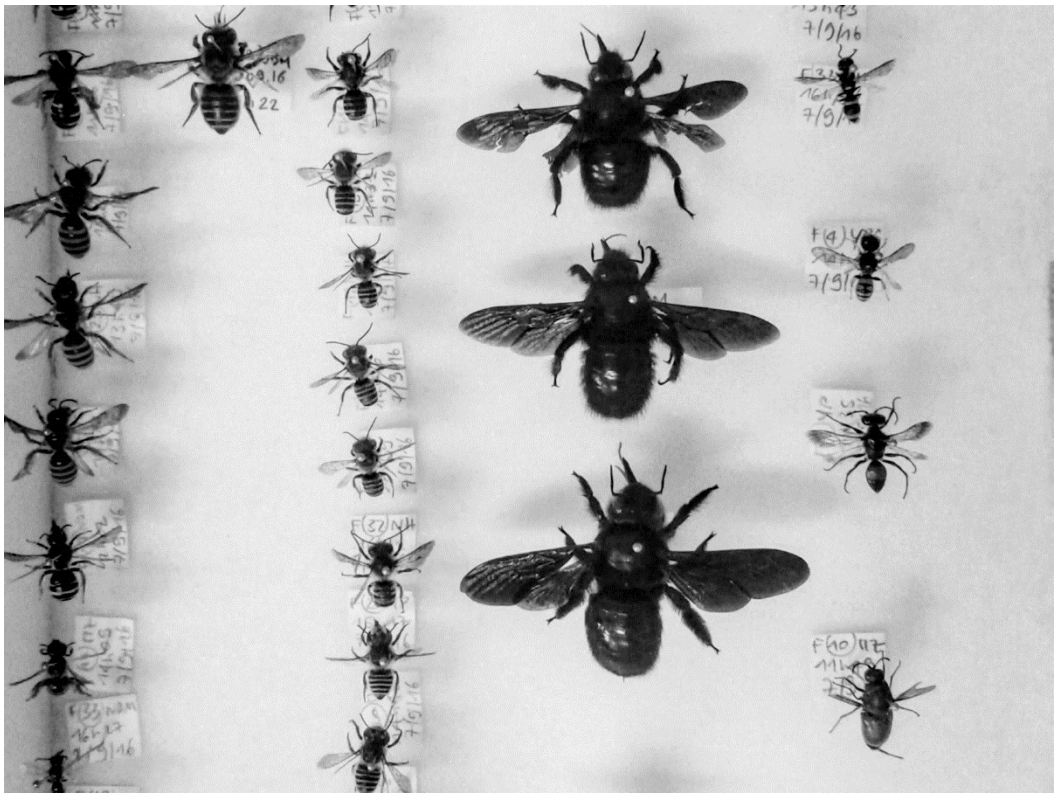
Chapter I

Characterization and diversity of plant-pollinator community in calcareous grasslands



Abstract

Studies along environmental gradients are widely used in evolutionary ecology to assess phenotypic and genotypic variability of traits in plants and animals and to understand their environmental factors. In the context of the current global change, synchronic studies along a latitudinal gradient (space-for-time substitution) are considered as an easier way to infer the potential for genetic change in the near future compared with allochronic studies. Since the latitudinal gradient influences not only species traits, but also species diversity and biotic interactions, such as plant-pollinator associations, synchronic studies along environmental gradients might therefore be a promising tool to investigate how ecological and evolutionary constraints shape network structure and species assemblages. The number of empirical studies that consider such a gradient is, however, still limited. In Europe, wild bee and vascular plants species richness decrease with latitude. However, the lack of data in most European countries, especially in Mediterranean regions, prevents an exhaustive knowledge of the local and regional diversity. New empirical studies are thus needed to assess plant and pollinator species diversity and how their associations vary in space and time. In this study, we provide new datasets obtained in the course of large nation-wide observations of plant and pollinator communities in French calcareous grasslands over two years. We used Shannon diversity measures to assess how plant and pollinator diversities are structured among sites (within a region and among regions) and among different time periods during the same year and between successive years. The general β diversity between the two years was quite low. The classic latitudinal trend of diversity was observed in both plants and pollinators, with southern sites displaying the highest species richness and diversity for both groups. Species turnover among and within regions did not show any clear pattern; indeed, factors responsible for most of β diversity variation in both plants and pollinators were sampling month and site. The next step of this analysis will be to look at the spatio-temporal variations of plant-pollinator interactions.

Context

Understanding the functioning of ecological communities, as well as its spatio-temporal variation, is among the primary goals of community ecology and underlies our capacity to understand ecological networks and their dynamics. Indeed, knowledge of community composition is important to understand how species can interact and how these interactions could change in space and time (Pellissier *et al.* 2017). The use of environmental gradients is a promising tool to investigate how ecological and evolutionary constraints can shape network structure and species assemblages under changing environments (Pellissier *et al.* 2017). In the context of global change, using "space-for-time" substitution should even also help us predict what will happen to current networks in the near future. Species richness in most groups of organisms decreases with latitude (Gaston 2000; Schemske *et al.* 2009). This phenomenon has been observed in Europe, with Mediterranean countries displaying a higher diversity than northern countries (Nieto *et al.* 2014). However, global changes, such as climate change, have the potential to modify habitat conditions and, to influence species ranges and, thus, local diversity. In the long run, this can force species to move polewards, as already documented for some insect and plant species (Davis & Shaw 2001; Crozier 2004; Parmesan 2006; Menendez 2007; Willmer 2012; Rasmont *et al.* 2015). Moreover, changes in species life cycles induced by global changes can cause local phenological mismatches between interaction partners, which can significantly compromise species interactions (Memmott *et al.* 2007; CaraDonna *et al.* 2014; Forrest 2015; Schenk *et al.* 2018). In this context, pollination studies along altitudinal or latitudinal gradients can help assess how plant-pollinator networks could be affected by climate change (Hegland *et al.* 2009). However, species respond differently to perturbation caused by climate change, thus it is possible that the impact of disturbance is more easily seen at the species level that at the network level (Hutchings *et al.* 2018). Nevertheless, impacts of climate change on individual species can cause a cascading effect and finally propagate through ecological communities and species interactions (Hegland *et al.* 2009; Schleuning *et al.* 2016)

Few studies have precisely dealt with this aspect through the study of network variation along latitudinal gradients, instead mostly focusing on altitudinal gradients (Benadi *et al.* 2014; Norfolk *et al.* 2015). Similarly, few studies have compared the plant-pollinator networks considering the entire flowering season for more than one year (Petanidou *et al.* 2008; Cirtwill *et al.* 2018). Overall, there is a scarcity of data on how plant and pollinator communities co-vary in space (among sites along a gradient) and time (along the flowering season). However, analysing such data could provide key insights as to how plant-pollinator networks might cope with environmental changes in the short term.

Here we provide new data obtained in the course of large nation-wide observations of plant and pollinator communities of French calcareous grasslands along a 1000 km latitudinal gradient. This dataset is then explored using Shannon diversity measures to assess how plant and pollinator diversities are structured among sites (within a region or among regions) and among different time periods during the same year and between successive years.

Methods

To study how spatial and temporal variation can affect the diversity of both plants and pollinators, we focused on calcareous grassland communities along a latitudinal gradient in France. We selected six calcareous grasslands in three different regions (Occitanie, OCC, Normandie, NOR, and Hauts-de-France, HFR) and in these sites we assessed the temporal variation of diversity, following plant and pollinator communities from April to October and for two consecutive years (see Materials and Methods in the Introduction chapter). Each site was sampled once each month. Our aim is to compare plant and pollinator taxonomical diversity (i) along the environmental gradient, between and within region, and (ii) along the season, from April to October, and in the two years of sampling. In particular, we want to understand whether plant and insect diversities follow the latitudinal gradient and how these diversities co-vary along the season.

Beside species richness, we used Shannon's entropy (logarithm of the order q=1 Hill number, Jost 2007, Marcon *et al.* 2014) and calculated the entropy of each community (α diversity), between communities (β diversity) and of aggregated communities (γ diversity) using the function *DivPart* and *BetaEntropy* in the R package *entropart* (Marcon & Hérault 2018a, b). Since Shannon's entropy allows the calculation of a partial β diversity measure for each site (Lande 1996), we also calculated partial β diversity as a measure of a site's originality.

We calculated diversity indices separately for insect and plant species and we separated insects sampled with hand nets from those sampled with pan traps since the two methodologies are not comparable in terms of abundances. We applied Chao *et al.*'s (2013) bias correction to the estimation of insect species entropies, but not for plant species since plant individuals were not counted (abundance measures are based on Braun-Blanquet's dominance instead, which can yield an estimation of species frequencies, but not absolute abundances).

We tested which factors were important to explain β diversity between and among regions, sites, months and years using the PERMANOVA approach of Anderson (2001). In brief, this method

"regresses" the matrix of β diversities between all pairs of sites against explanatory factors and assess the significance of these factors using permutation of matrix rows and comparing pseudo-F values obtained with and without each factor (marginal comparison) to those obtained with permuted matrices. Here, we performed 9,999 permutations and used year, month, region and site as potential explanatory factors. This analysis was performed using the function *adonis2* implemented in the package *vegan* in R (Oksanen *et al.* 2019).

Results and Discussion

Species richness and abundances

After two years of fieldwork, we sampled 15702 insects (12171 Anthophila and 3531 Syrphidae) corresponding to 318 bee species (corresponding to about 33% of the French bee fauna) and 109 hoverfly species (corresponding to 20% of the French hoverfly fauna), using both sampling techniques (hand net and pan traps). We recorded 288 plant species, 255 species in 2016 and 229 species in 2017. We excluded trees, ferns and anemophilous grasses (such as *Poaceae, Cyperaceae* or *Juncaceae*) from the analysis, and we only considered flowering species.

We observed the highest species richness in southern sites, both for plant and insect species in both years (Fig. 1.1b and Fig. 1.2). Insect richness decreased along the latitudinal gradient (Fig. 1.1b); however, the pattern was less clear for plants (Fig. 1.2), where richness was similar between individual sites from Normandie (CG and FAL) and Hauts-de-France (LAR and R).

61 % of insect species were shared between the two years, while 15% were unique to 2016 (41 unique bee species and 22 unique hoverfly species), and 24% were unique to 2017 (78 and 24 unique species for bees and hoverfly, respectively). 68% of plant species were shared between the two years (n = 196 plant species), while 20.5% were unique to 2016 (n = 59 species) and 11% to 2017 (n = 33 species).

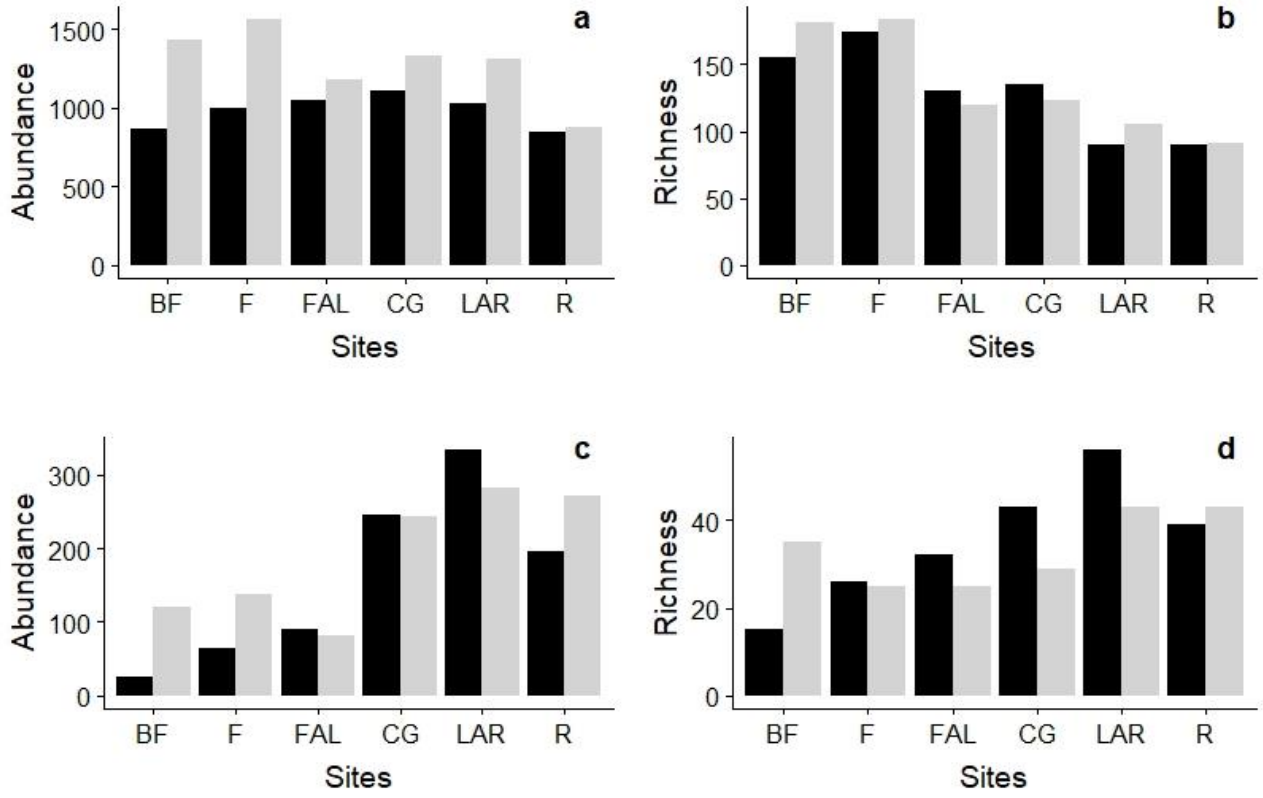


Figure 1. 1. Total insect abundance and insect species richness recorded in each site (BF, Bois de Fontaret; F, Fourches; FAL, Falaises; CG, Chateau Gaillard; LAR, Larris and R, Riez), using hand nets (a,b) and pan traps (c,d), in 2016 (black bars) and 2017 (grey bars).

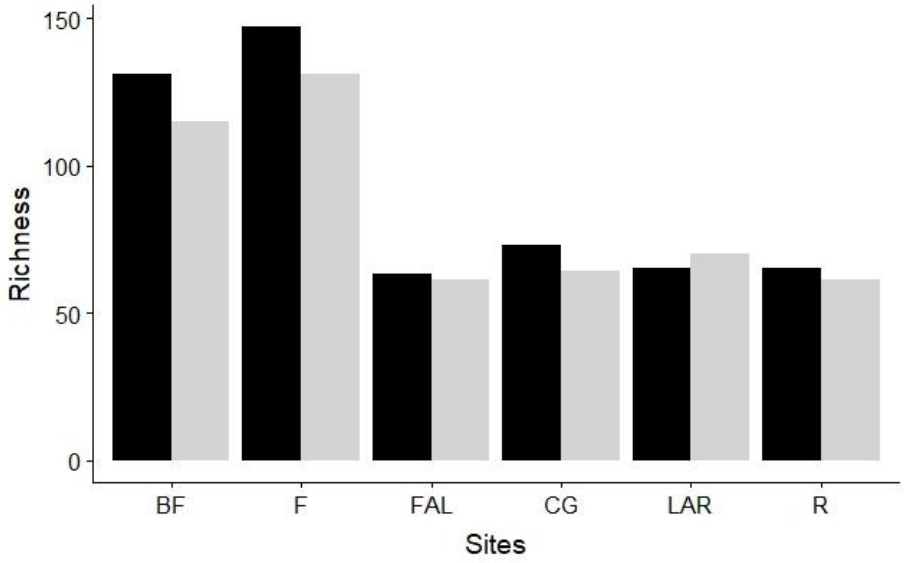


Figure 1. 2. Plant species richness recorded in each site (BF, Bois de Fontaret; F, Fourches; FAL, Falaises; CG, Chateau Gaillard; LAR, Larris and R, Riez) in 2016 (black bars) and 2017 (grey bars).

We recorded the highest number of insect species in 2017, both for bees (Anthophila = 278 species in 2017 and 239 in 2016) and hoverflies (Syrphidae = 87 species in 2017 and 85 species in 2016). The difference in species richness between the two years might be due to the higher number of individuals sampled in 2017 (n = 8849) than in 2016 (n = 6853). Even if the sampling effort was the same in all sites, insect abundances differed between regions and between sites (Fig. 1.1a). For example, in 2016, we recorded the highest insect abundance in the site of CG and FAL (Normandie), while in 2017 we sampled the highest insect abundance in the two sites of Occitanie (BF and F, Fig. 1.1a). In both years, we sampled the lowest number of insects in the site of Riez (R, Hauts-de-France, Fig. 1.1a). In 2016 it was not possible to perform the first field session (April) in the site of Riez because of the weather conditions. However, we recorded the same number of species (considering the whole year 2016) as in the site of Larris (LAR, Hauts-de-France, Fig. 1.1b). Despite differences in the species abundance recorded in 2016 and in 2017 with the hand net, insect richness followed the latitudinal gradient, with southern sites displaying the highest richness and northern sites the lowest one (Fig. 1.1b).

Pan traps were more attractive in Normandie and northern sites than in the southern sites in both years (Fig. 1.1c), showing a “reverse” latitudinal gradient of insect richness (Fig. 1.1d). The attractiveness of pan traps in northern sites, especially at the begin of the season (Fig 1.3c, d, blue lines), can probably be linked to the lower plant diversity and floral abundance recorded in these sites (Fig. 1.2). However, the attractiveness of pan traps is highly variable among sites and among months, and it did not show a clear pattern along the season (Fig. 1.3c, d).

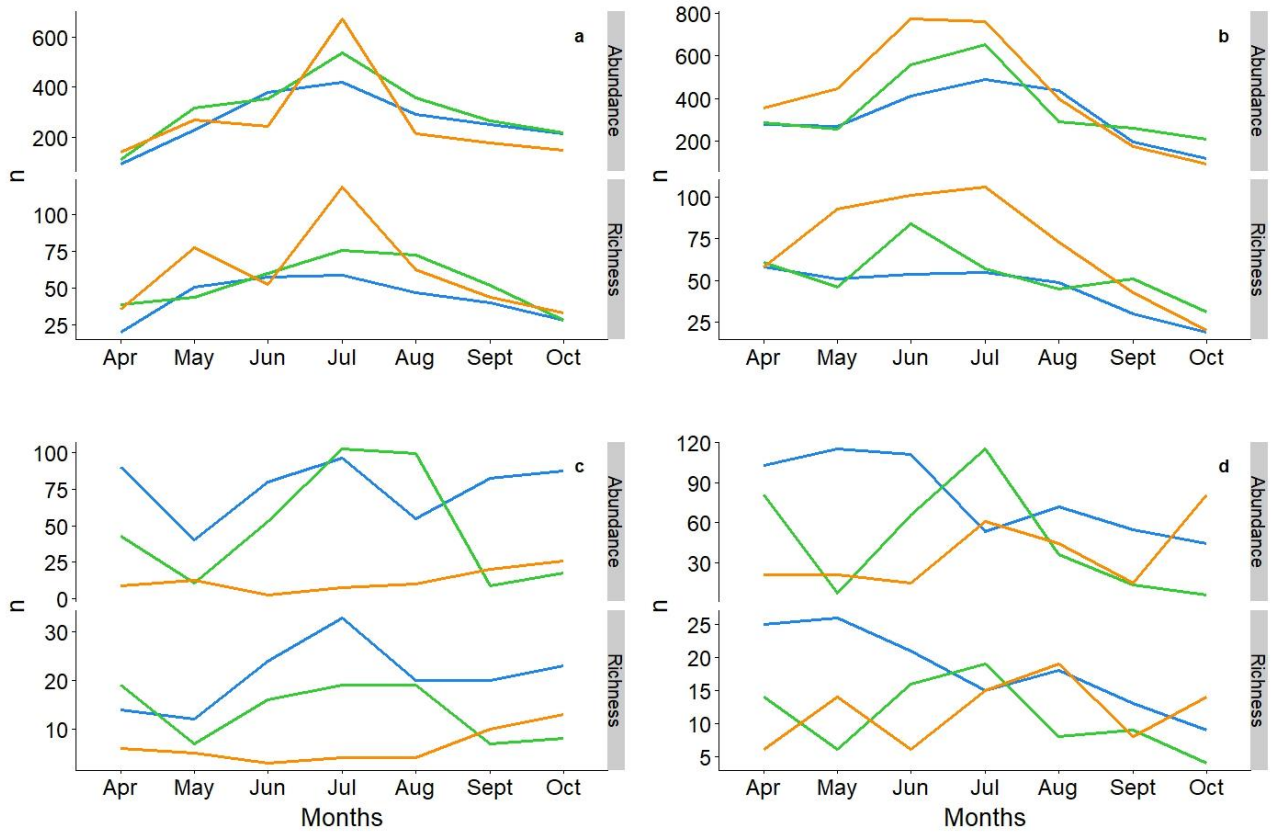


Figure 1. 3. Seasonal insect abundances and richness displayed in the three regions (Occitanie in orange, Normandie in green and Hauts-de-France in blue) in 2016 (a,c) and 2017 (b,d), recorded using hand nets (a,b) and pan traps (c,d).

Insect species richness sampled with hand nets increased along the season from April to July and decreased from August to October in 2016 and 2017 (Fig. 1.3a,b). In 2016 in the region Occitanie (in orange, Fig. 1.3a), insect species richness peaked in the month of May and July and decreased in June. June 2016 was very rainy and thus we sampled a lower number of insects in southern sites (BF = 120; F = 126) than in the other four sites (CG = 239; FAL = 168; LAR = 213; R = 246).

Insect abundance and richness recorded along the season using pan traps showed variable patterns among regions and years. In 2016 in the region Occitanie pan traps had a low sampling success along the season (Fig. 1.3c), while in the regions Normandie and Hauts-de-France sampling success followed the pattern observed with the hand net, with lower numbers both for abundance and species richness (Fig. 1.3a, c). In 2017 sampling success was slightly higher than in 2016 in all regions, with a high temporal variability within and among regions (Fig. 1.3d).

Plant and pollinator communities

Pollinator communities – Insect species of the group Anthophila were separated in 6 Families, while hoverfly species are part of the same family (Syrphidae). Overall, we recorded 318 bee species, part of 36 genera, and 109 hoverfly species of 35 genera overall. Not all the genera, and species, were present in all sites and in all regions. The pollinator communities in the three regions differed significantly, both in term of insect abundance and number of species (species richness, Fig. 1.4). The three regions had a characteristic pollinator community for sites of their latitude, for both bee and hoverfly species. In the group Anthophila only the 18% of the species were shared among the three regions and the 56% of the species (n = 177) were unique to one region. The region Occitanie showed the highest number of unique species (n = 128), compared to the region Normandie (n = 28) and Hauts-de-France (n = 21). In the family Syrphidae we observed a similar trend, with 54% (n = 59) of species that were recorded only in one region and 20% of common species (shared by the three regions). Also for hoverfly species, the region accounting for the highest number of unique species was Occitanie (n =33).

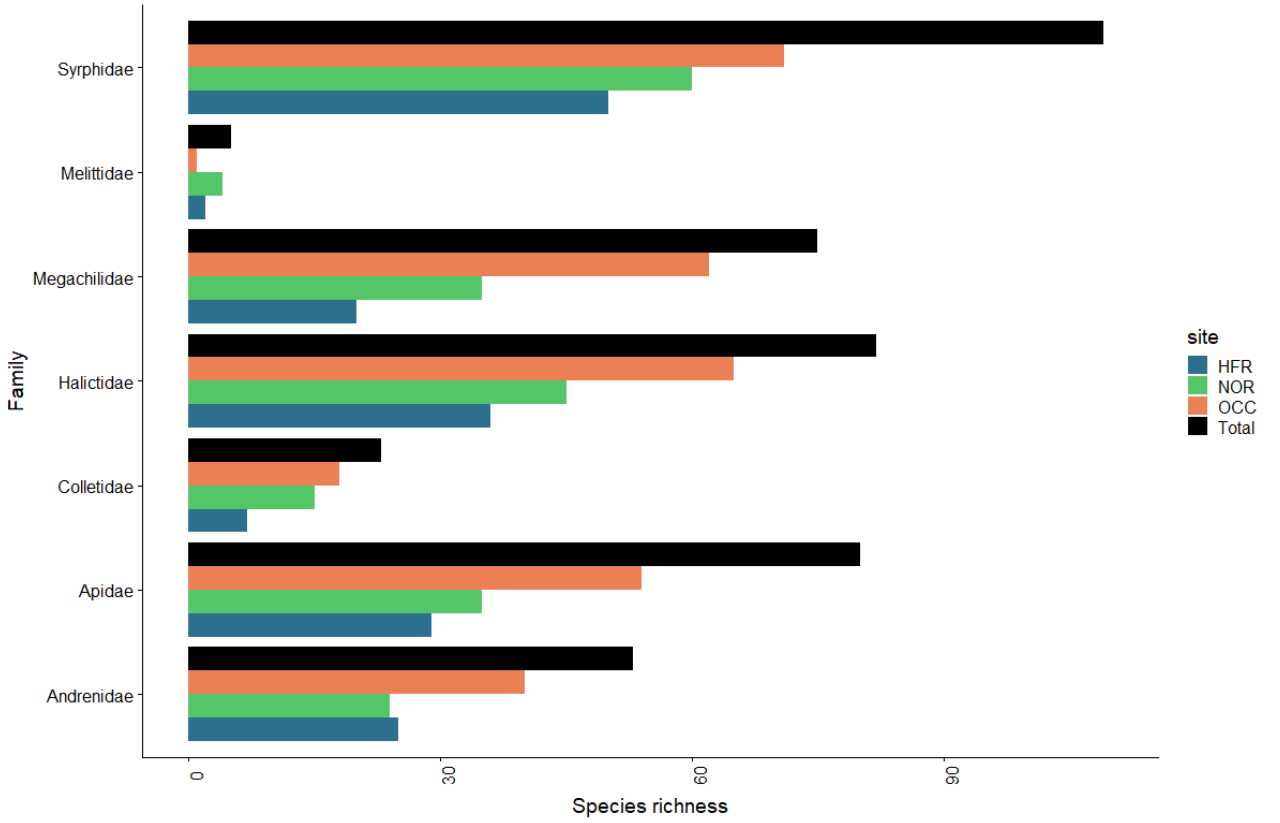


Figure 1. 4. Pollinator families and species richness in the three regions Hauts-de-France (HFR), Normandie (NOR) and Occitanie (OCC).

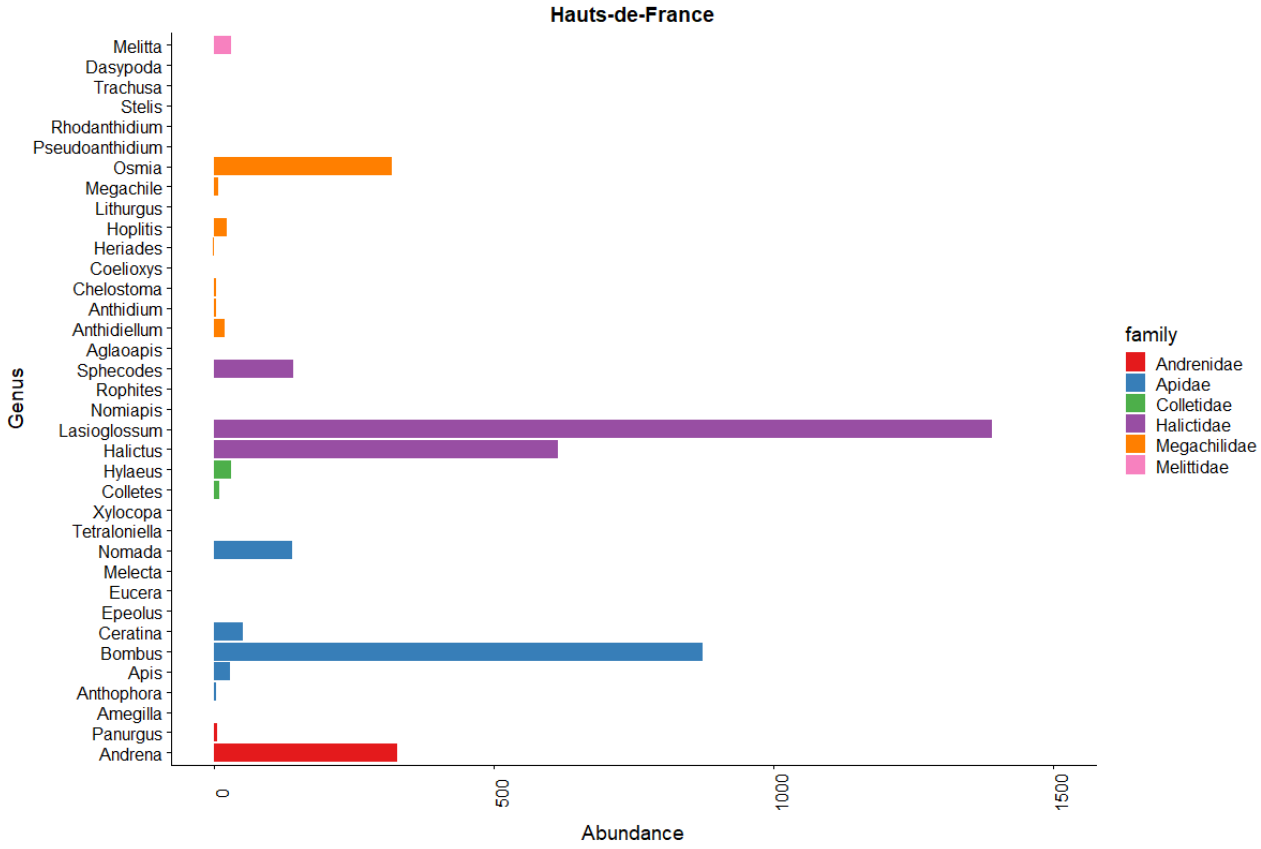
In particular, for the Anthophila group, we identified 20 genera and 119 species in Hauts-de-France (HFR), 30 genera and 158 species in Normandie (NOR) and 32 genera and 240 species in Occitanie (OCC) in the two years of sampling, using both techniques (Fig. 1.5). For the Syrphidae family we identified 26 genera corresponding to 50 species in Hauts-de-France and 71 species in Occitanie, and 28 genera corresponding to 60 species in Normandie (Fig. 1.6).

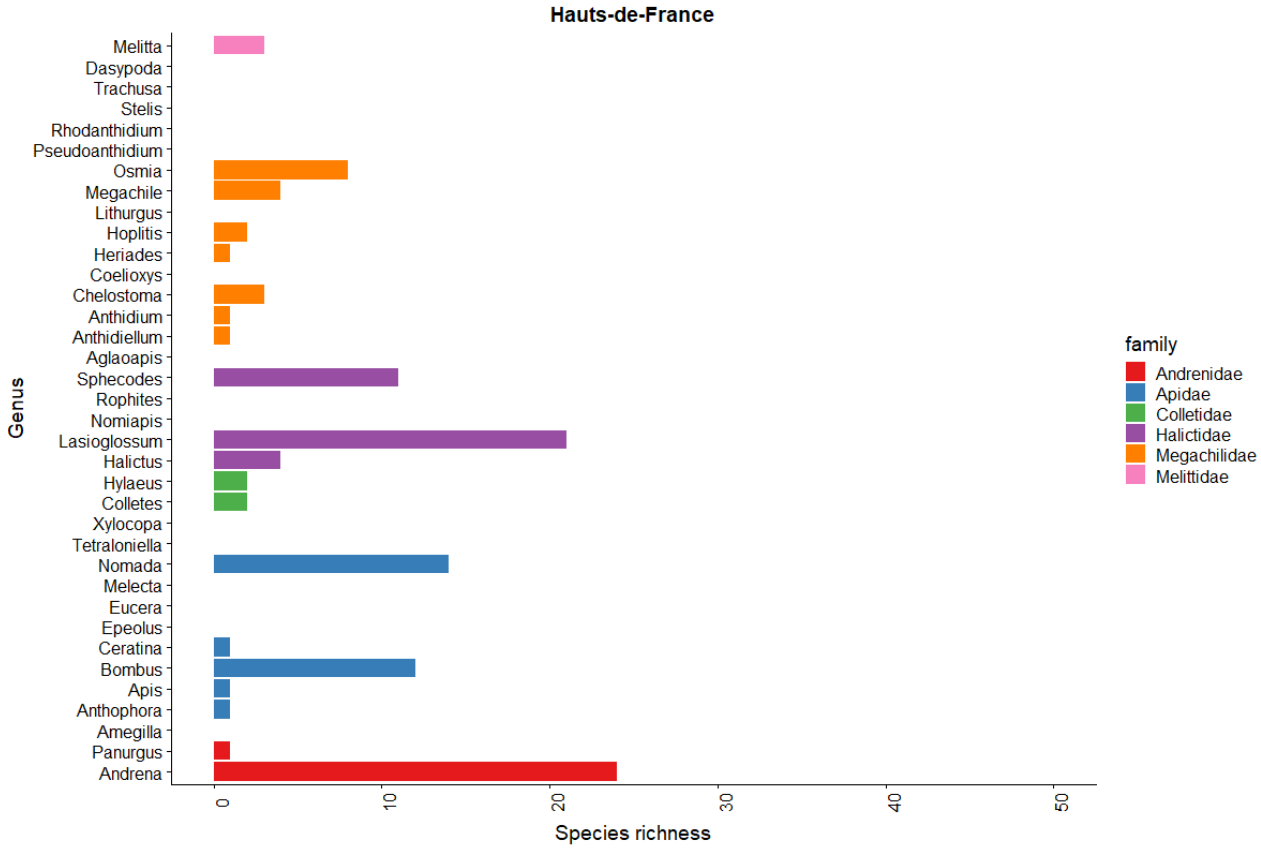
The most remarkably family-level differences that we can observe among regions are:

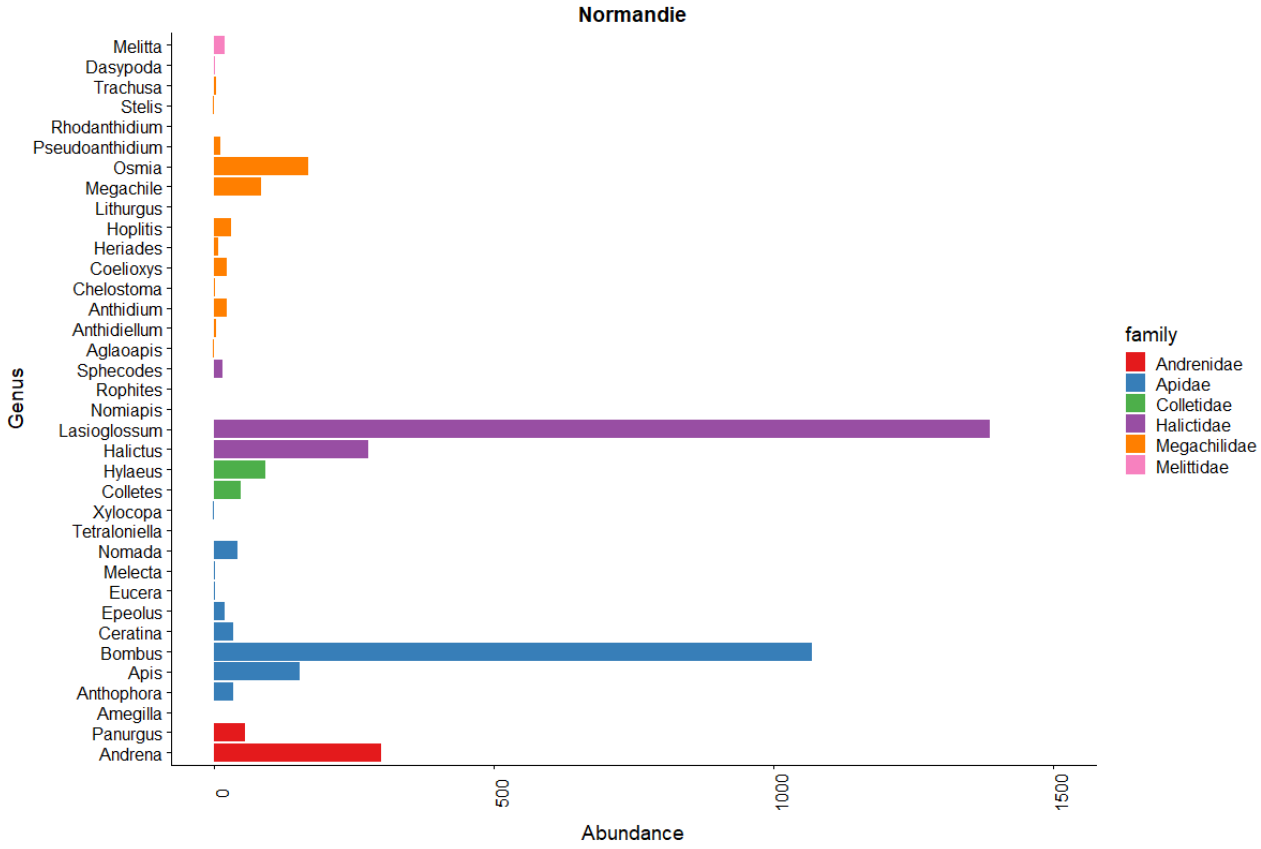
- **Andrenidae** (red colour in Fig. 1.5): the genus *Andrena* was the one with the highest number of sampled species (51 species overall). In the OCC region we sampled the highest diversity (40 species), doubling the number of species recorded in the other two regions. Conversely, the genus *Panurgus* (represent by 2 species) was never recorded in the OCC region.
- **Apidae** (blue colour in the Fig. 1.5): we sampled 11 genera overall. However, some genera such as *Tetraloniella* and *Amegilla* were only found in the OCC region. Also, the group *Eucera* was mostly present in the southern region, with only 1 species (*E. nigrescens*) also present in Normandie. We sampled individuals of the genera *Anthophora*, *Bombus*, *Ceratina* and *Nomada* in the three regions, but we found differences at the species level. For the genus *Bombus* we recorded the highest abundance in the HFR and NOR regions, but we recorded almost the same number of species in the three regions.
- **Colletidae** (green colour in the Fig. 1.5): the number of species for the two genera of this family, *Colletes* and *Hylaeus*, decreased along the gradient, with the higher number of species recorded in the OCC region.
- **Halictidae** (purple colour in Fig.1.5): was the family with the highest number of individuals sampled in all regions and the highest number of species considering all genera (n = 82 species), especially in the case of genus *Lasioglossum* which showed the highest number of individuals sampled overall (n = 4224), accounting for 50 species. Two genera of this family, *Nomiapis* and *Rophites*, were only found in the OCC region. For the genera *Halictus* we sampled the highest number of individuals in the HFR region, but in this region we also recorded the lowest number of species, only 4 species. In HFR, the species *Seladonia tumulorum* was the most abundant (n = 478 sampled individuals) followed by the species *Halictus rubicundus* (n = 133 individuals). Both of these species were absent or nearly absent in the OCC region.
- **Megachilidae** (orange colour in Fig. 1.5): was the family with the highest number of sampled genera (n = 14). For this family we sampled 75 species overall (11 species were shared among the three regions), and the number of genera and species decreased along the gradient (OCC

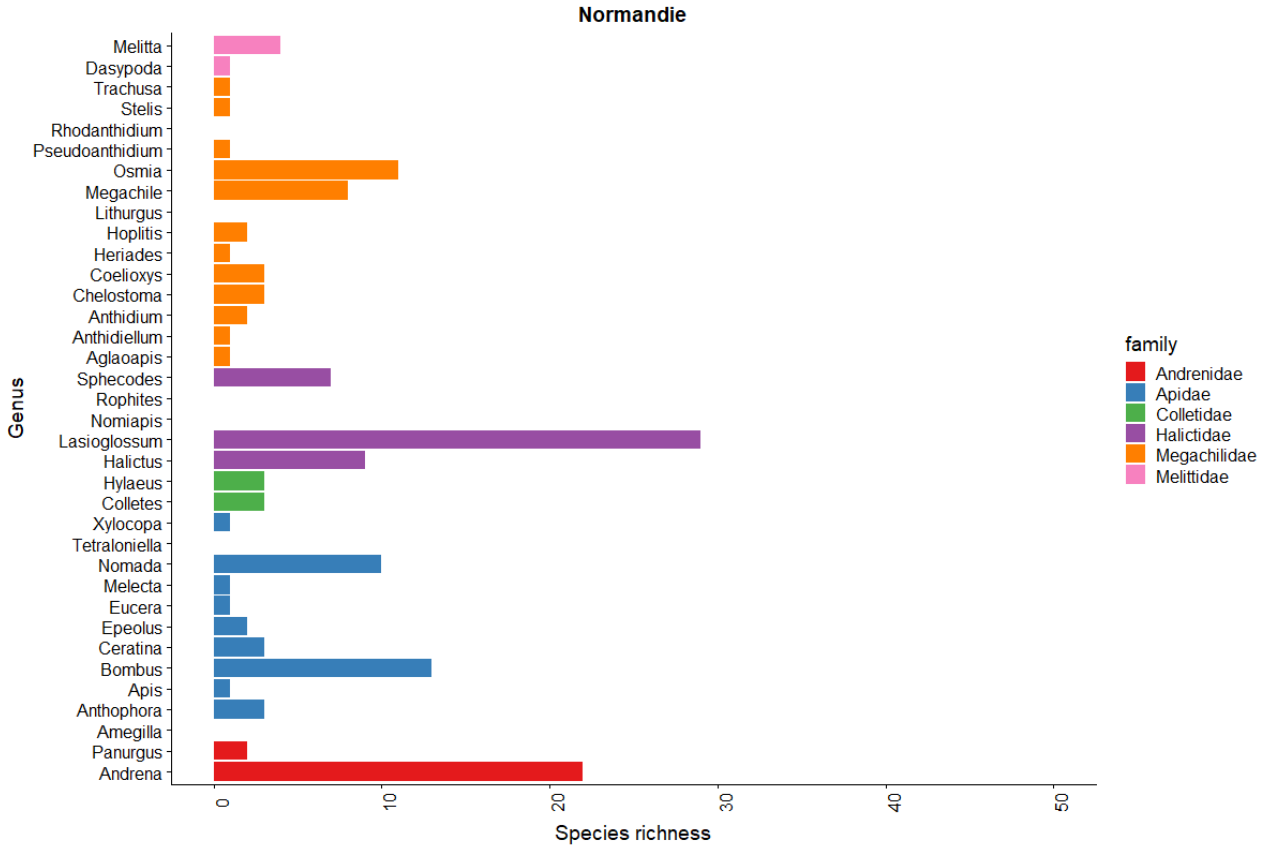
= 13 genera, 62 species; NOR = 12 genera, 35 species and HFR = 7 genera, 20 species). The species *Osmia bicolor*, was the most abundant species recorded in HFR that we did not found in OCC.

- **Melittidae** (pink colour in Fig. 1.5): we sampled two genera of this family, the genera *Dasypoda*, with only 2 individuals of *D. hirtipes* sampled in Normandie, and the genus *Melittidae*. This last genus was "underrepresented" in the OCC region. Indeed, we only sampled one species, *Melitta dimidiata*, which were not found in the other two regions. Most of the species in this family are oligolectic, which means that they are specialised in one single genus of flowering plants. For example, *Melitta haemorrhoidalis* is specialised in the genus *Campanula*, while *M. dimidiata* is specialised in the genus *Onobrychis* and *M. tricincta* in the genus *Odontites*. Even if *M. leporina* is one of the most widespread species of this genus, with *M. tricincta* and *M. haemorrhoidalis*, we only sampled one individual in the NOR region.
- **Syrphidae** (Fig. 1.6): for this family we sampled a similar number of genera among the three regions, but we recorded the highest diversity in the OCC region. The most remarkably difference with the other two regions is for the species of the genus *Merodon*. We sampled 11 species of this genus in the OCC region, in contrast with 1 species in HFR and 3 species in NOR. However, 3 genera (*Xylota*, *Orthonevra* and *Eristalinus*) were only found in the HFR region.









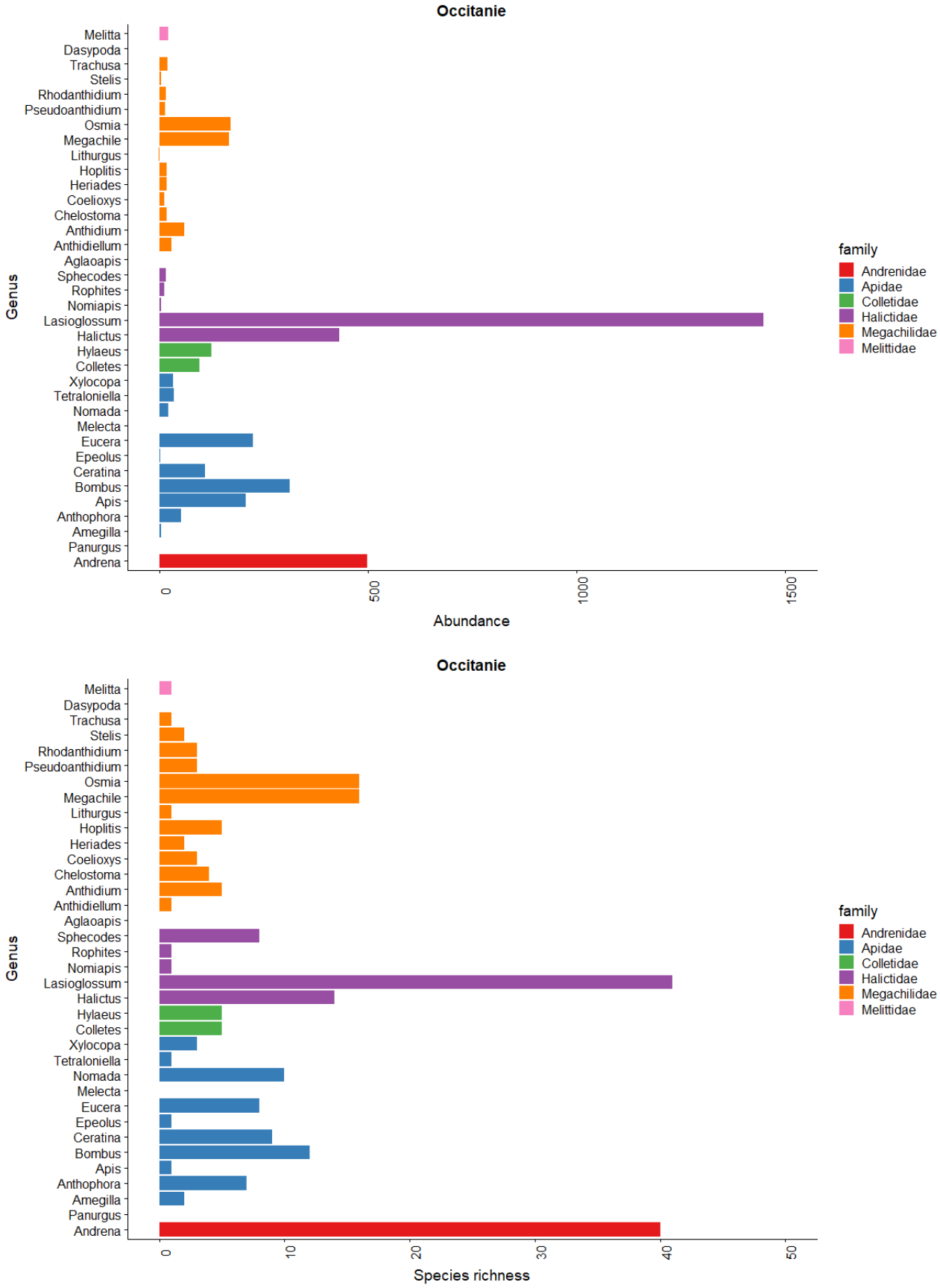
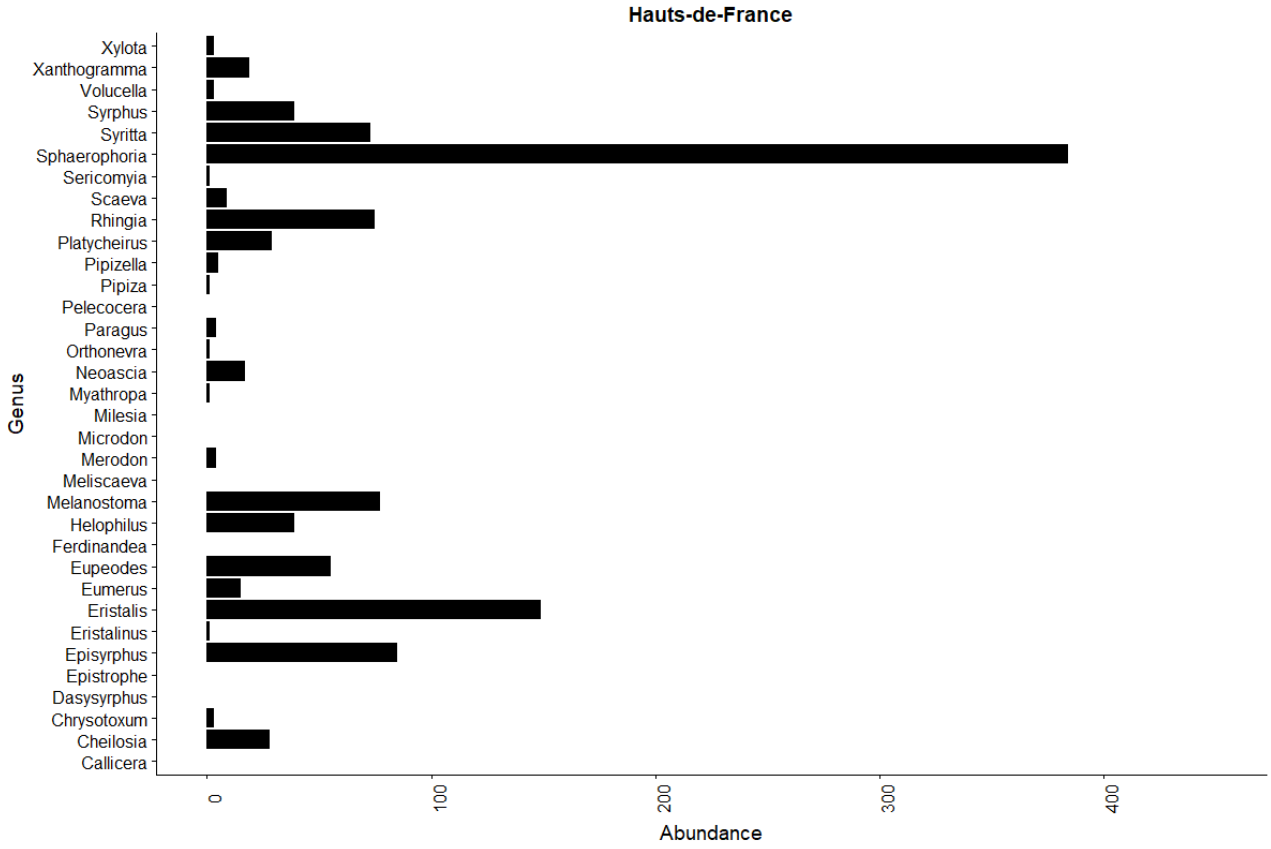
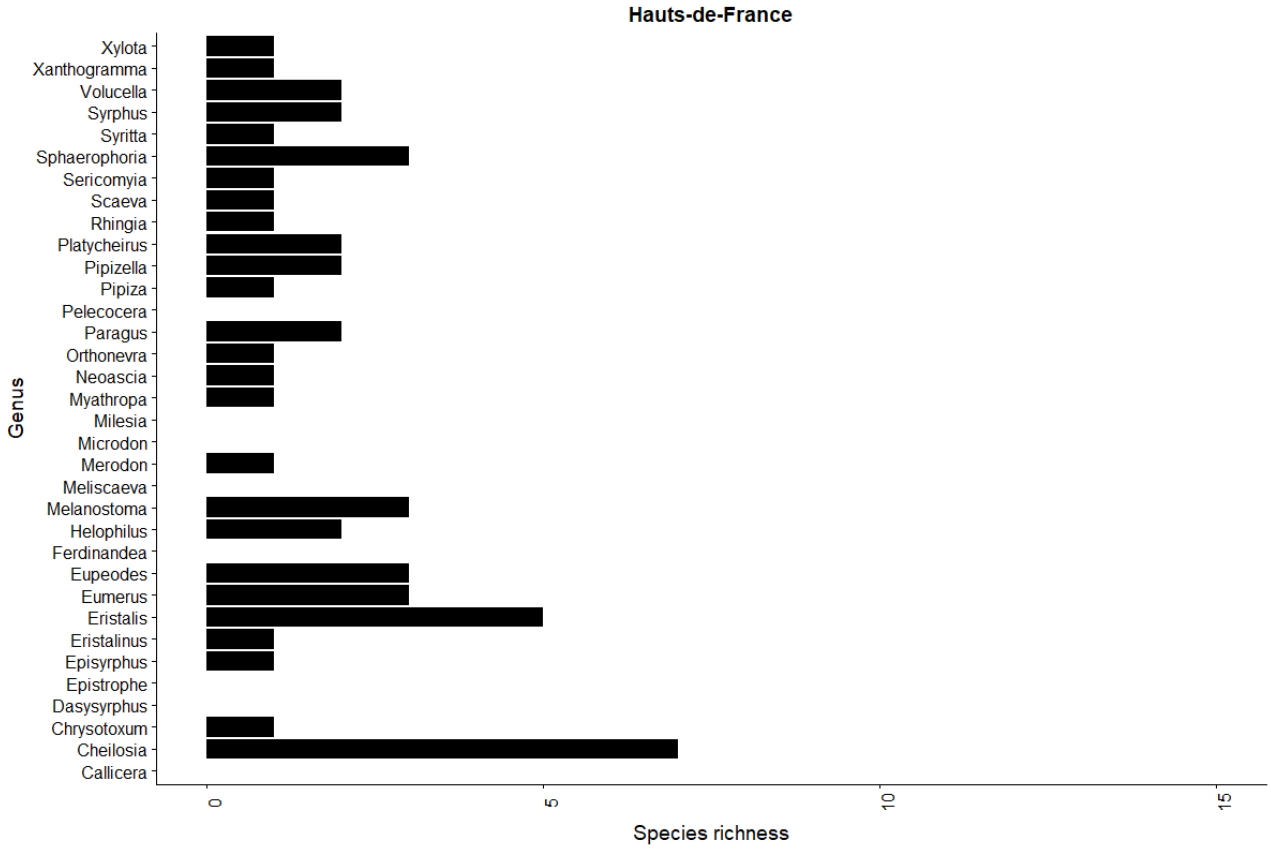
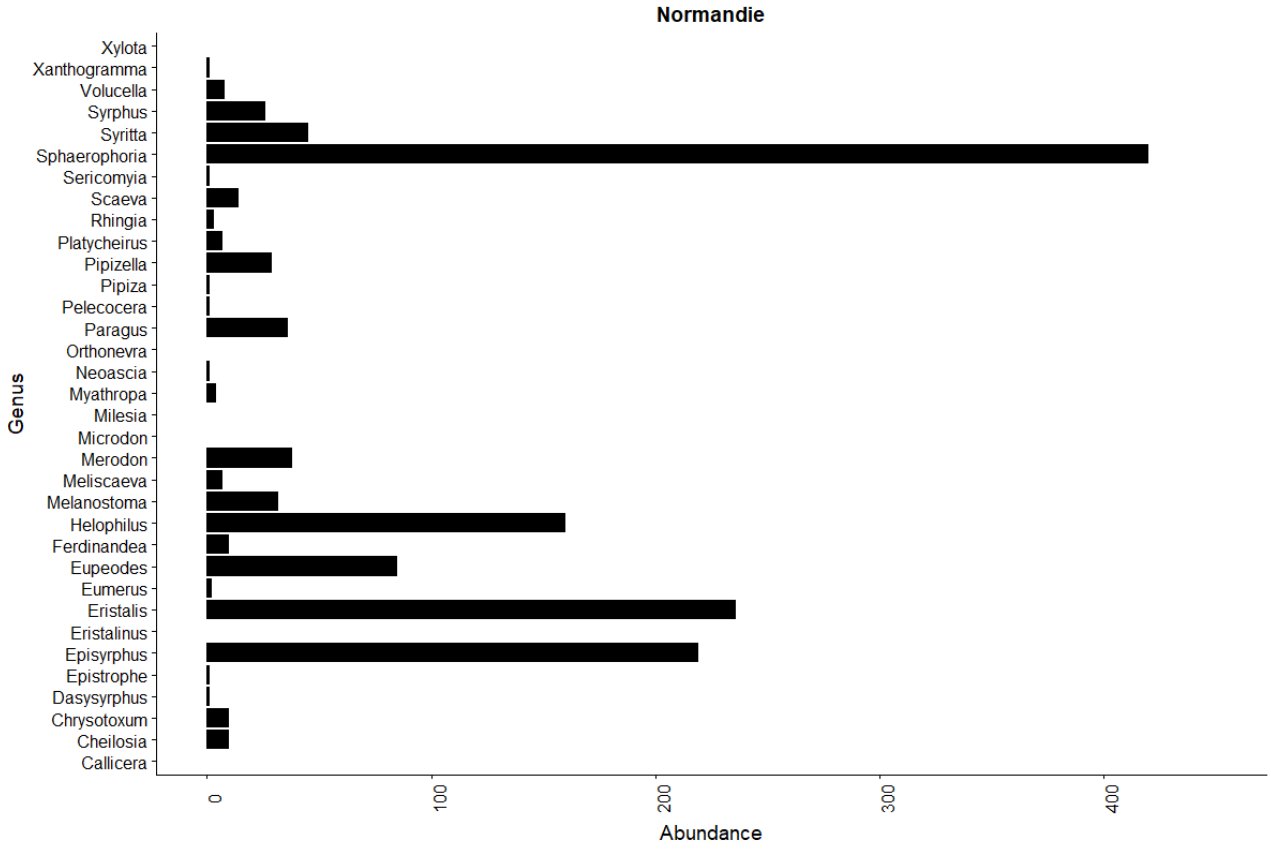
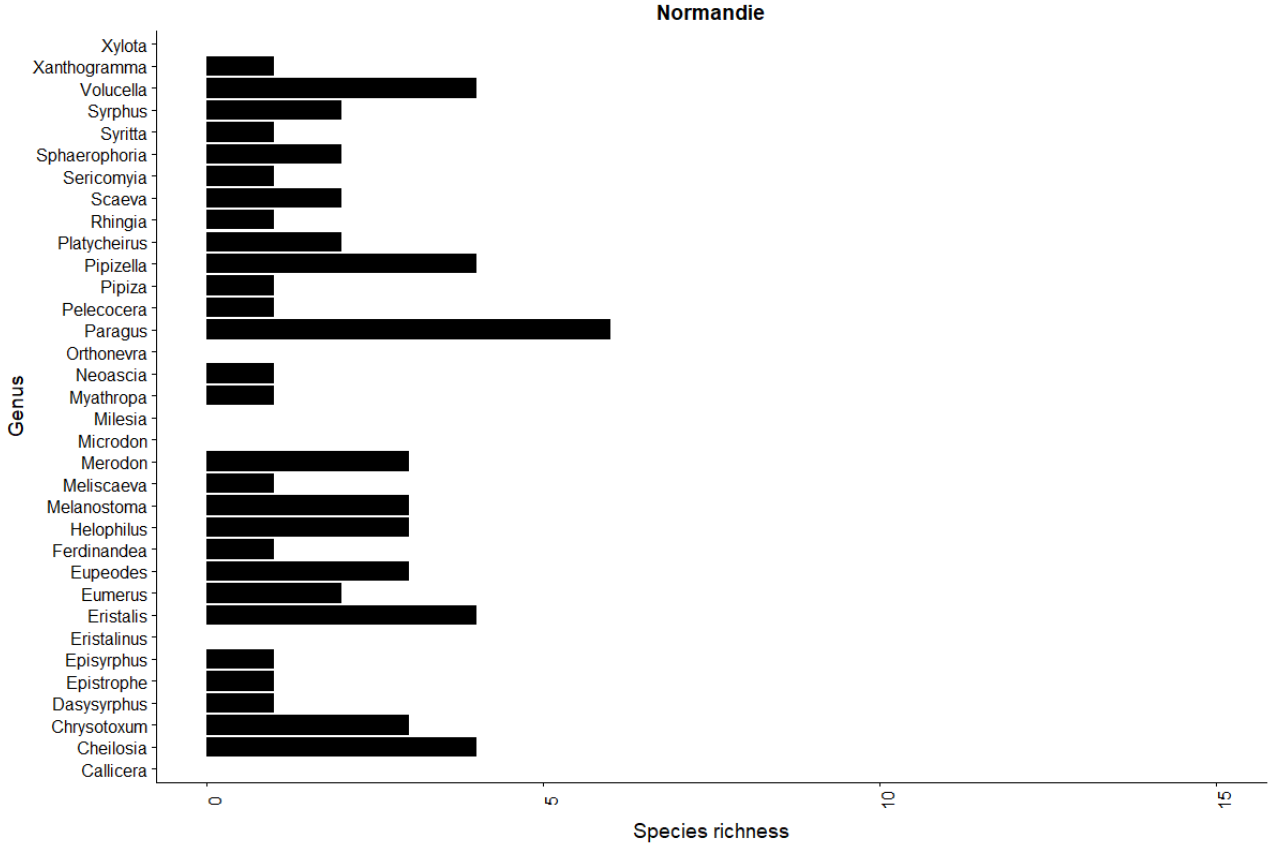


Figure 1. 5. Anthophila families, genera and species abundance (upper panel) and richness (lower panel) observed in the three regions Hauts-de-France, Normandie and Occitanie.









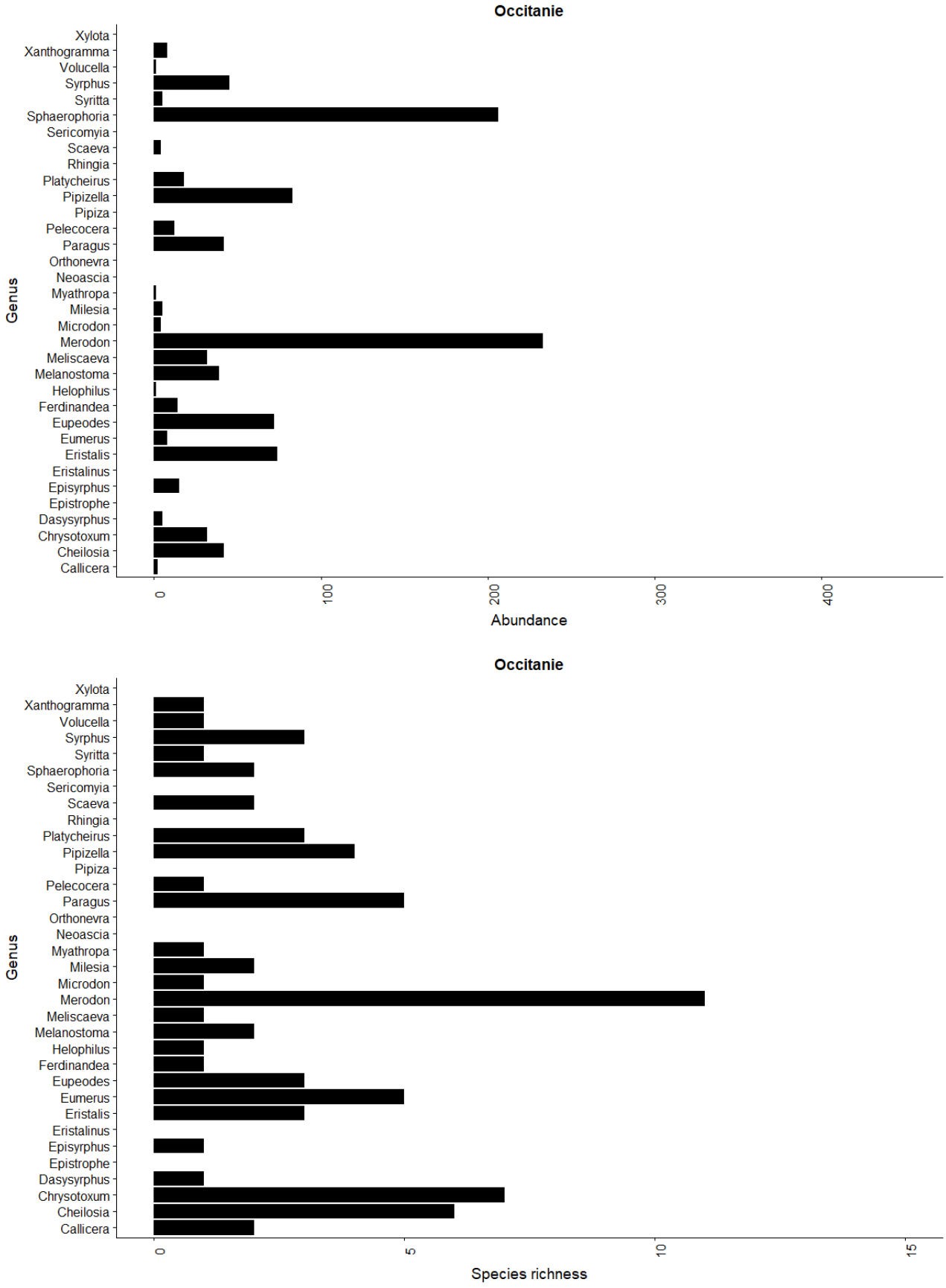


Figure 1. 6. Syrphidae genera and species abundance (upper panel) and richness (lower panel) observed in the three regions Hauts-de-France, Normandie and Occitanie.

Plant communities – We separated the plant species in 38 families and 164 genera, overall. We recorded the highest number of plant species in Occitanie (n = 221 species), followed by the Hauts-de-France (n = 94 species) and Normandie region (n = 92 species). The plant families for which we recorded the highest number of species (> 10) were (Fig. 1.7): Asteraceae (n = 54 species), Fabaceae (n = 38 species), Orchidaceae (n = 22 species), Lamiaceae (n = 20 species), Caryophyllaceae (n = 14 species), Brassicaceae (n = 13 species), Plantaginaceae (n = 12) and Apiaceae (n = 11). Even if most plant families were present in all regions, 13 families were unique to one region (Fig. 1.7). Only two plant families were unique to HFR (Scrophulariaceae and Cyperaceae), 4 families were only recorded in NOR and most of them were unique to OCC.

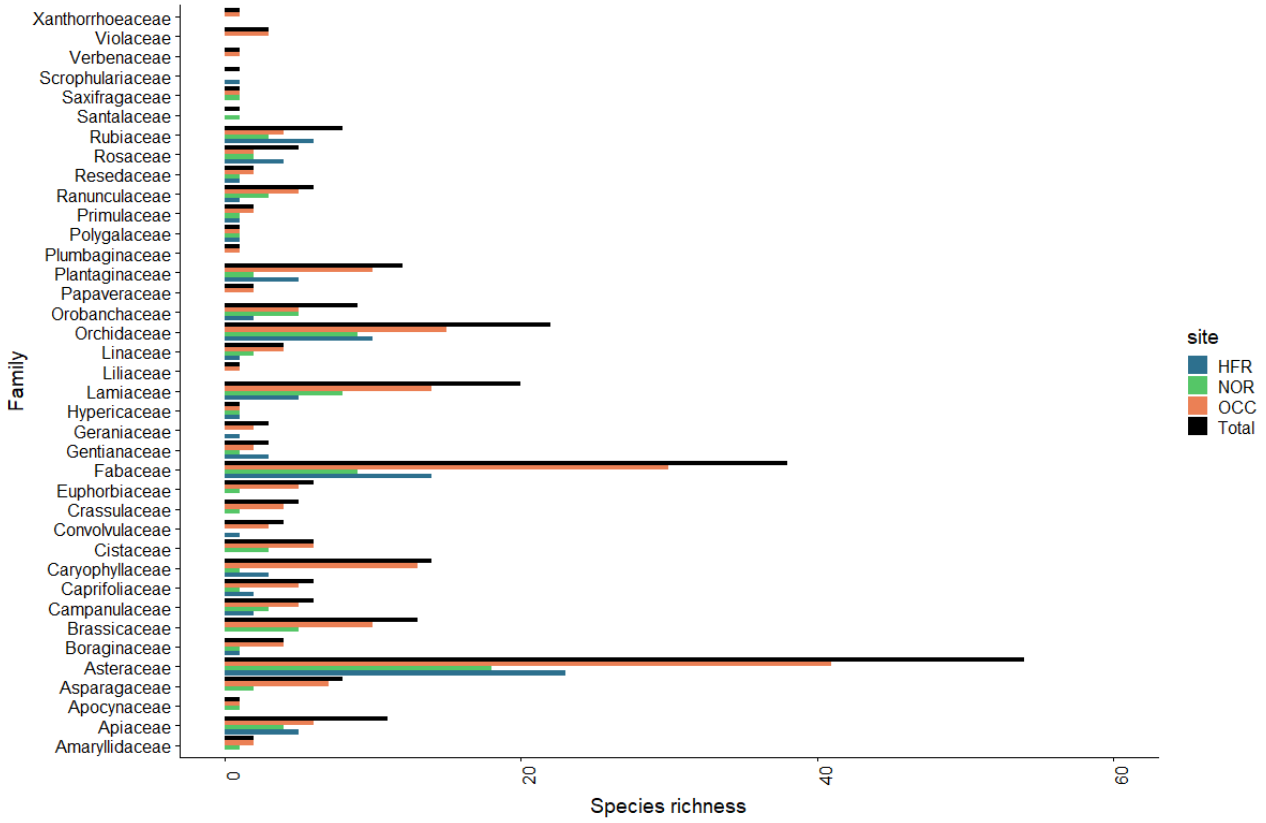
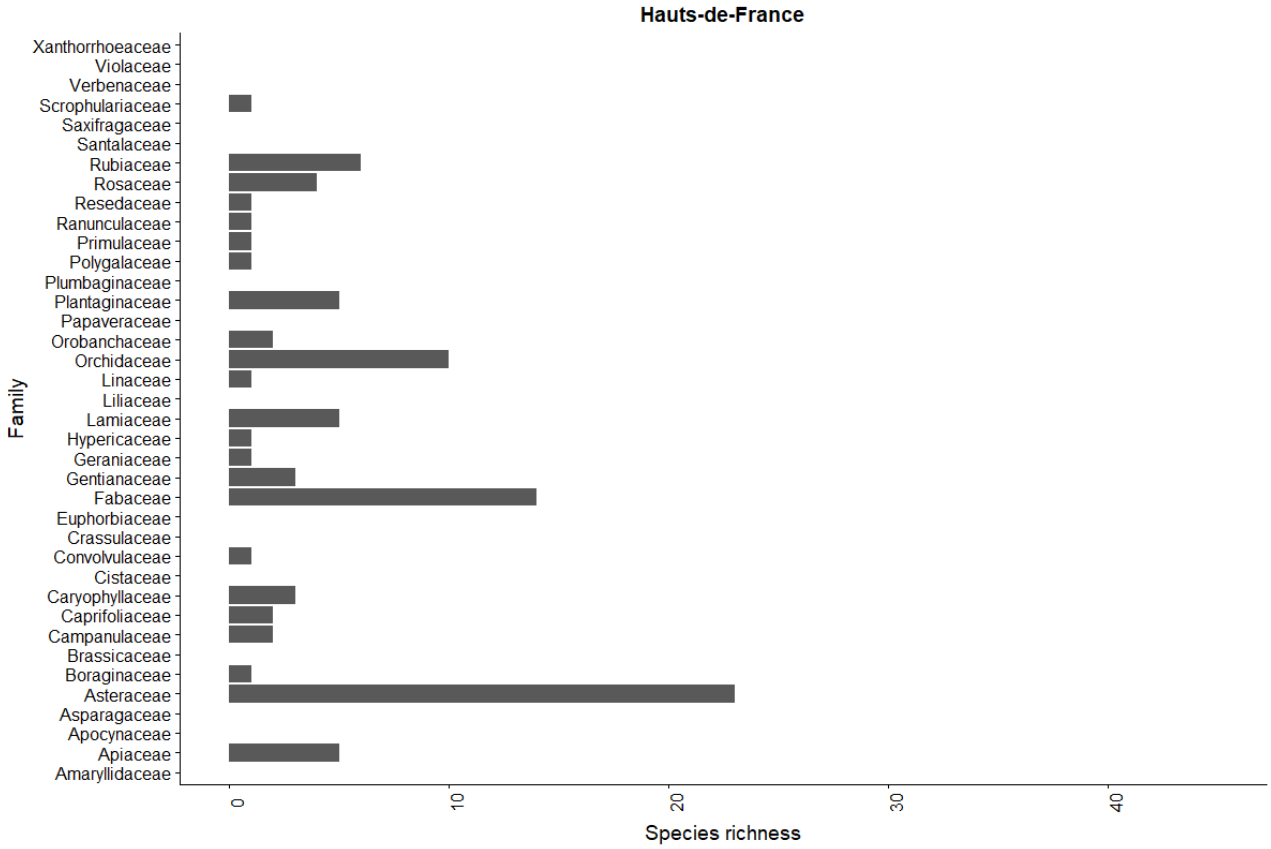
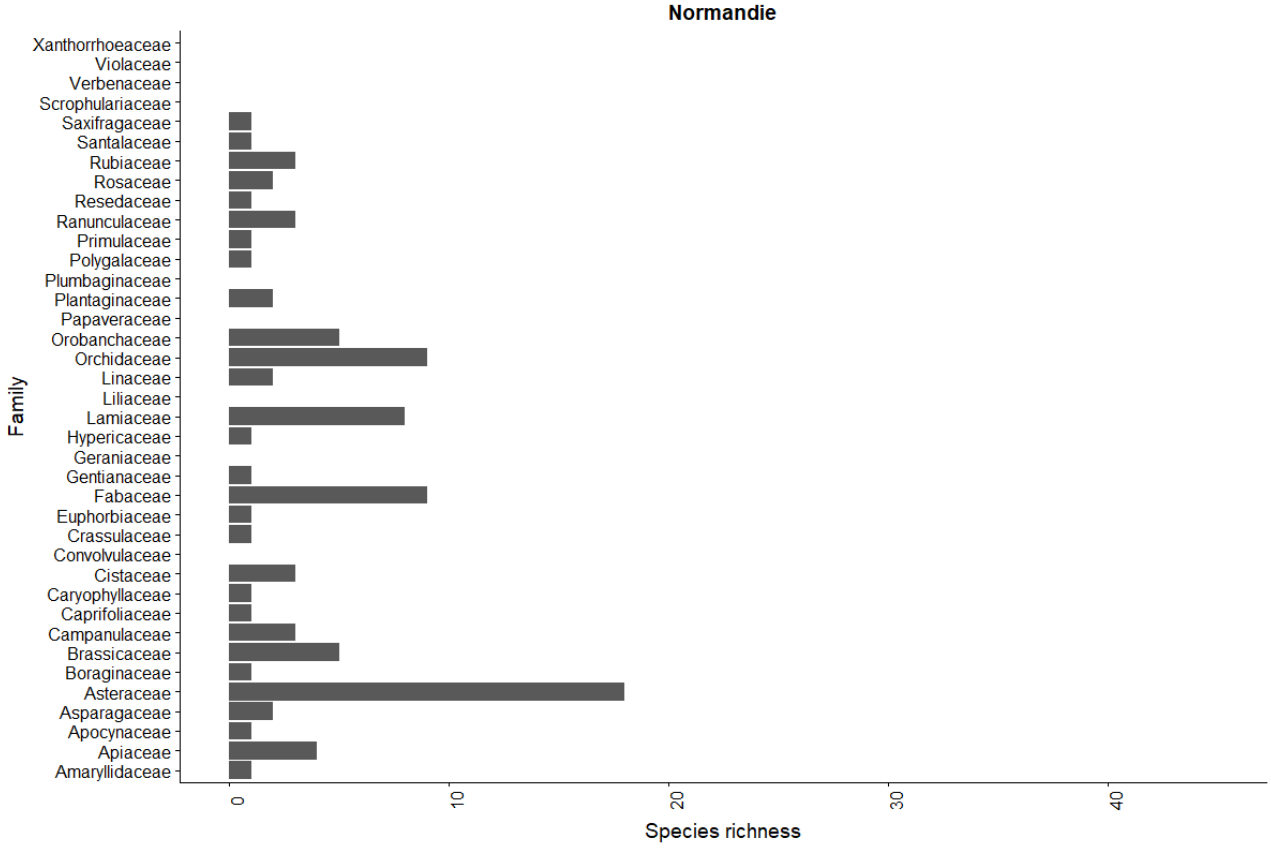


Figure 1. 7. Plant families and species richness in the three regions Hauts-de-France (HFR), Normandie (NOR) and Occitanie (OCC).

Plant communities differed greatly among regions (Fig. 1.8): among the 288 recorded species, only 9% (n = 26 species) were shared among the three regions, while 68% were unique to one region. Particularly, 26 species were unique to HFR, 21 to NOR and 148 species to OCC. Some species were indeed typical of the southern region, such as *Narcissus juncifolius, Aphylllanthes monspeliensis, Linum narbonense*.





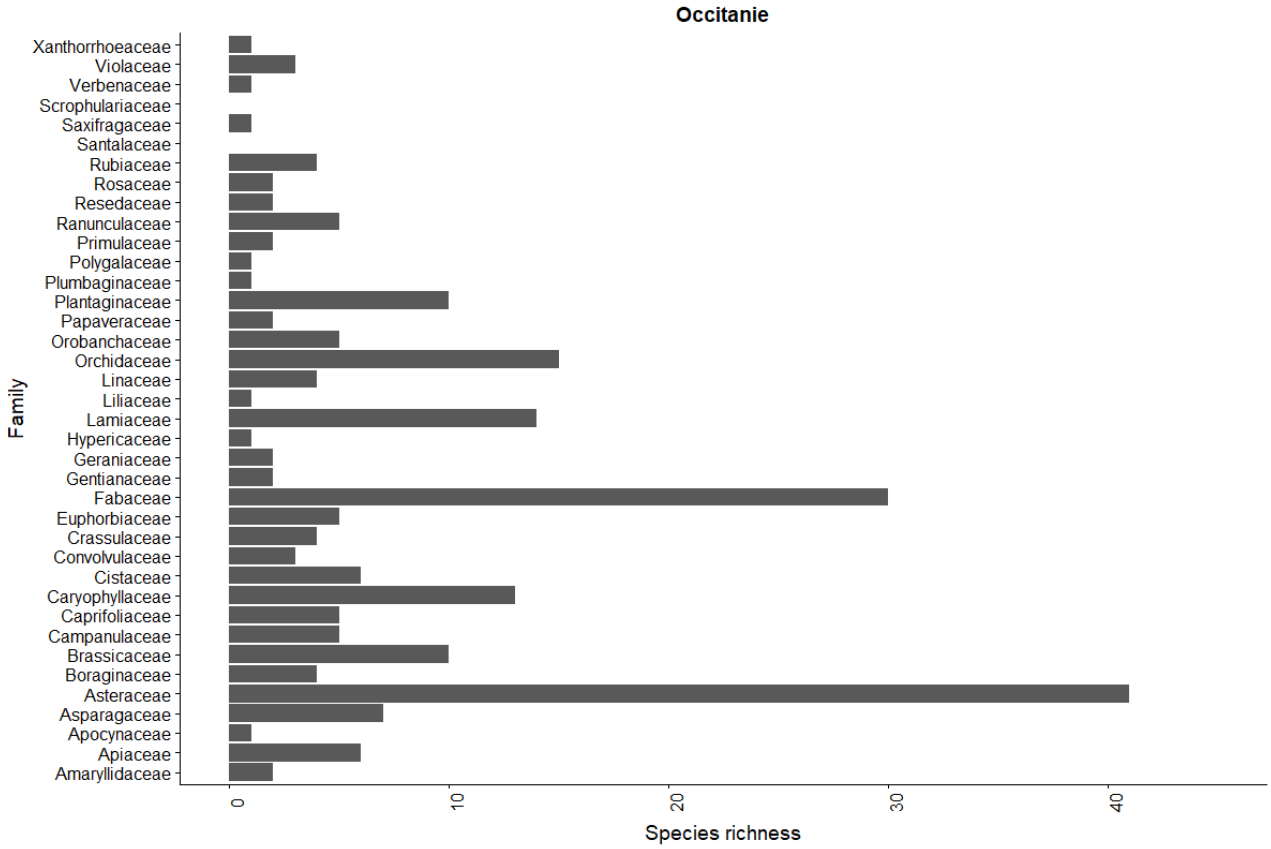


Figure 1. 8. Plant families and species richness observed in the three regions Hauts-de-France (upper panel), Normandie (middle panel) and Occitanie (lower panel)

Plant and pollinator phenologies

In each region, plant and pollinator communities also differed greatly among months (Fig. 1.9), due to differences in species phenologies (Fig. Supplementary materials). In general, plant species with longer phenologies are uncommon, and a highest proportion of species display short phenologies. In the region Occitanie most plant species had shorter phenologies, with some flowering only one month (n = 89 species), two months (n = 76), or three months (n = 38). Only two species, *Crepis foetida* and *Biscutella laevigata*, were flowering for a longer period (6 or 7 months). The richest month was June, during which we recorded 121 flowering plant species in the two years of sampling. In Normandie most plant species were flowering for two months and few species (15 species) were flowering almost all the season (between 5 and 7 months). The richest month was also the month of June with 53 recorded plant species for the two years of fieldwork. A similar pattern was also observed in Hauts-de-France, but the richest month was August with 54 species recorded when we pooled the two years of sampling.

As observed for plant species, pollinators mostly showed short phenologies, especially in OCC, in both bee and hoverfly species. Among bees with longer phenologies, we observed several species of the genera *Bombus* and *Lasioglossum*, both in Normandie and Hauts-de-France.

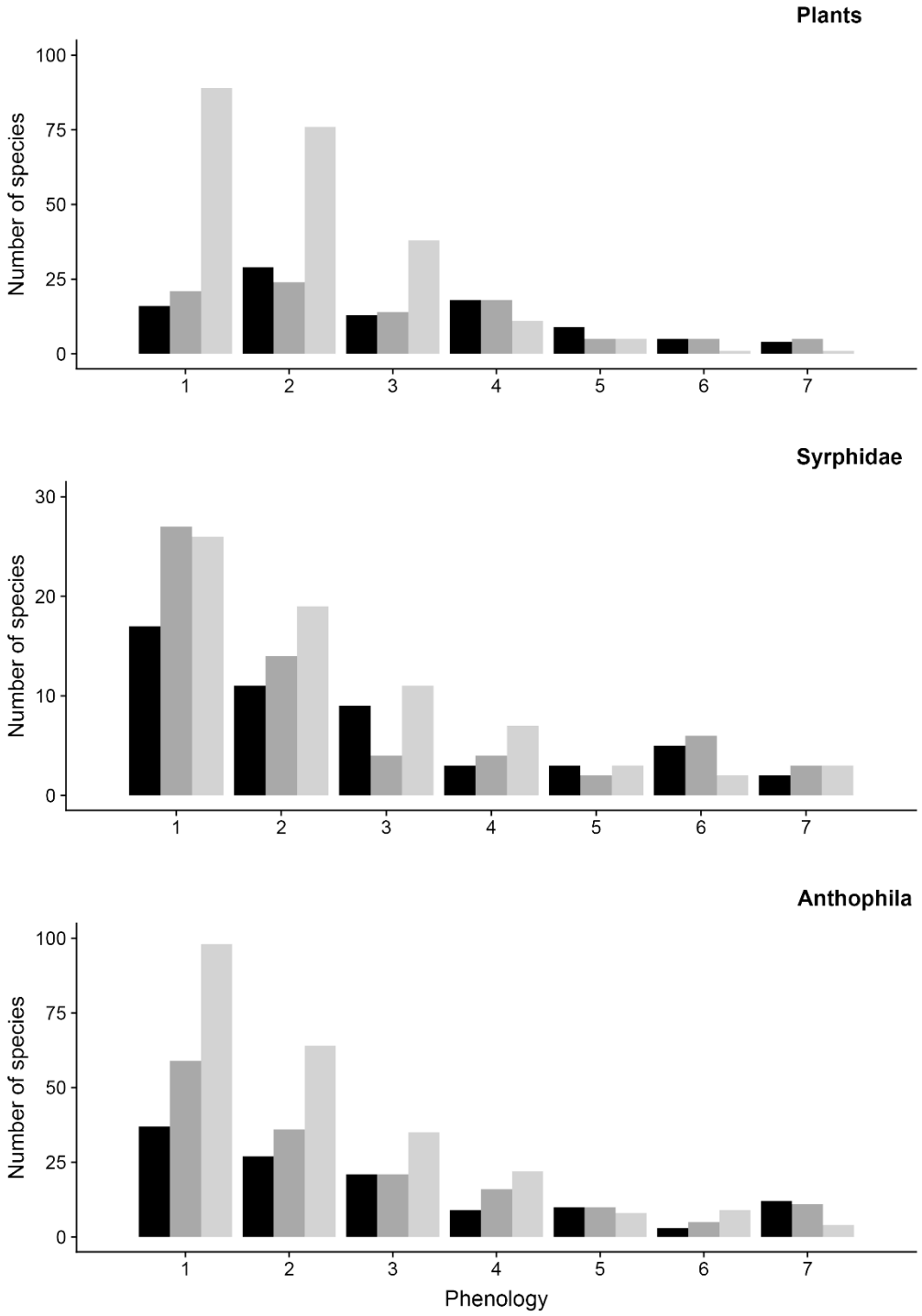


Figure 1. 9. Plant and pollinator (Syrphidae and Anthophila) species phenology. Phenology indicates the number of months in which a plant species flowered, and an insect species was sampled, e.g. 1 = observed only in one month, 2 = observed in two months, etc.

Species diversity

Regional γ diversity also reflected the latitudinal gradient when we pooled all samples, in both plants and insects (Fig. 1.10). We observed that between-region β diversities between Occitanie and Normandie and Occitanie and Hauts-deFrance were higher than between Normandie and Hauts-de-France. Between-site β diversity within a given region was always lower than between-region β diversities.

Interannual variation of species diversity

Despite the differences in abundances between 2016 and 2017, between-year β diversity was quite low for both plants and insects (Fig. 1.10). Moreover, diversity trends were constant in both years, with the two southern sites displaying the highest diversity of both plant and insect species. Several studies at the landscape level have found a positive relationship between the diversity of floral resources and the diversity and abundance of pollinators (Hegland & Boeke 2006). For insects, between-year β diversity was higher for pan trap captures (β diversity = 0.15) than that observed with hand nets (β diversity = 0.09). Passive sampling was indeed more variable than active sampling and not all insects are equally attracted by pan traps in general (Westphal *et al.* 2008). For example, with the pan traps we sampled 161 hoverflies in 2016 and only 27 in 2017, overall. Since the number of insect individuals sampled using pan traps was too low to allow calculating most β diversity measures between and within regions, in the following we refer to "pollinator diversity" as the diversity calculated using only insects sampled with hand nets.

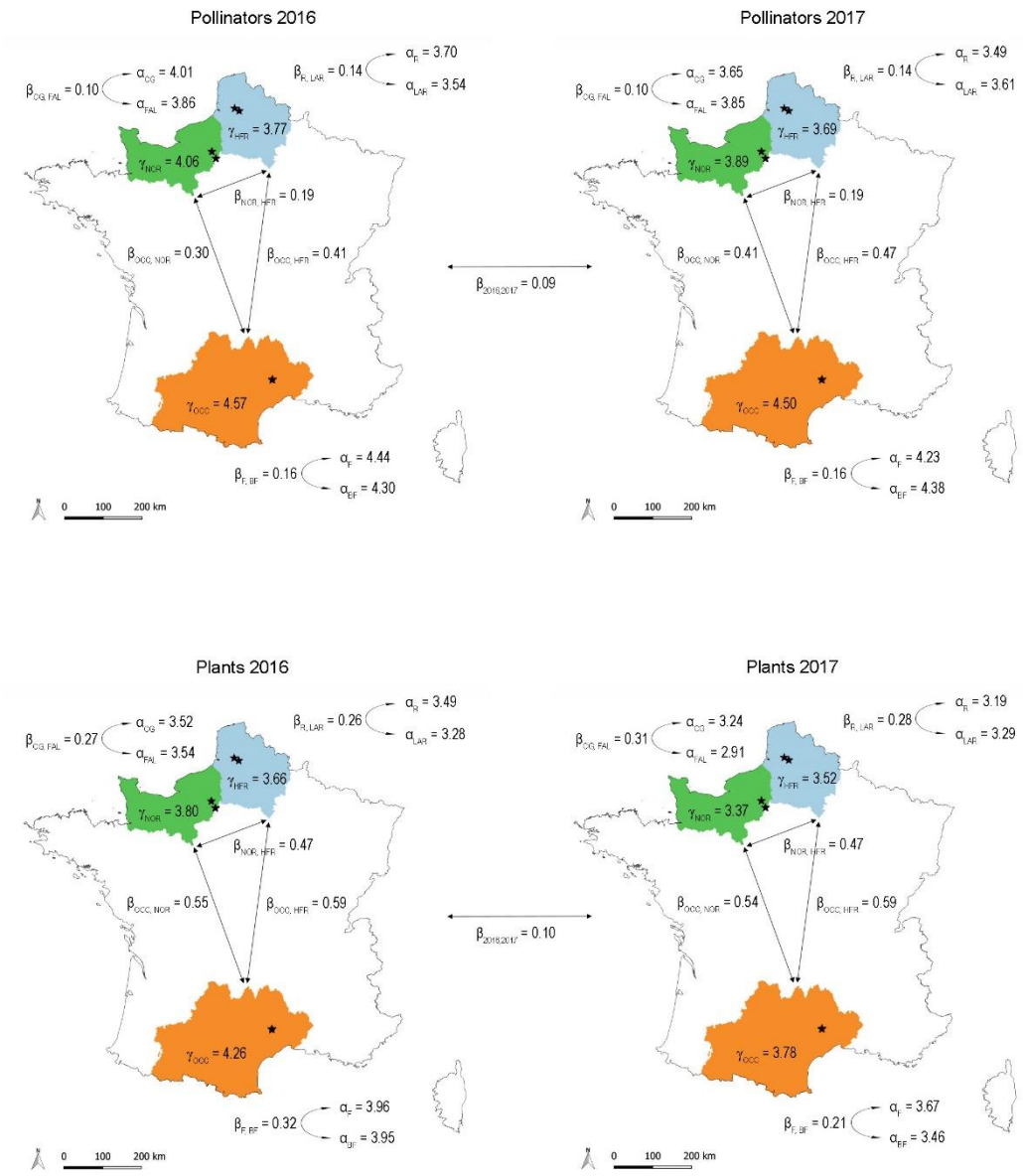


Figure 1. 10. Pollinator and plant α, β and γ diversities in and between the three regions (OCC, Occitanie in orange; NOR, Normandie in green and HFR, Hauts-de-France, in blue) and six locations (BF, Bois de Fontaret; F, Fourches; CG, Chateau Gaillard; FAL, Falaises,; LAR, Larris and R, Riez) in 2016 and 2017. Overall β diversity between years is shown for plant and pollinators.

Year-long variation of species diversity patterns

If we compare α and γ diversities along the season, we can observe temporal fluctuations of diversity between and within regions in both years, for both insects (Fig. 1.11 and 1.12) and plants (Fig. 1.13 and 1.14).

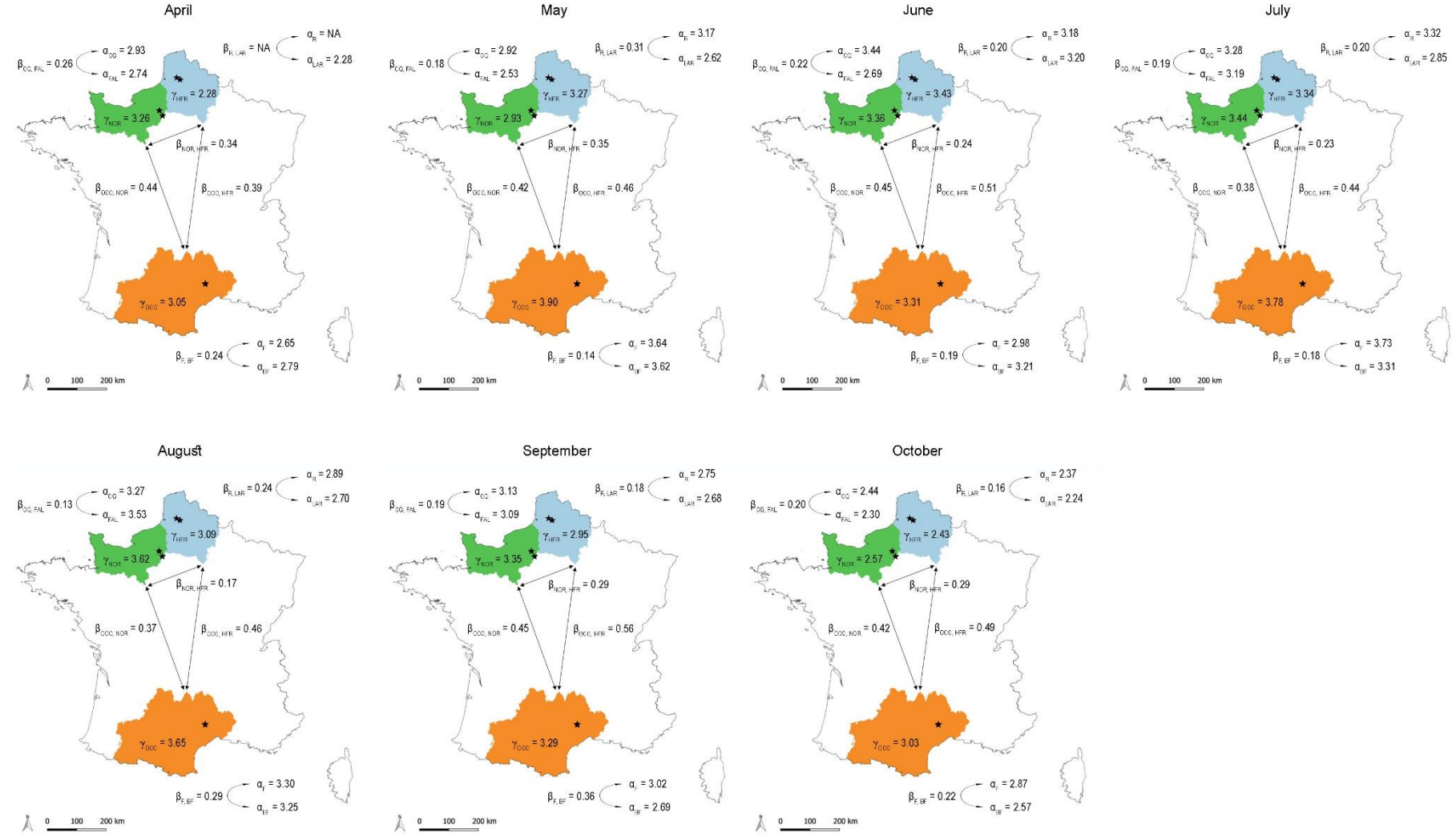


Figure 1. 11. Pollinator diversity along the season, from April to October 2016.

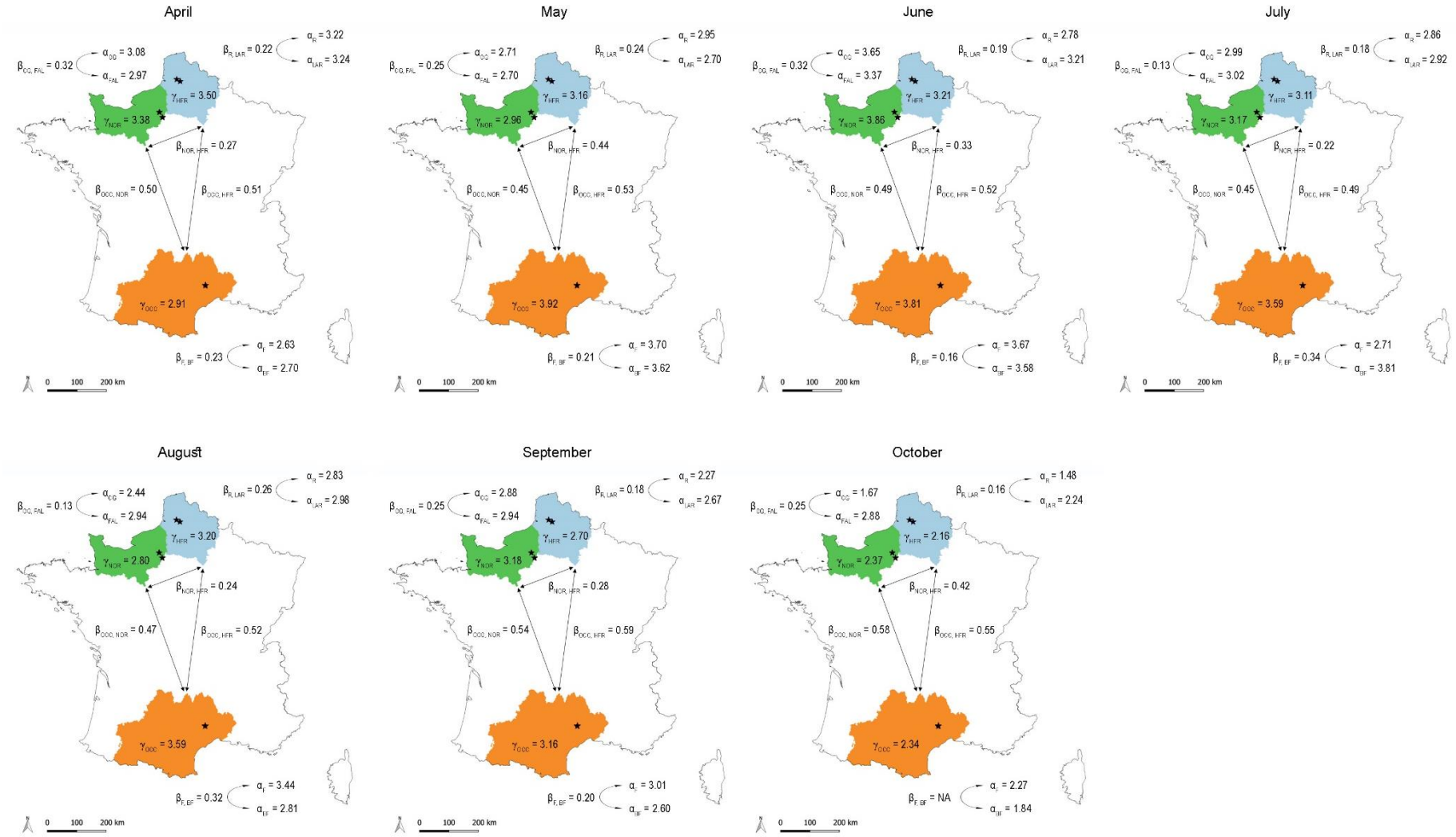


Figure 1. 12. Pollinator diversity along the season, from April to October 2017.

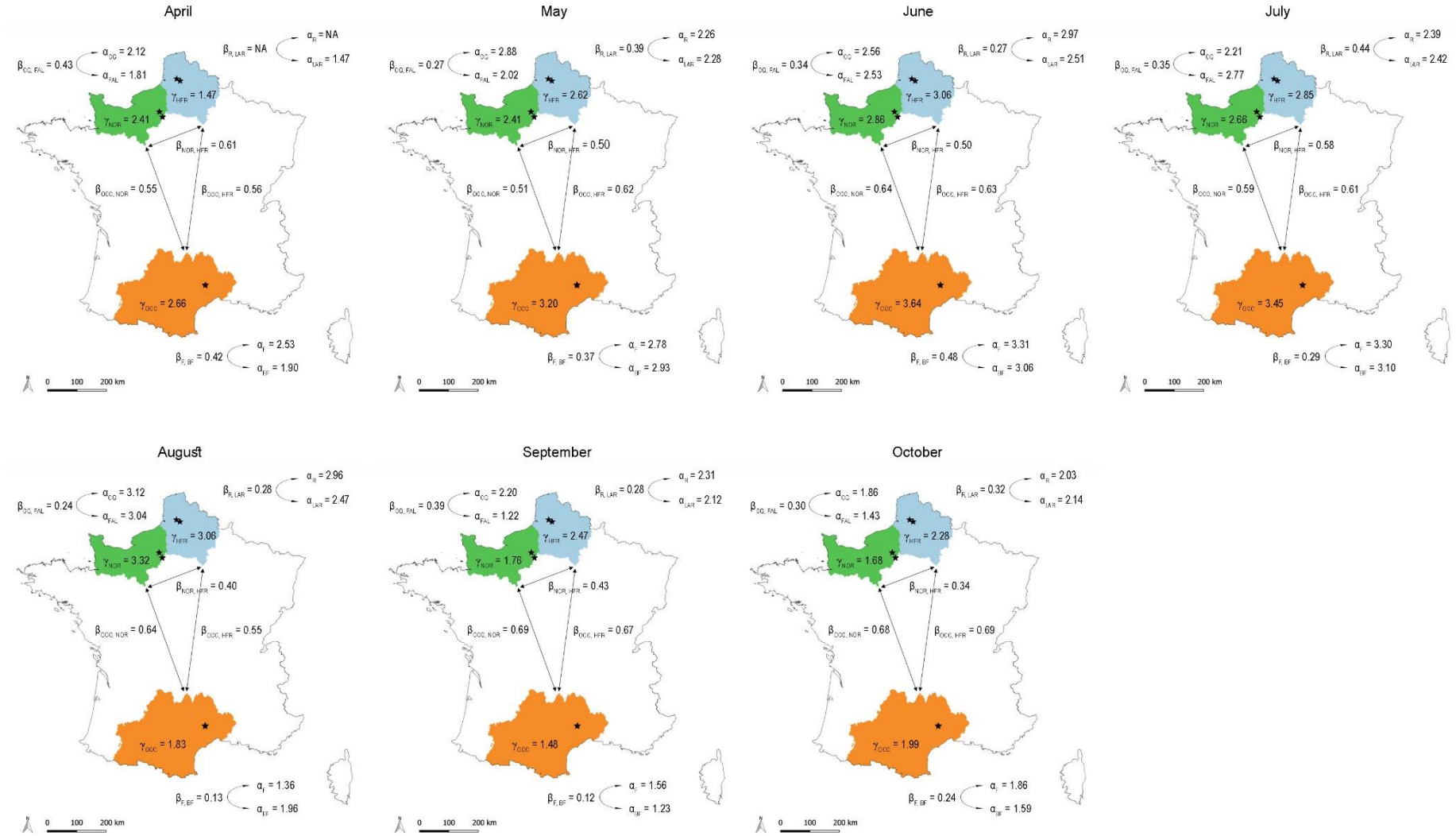


Figure 1. 13. Plant diversity along the season, from April to October in 2016.

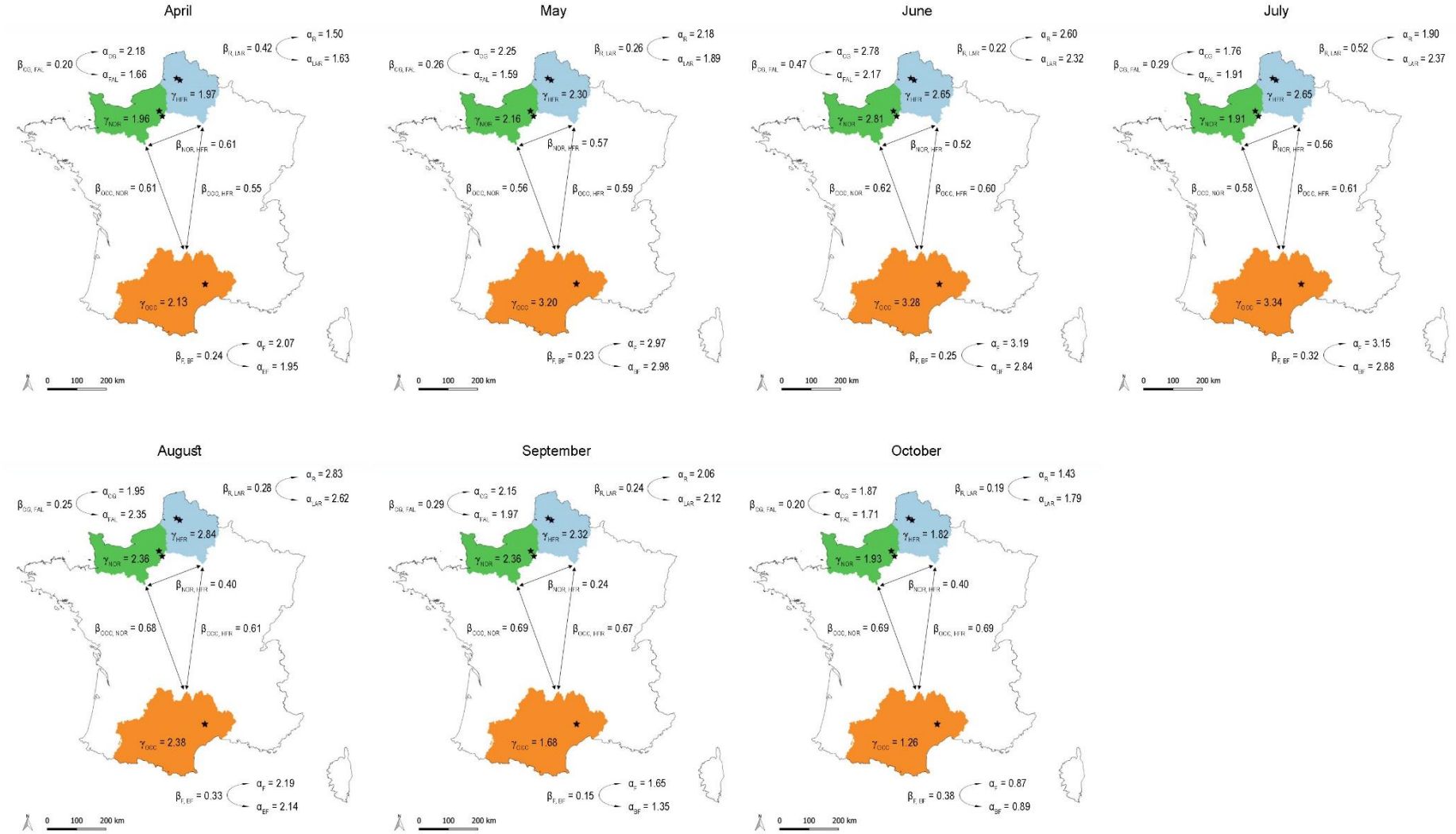


Figure 1. 14. Plant diversity along the season, from April to October 2017.

In 2016, regional and within-site plant diversity progressively increased, with a peak in June and July in Occitanie, which continued until August in Normandie and Hauts-de-France (Fig. 1.13). Plant diversity decreased in September and was lowest in October (end of the fieldwork season), except in Hauts-de-France. In the same year, regional and within-site pollinator diversity increased along the season with a peak in spring and summer, in particular during the month of May for the Occitanie region, August in Normandie, and June for the Hauts-de-France region. The lowest pollinator diversities were observed in the month of October.

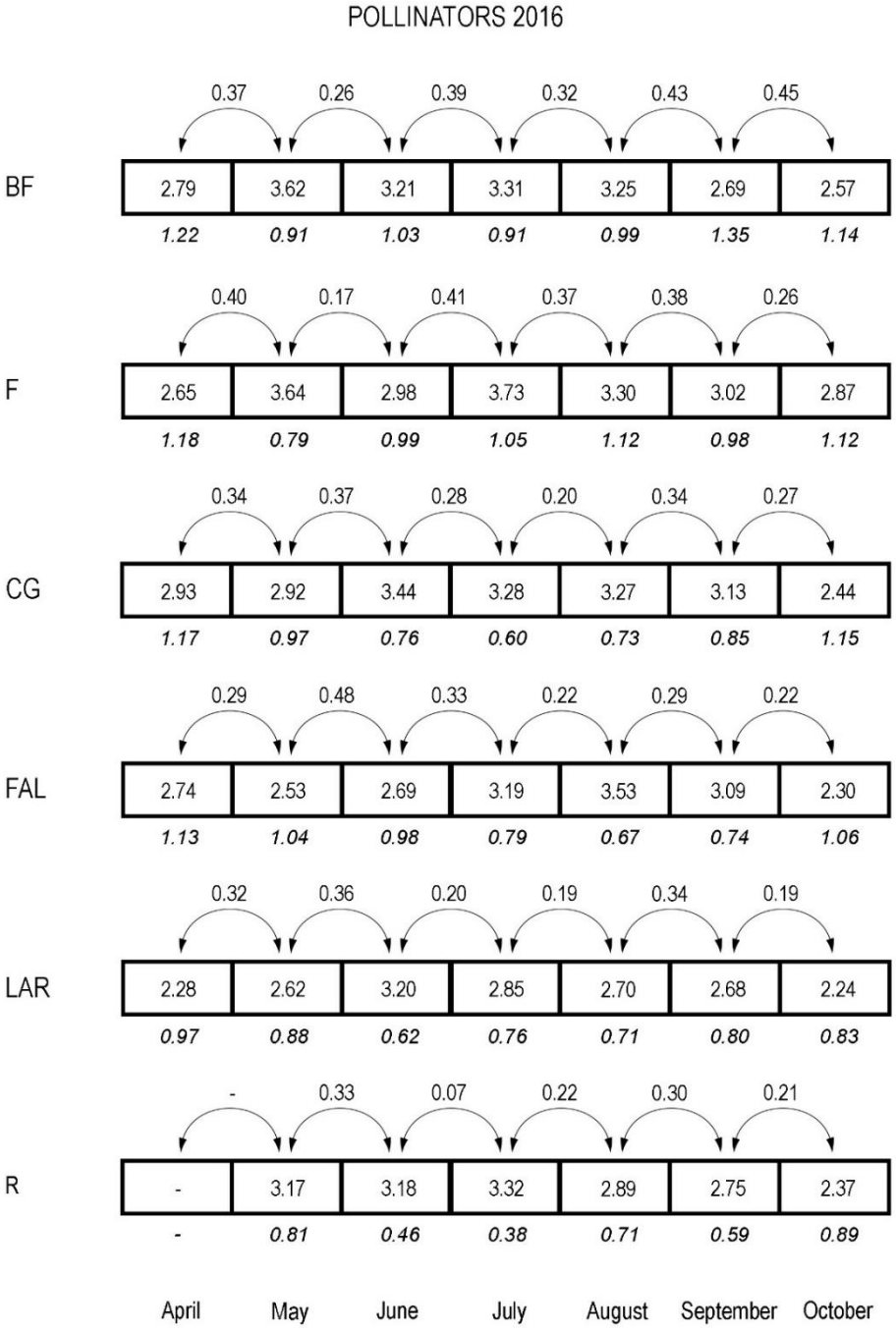
In 2017, trends were similar to those of 2016 for plant diversities at the regional and site scales (Fig. 1.14), but not for insect diversities (Fig. 1.12). We observed earlier peaks of insect γ diversity in Hauts-de-France and Normandie, *i.e.* in April and June, respectively. Pollinator diversity patterns did not change in the region Occitanie.

Monthly between-region β diversities for plants and pollinators were always higher when comparing the regions Occitanie and Normandie and the regions Occitanie and Hauts-deFrance than between Normandie and Hauts-de-France, in both 2016 and 2017 (Fig. 1.12 to 1.14). However, within-region β diversity (*i.e.* between sites) did not show any clear pattern, either for plants or pollinators, either in 2016 or 2017 (Fig. 1.12 to 1.14).

Temporal beta diversity

Pollinator temporal turnover (β diversity between successive months in a given site) showed higher values between May and June in the northern (LAR and R) and mid-latitude sites (CG and FAL) than in the southern sites (BF and F), in both years (Fig. 1.15). Plant turnover (Fig. 1.16) and monthly partial β diversities of both plants and insects showed a first peak of originality in the month of April which continued in months of May and June in both Normandie and Hauts-de-France regions, and a second peak in July for both the southern sites (Figs. 1.15 and 1.16).

For both insects and plants, the most important factors affecting β diversities between sampling sessions were the identity of the site and the sampling month (Table 1.1). Year effect was less important than effects of site and sampling month when tested in insects and was not significant in plants. In fact, in our study between-year β diversity was very low in both insects (β diversity = 0.09) and plants (0.10), compared to other studies which found a high temporal variation between years (Petanidou *et al.* 2008).



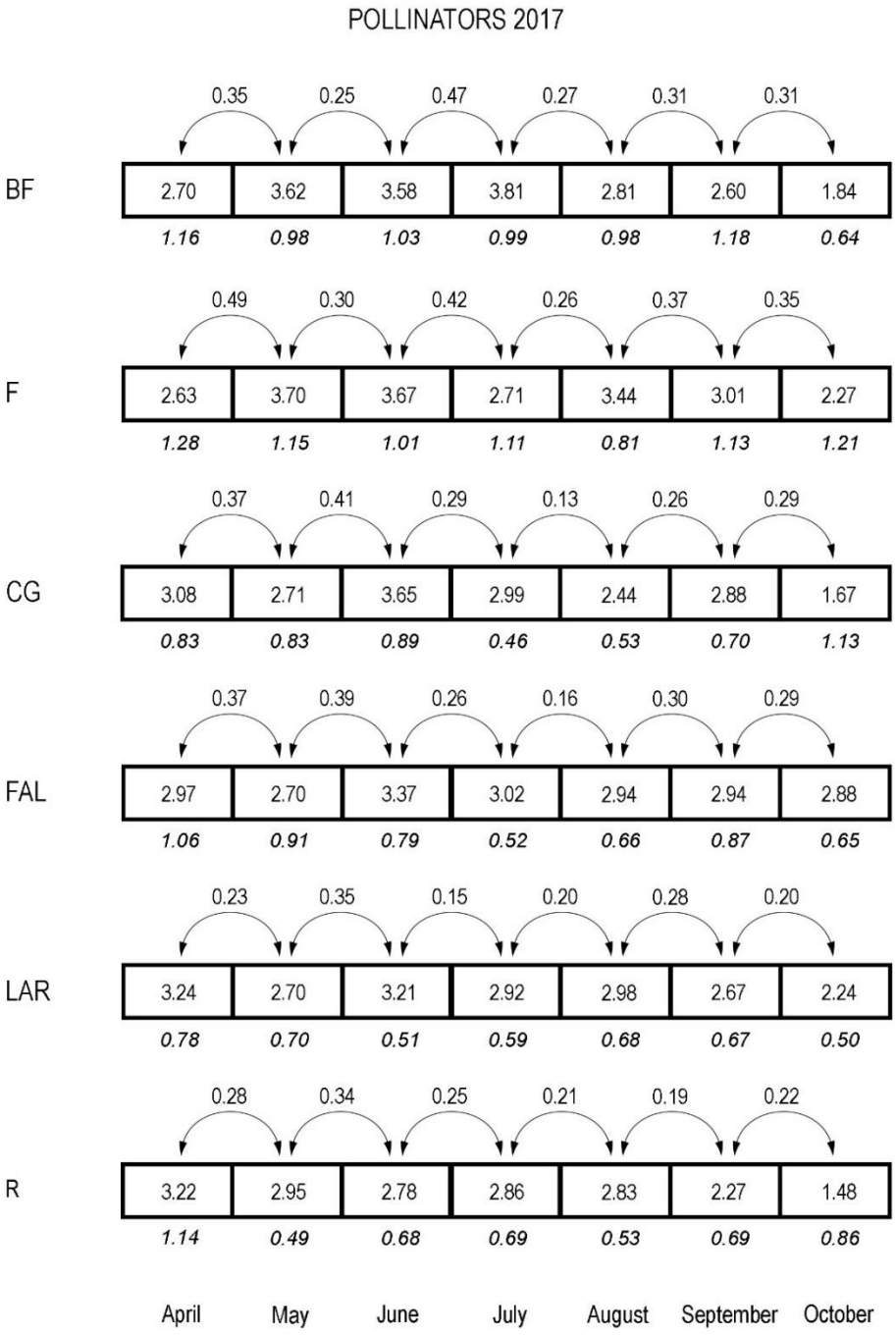
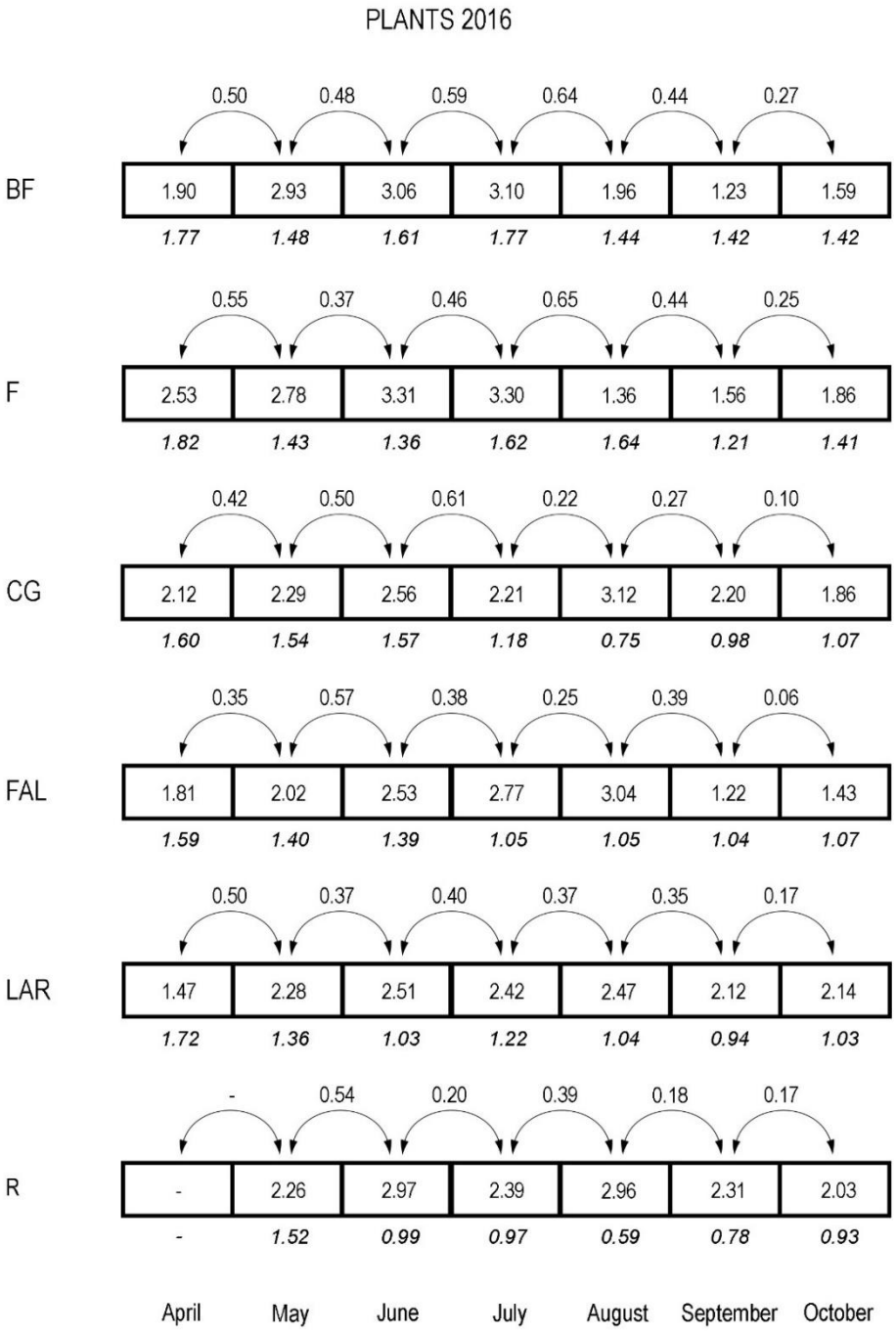


Figure 1. 15. Pollinator species temporal turnovers in each site (BF, Bois de Fontaret; F, Fourches; CG, Chateau Gaillard; FAL, Falaises; LAR, Larris and R, Riez) in 2016 and 2017. Values above the arrows represent the β diversity between months, values in the rectangles represent the α diversity within each site each month, and values in italic represent the partial β diversity (originality) of each month when compared to the whole year in that site.



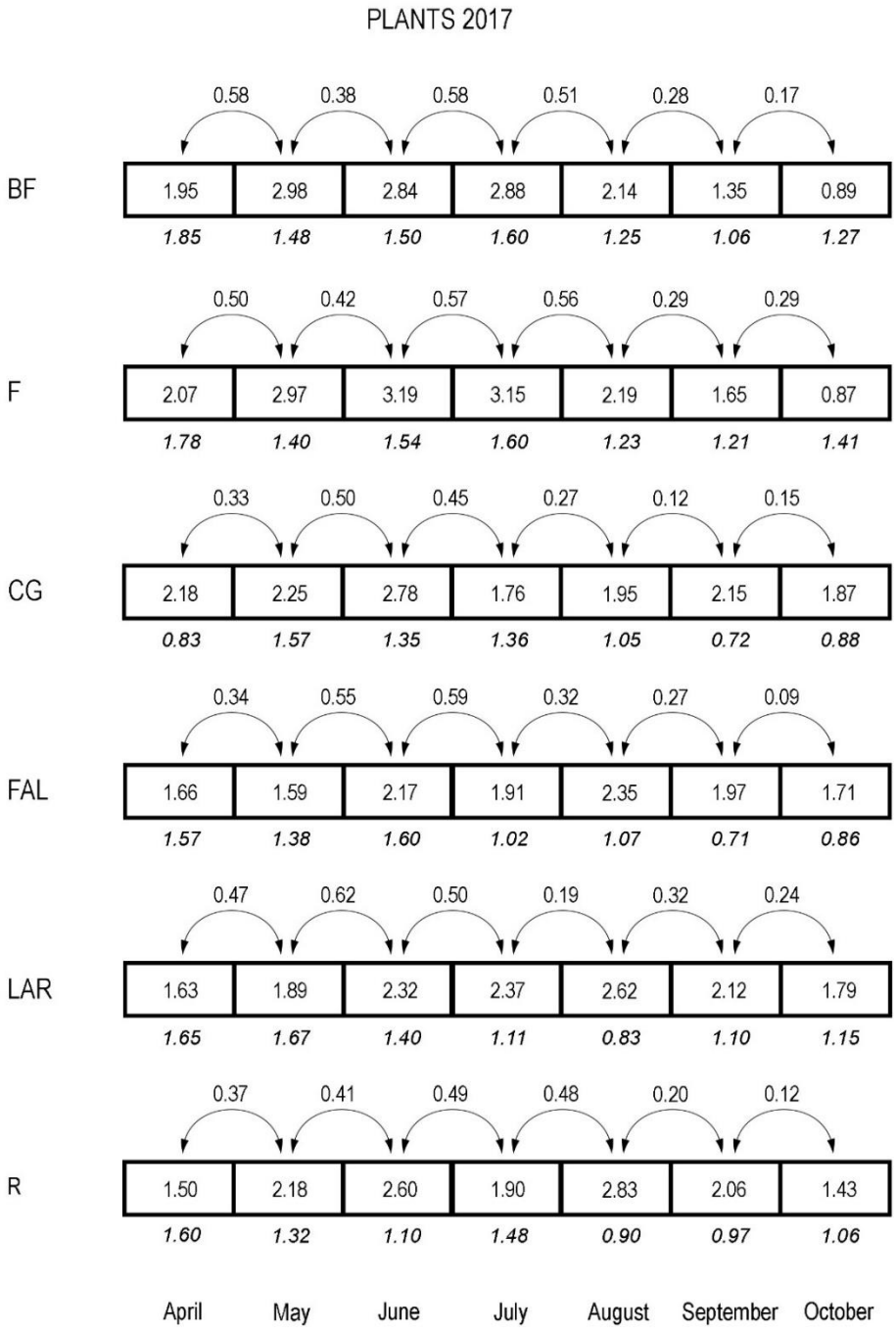


Figure 1. 16. Pollinator species temporal turnovers in each site (BF, Bois de Fontaret; F, Fourches; CG, Chateau Gaillard; FAL, Falaises; LAR, Larris and R, Riez) in 2016 and 2017. Values above the arrows represent the β diversity between months, values in the rectangles represent the α diversity within each site each month, and values in italic represent the partial β diversity (originality) of each month when compared to the whole year in that site.

Type	Model	Effect	Df	SumsOfSqs	R2	F.Model	Pr(>F)
Pollinators (hand net)	site %in% region + year + month	year	1	0.24	0.02	3.47	7e-04 ***
		month	6	3.09	0.27	7.35	1e-04 ***
		site:region	5	3.32	0.29	9.46	1e-04 ***
	year * (site %in% region) + year * month + year	year:month	6	0.59	0.51	1.49	0.0116 *
		year:site:region	5	0.42	0.036	1.28	0.09 n.s.
	site + month:site + month:year	site:month	36	5.09	0.44	3.35	1e-04 ***
		month:year	7	0.83	0.07	2.8	1e-04 ***
Plants	site %in% region + year + month	year	1	0.05	0.003	0.51	0.92 n.s.
		month	6	5	0.31	8.48	0.0001 ***
		site:region	5	3.97	0.25	8.07	0.0001 ***
	year * (site %in% region) + year * month + year	year:month	6	0.28	0.01	0.44	1 n.s
		year:site:region	5	0.16	0.01	0.3	1 n.s
	site + month:site + month:year	site:month	36	8.32	0.52	8.9	1e-04 ***
		month:year	7	0.33	0.02	1.84	3e-04 ***

Table 1. 1. Results of the PERMANOVA analysis on β diversities between sampling sessions, explained by site, region, month and year. For each community (pollinators or plants), we used three models, possibly accounting for nested factors and/or interactions between factors. For each effect, we report the explained sum of squares as well as the R^2, pseudo-F values and the significance of this pseudo-F when tested against 9,999 permutations of the matrix rows.

To conclude, our results evidenced that yearly α and γ diversities followed the latitudinal gradient, in both years. Between-region β diversities were highest between Occitanie and any of the two other regions than between Hauts-de-France and Normandie. However, these patterns were less clear when we levelled down to monthly records, probably due to the shorter phenologies of most plant and insect species. Factors responsible for β diversity variation in both plants and pollinators were monthly variability and differences among sites. Indeed, we observed that each region presents a great amount of unique species, especially for the southern region. A more intense sampling, repeated several times a month, could help clarify the variations related to environmental factors and compensate for any problems of sampling related to some sessions (such as the lack of the April session in the site of Riez or the weather problem in June in the south that affects the diversity of insects). The next step of this analysis will be to look at the diversity of plant-pollinator interactions in all regions to assess how ecological interaction networks vary in space and time (Ohlmann *et al.* 2019).

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Supplementary Figures

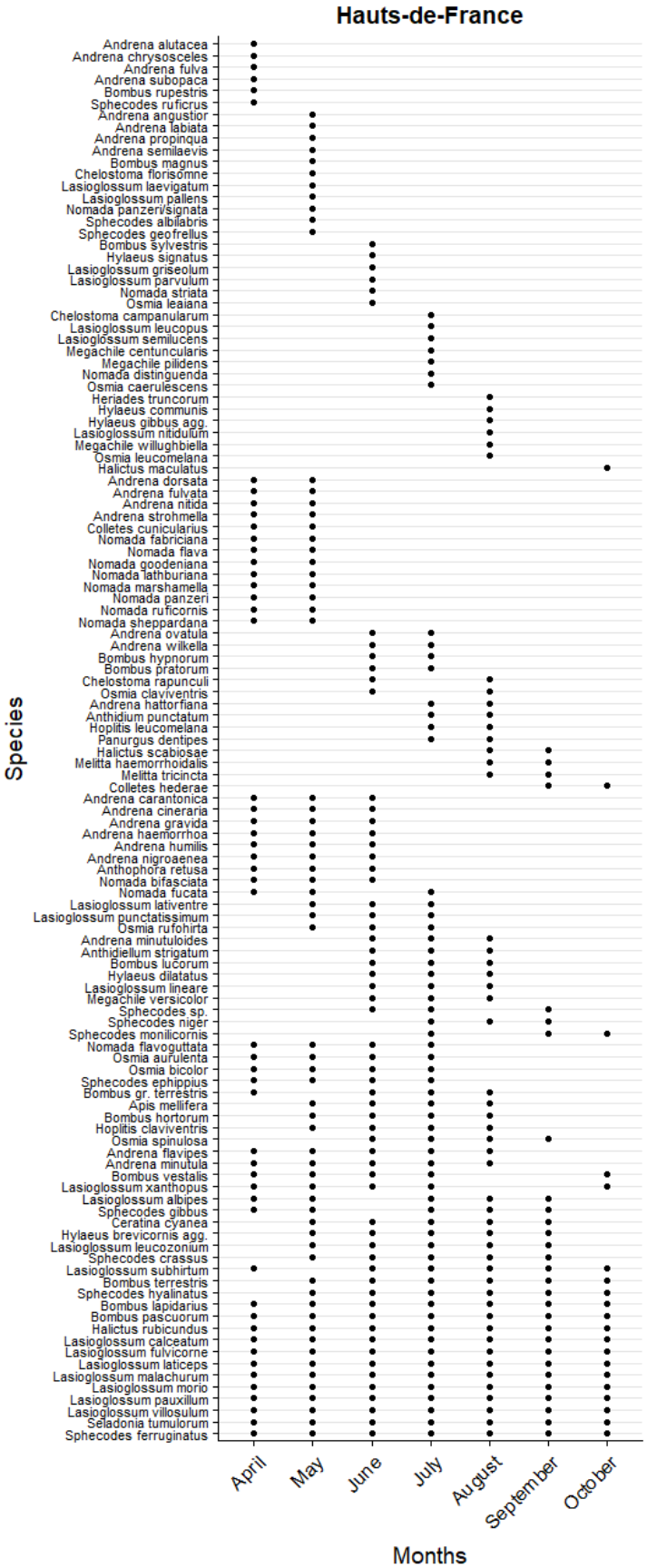


Figure S1. 1. Pollinator phenology of the group Anthophila in the region Hauts-de-France.

Figure S1. 2. Pollinator phenology of the group Anthophila in the region Normandie (part I).

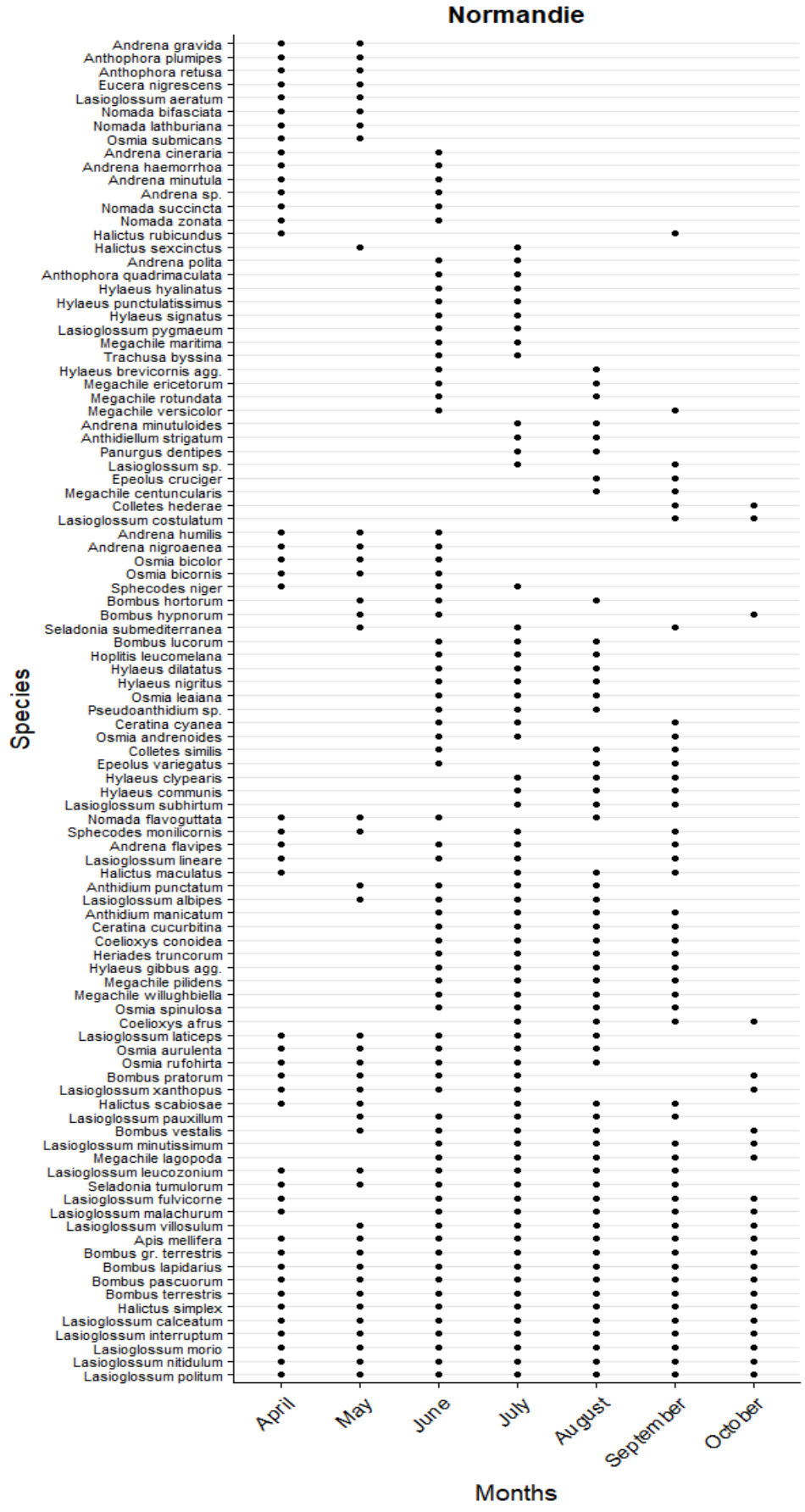
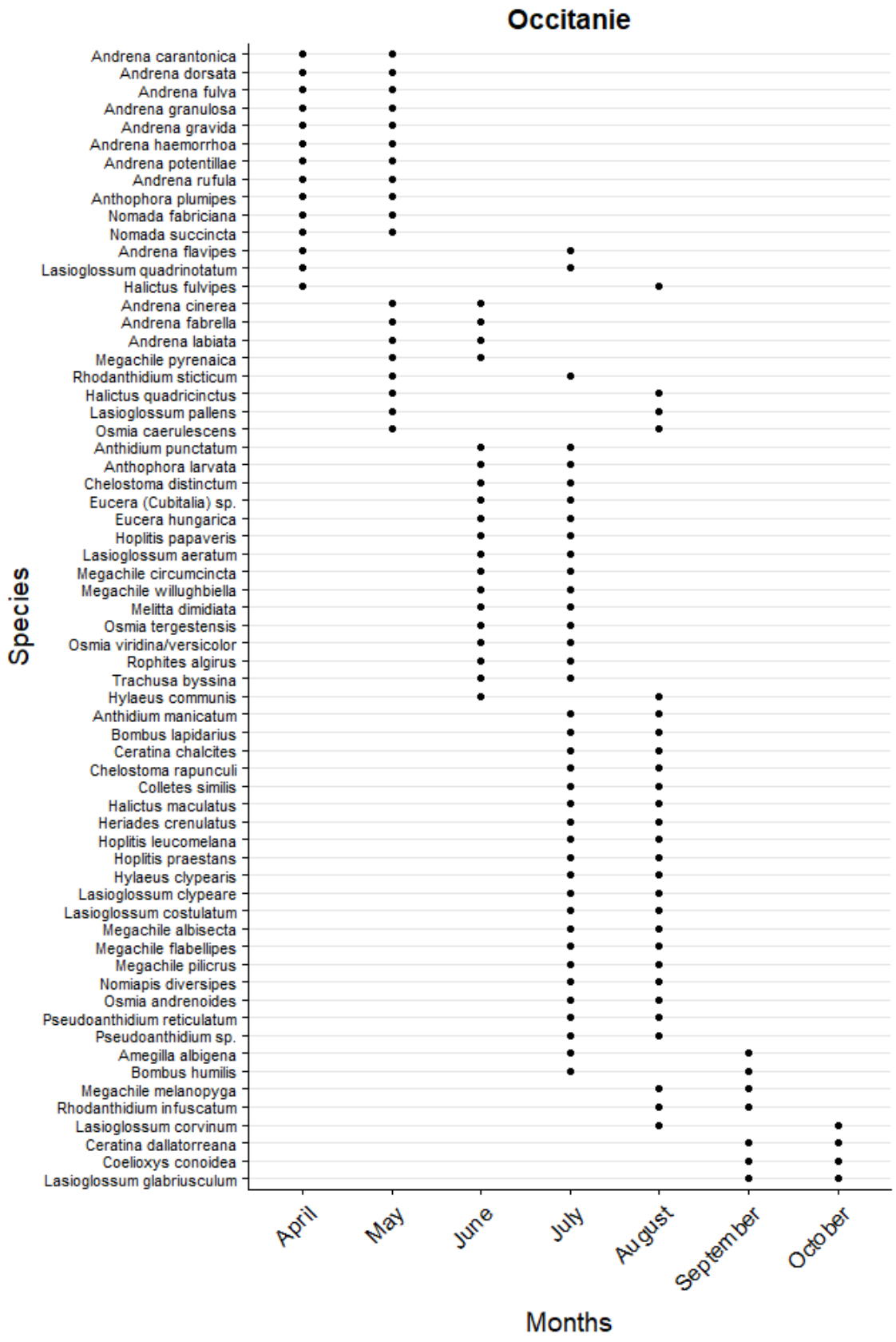


Figure S1. 3. Pollinator phenology of the group Anthophila in the region Normandie (part II).



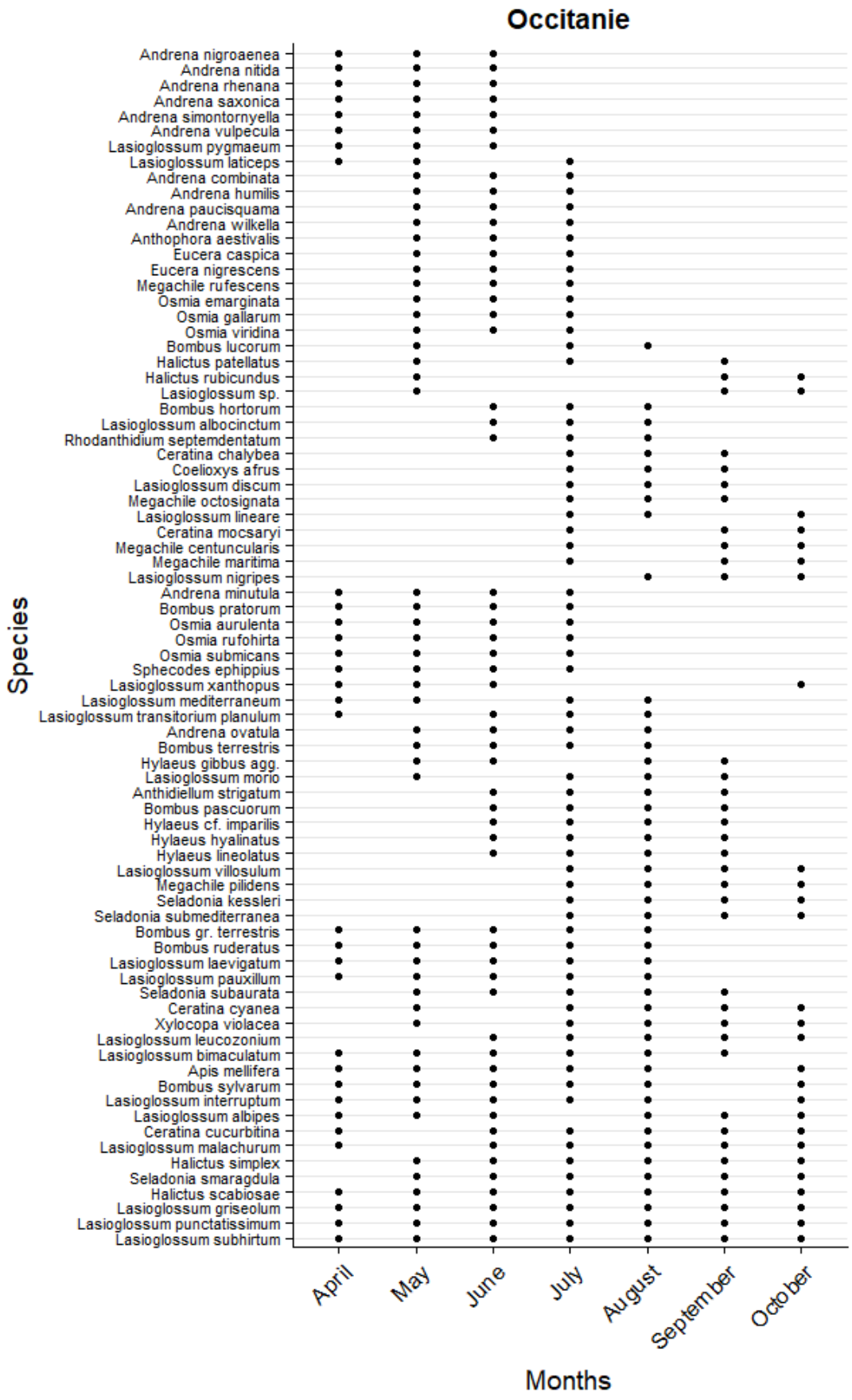


Figure S1. 4. Pollinator phenology of the group Anthophila in the region Occitanie.

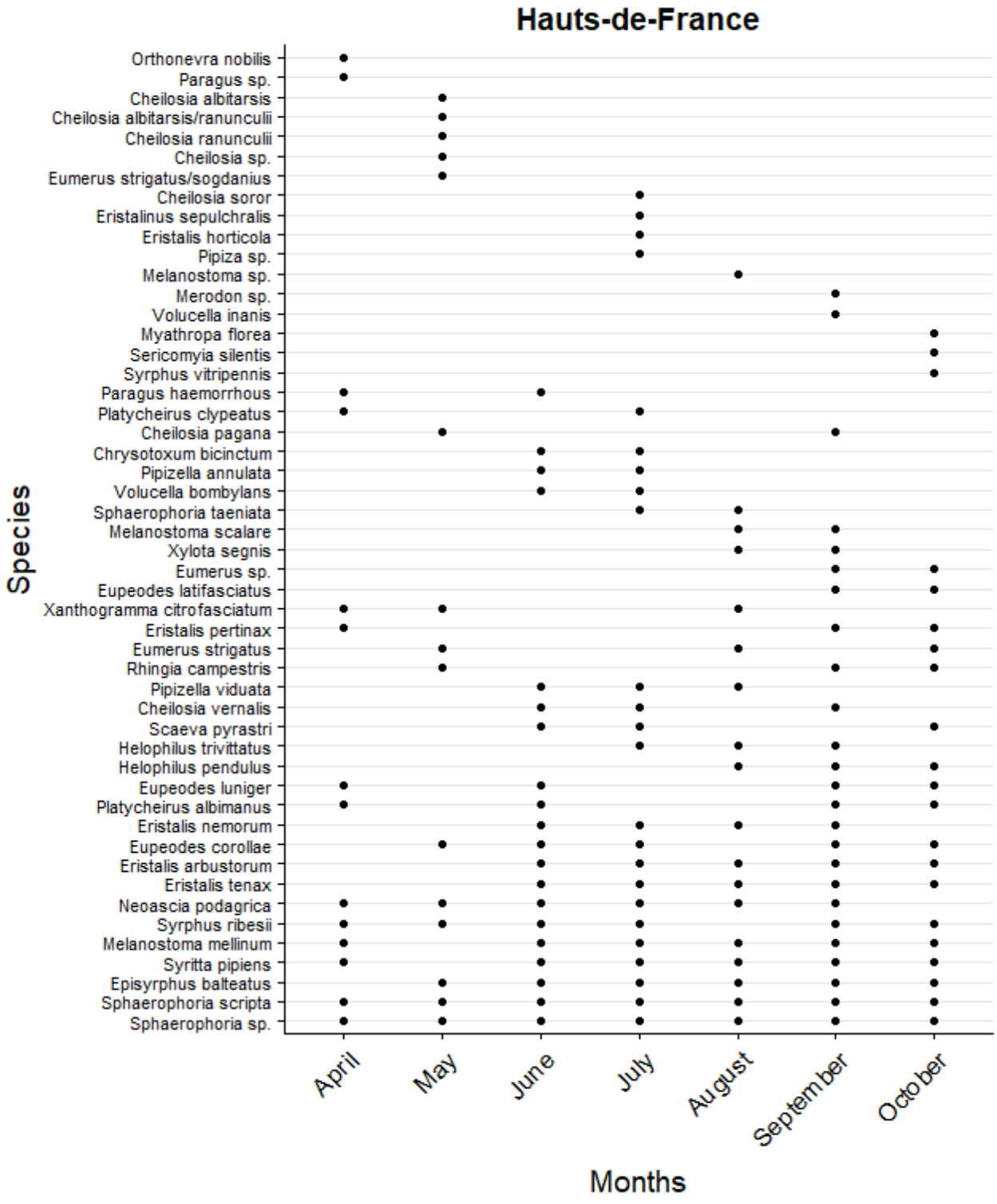


Figure S1. 5. Pollinator phenology of the family Syrphidae in the region Hauts-de-France.

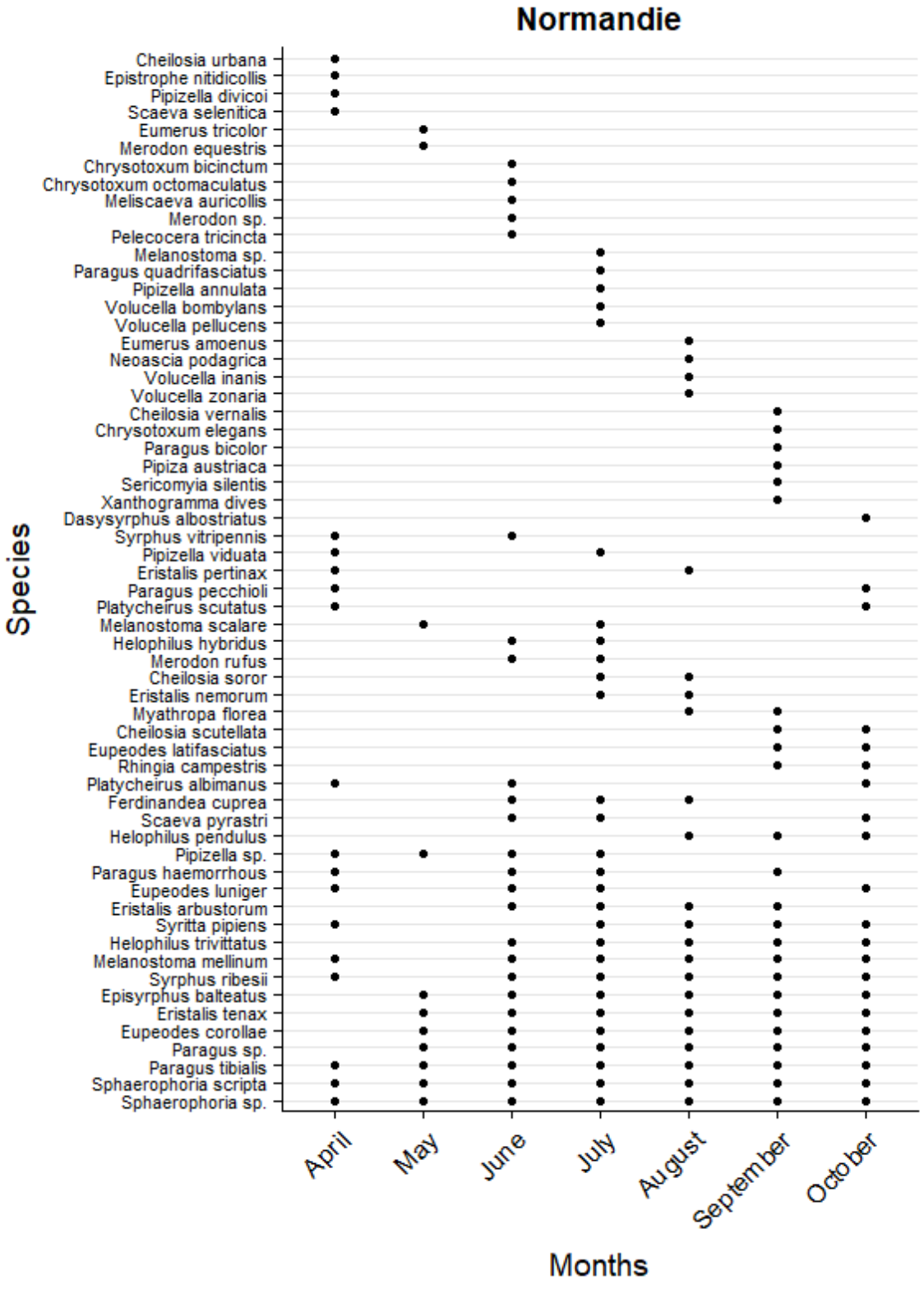


Figure S1. 6. Pollinator phenology of the family Syrphidae in the region Normandie.

Figure S1. 7. Pollinator phenology of the family Syrphidae in the region Occitanie.

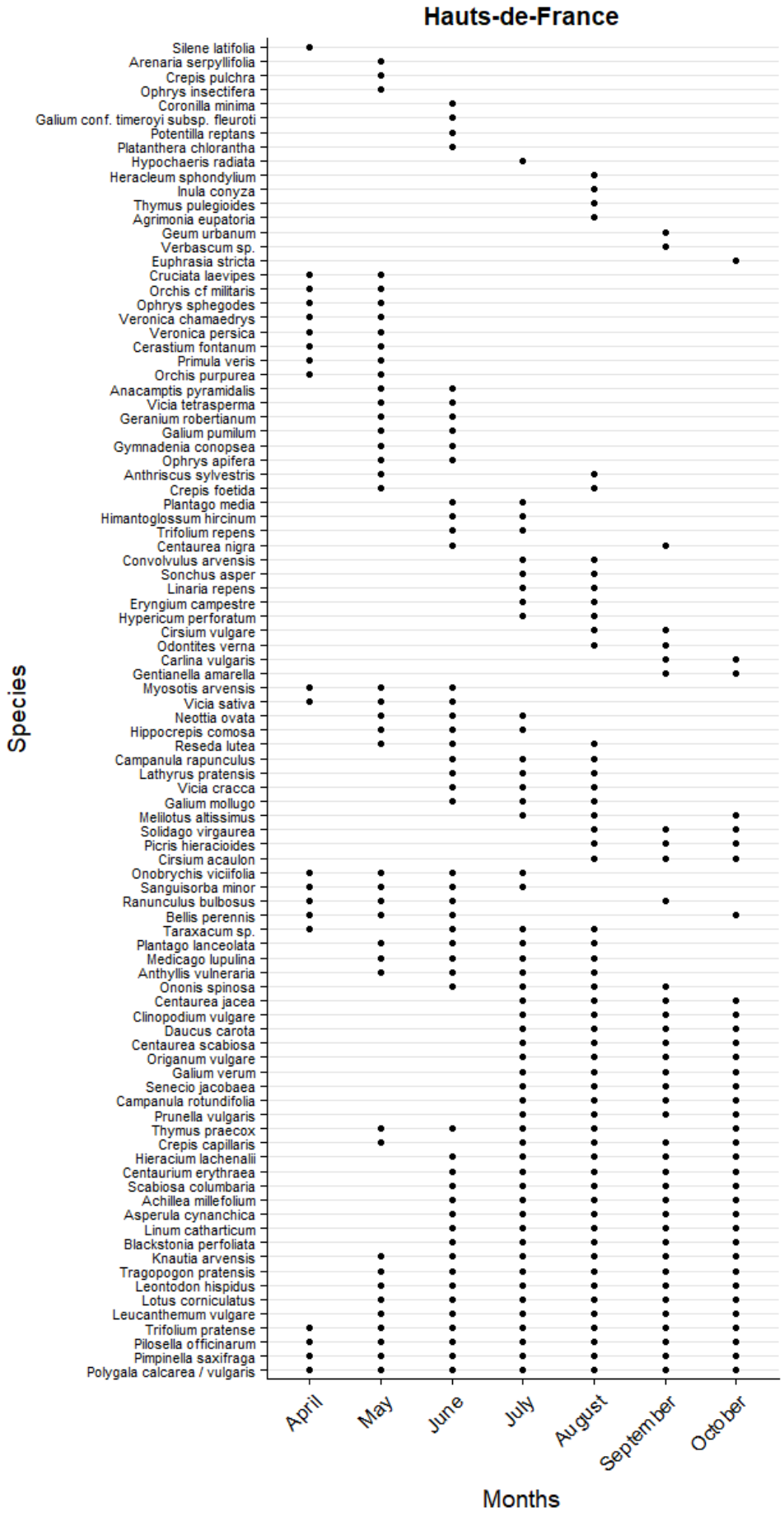


Figure S1. 8. Plant species phenology in the region Hauts-de-France.

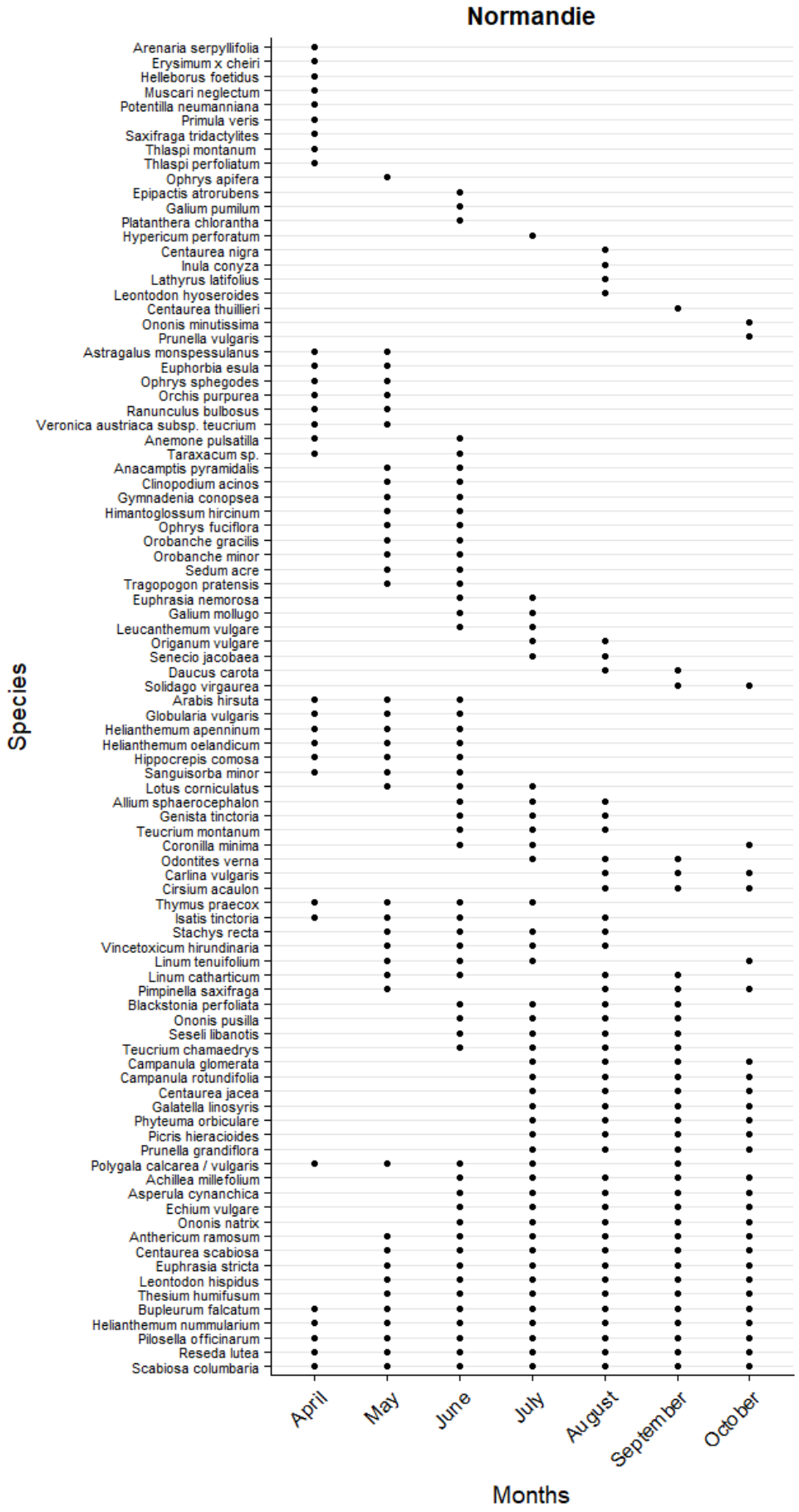
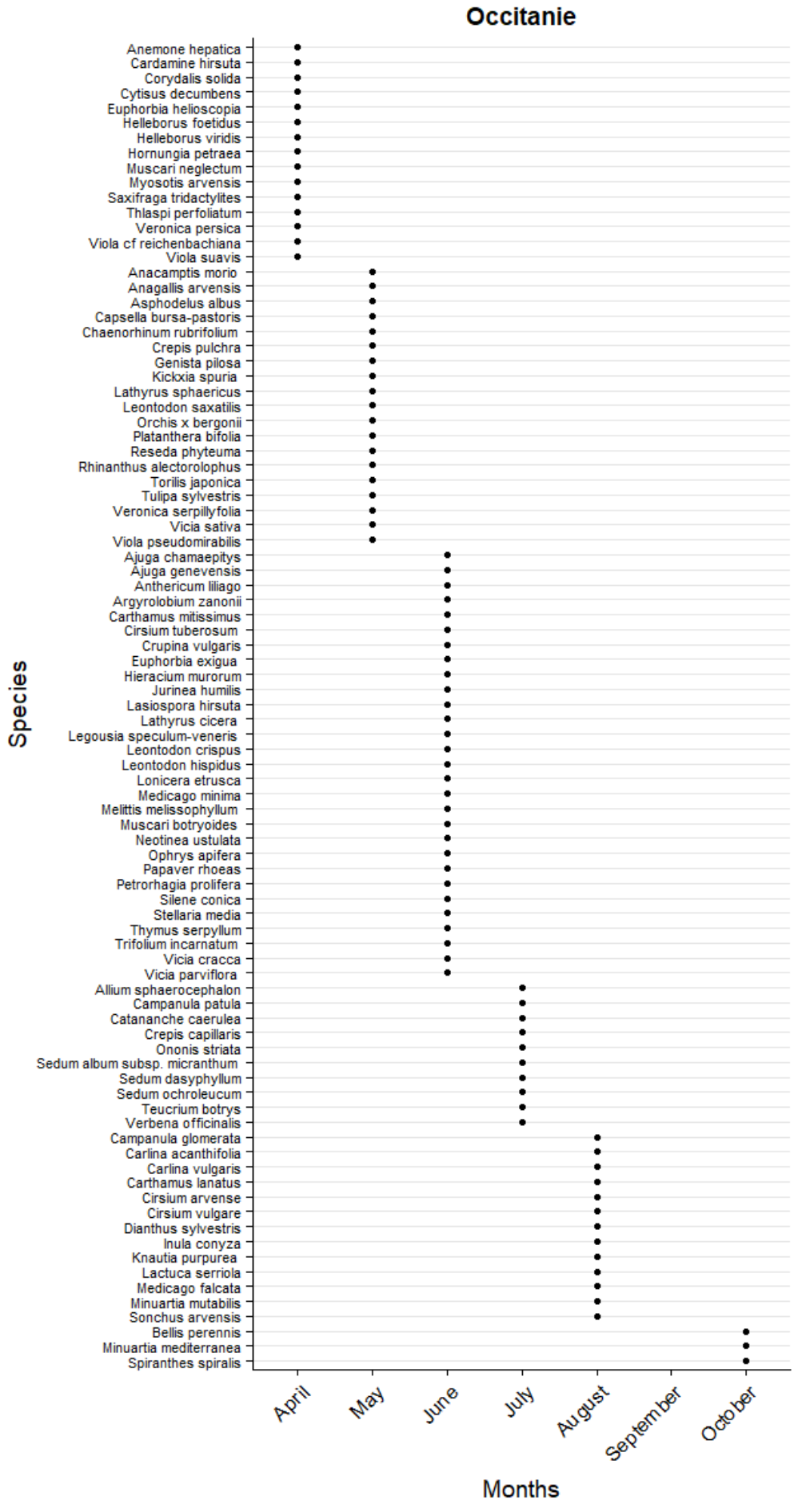


Figure S1. 9. Plant species phenology in the region Normandie.



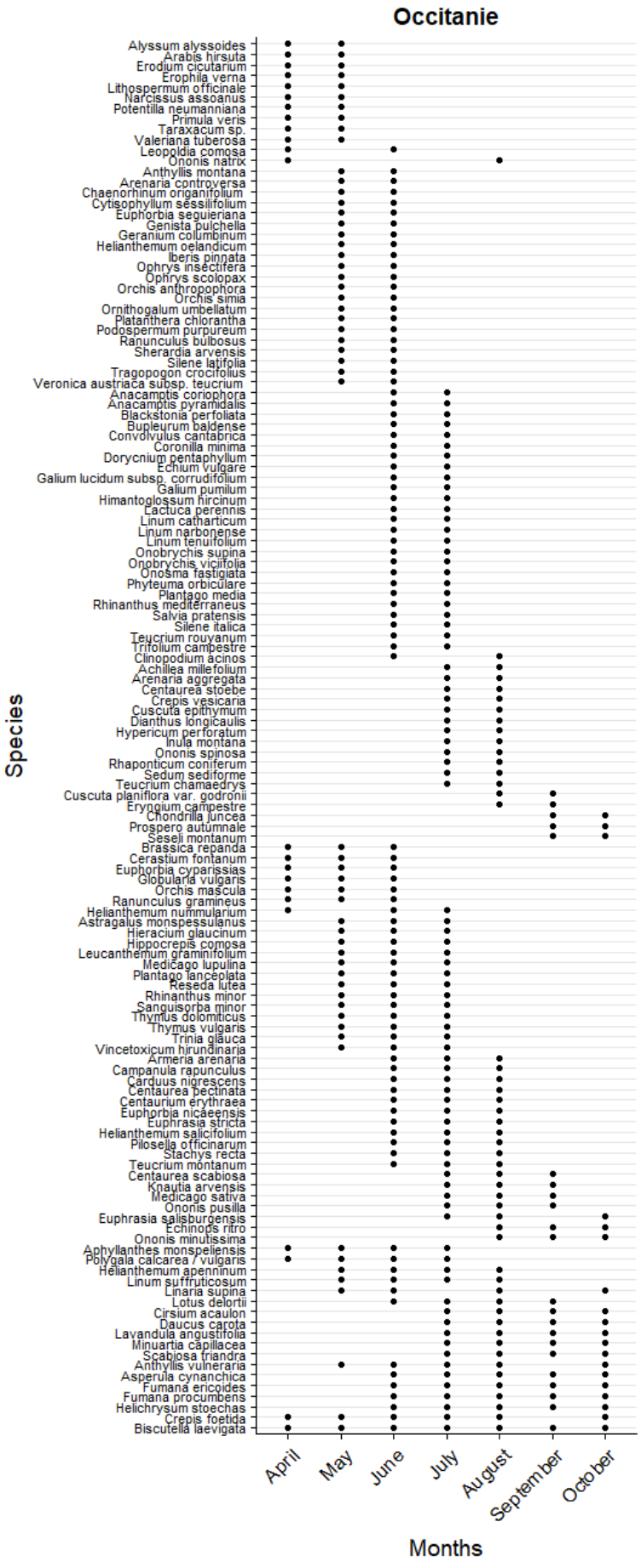


Figure S1. 10. Plant species phenology in the region Occitanie

Chapter II

Does phenology explain plant-pollinator interactions at different latitudes? An assessment of its explanatory power in plant-hoverfly networks in French calcareous grasslands

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Abstract

For plant-pollinator interactions to occur, the flowering of plants and the flying period of pollinators (i.e. their phenologies) have to overlap. Yet, few models make use of this principle to predict interactions and fewer still are able to compare interaction networks of different sizes. Here, we tackled both challenges using Bayesian Structural Equation Models (SEM), incorporating the effect of phenology overlap, in six plant-hoverfly networks. Insect and plant abundances were strong determinants of the number of visits, while phenology overlap alone was not sufficient, but significantly improved model fit. Phenology overlap was a stronger determinant of plant-pollinator interactions in sites where the average overlap was longer and network compartmentalization was weaker, i.e. at higher latitudes. Our approach highlights the advantages of using Bayesian SEMs to compare interaction networks of different sizes along environmental gradients and articulates the various steps needed to do so.

Introduction

Understanding how phenology determines species interactions is a central question in the case of mutualistic networks. In plant-pollinator networks, phenology shapes their temporal and spatial limits, thus defining the area and the period along the season in which interactions preferably occur (Olesen *et al.* 2011; Ogilvie & Forrest 2017). Since plant and pollinator phenologies are not equally affected by changes in environmental cues, partial or total phenological mismatches can occur as a result of environmental changes such as climate change (Parmesan 2007; Rafferty 2017). Phenological advances indeed increase at higher latitudes, as a response to the acceleration of warming temperature along the same gradient (Post *et al.* 2018), increase phenological mismatch, and have the potential to threaten the synchrony needed for effective pollination (Hutchings *et al.* 2018). Such environmental changes can thus drastically alter pollinator interactions through modified temporal overlap between pollinators and their floral resources leading, in extreme cases, to local extinctions (Memmott *et al.* 2007) and the ensuing absence of the partner species at the location and/or time at which the interaction should have taken place (Willmer 2012; Miller-Struttmann *et al.* 2015; Rafferty *et al.* 2015; Hutchings *et al.* 2018).

Because phenological match is crucial to plant-pollinator interactions, and thus ultimately to pollinators' fitness, pollinators have to adapt to phenological shifts either through interaction with other plant species (Rafferty *et al.* 2015) or through changes of their own phenology (Bartomeus *et al.* 2011). Phenology can then influence dynamical network properties, such as the stability and the coexistence of species, through changes in network topology (Encinas-Viso *et al.* 2012). Moreover, phenology predictably affects network compartmentalization as different phenophases likely correspond to different compartments when networks are considered on an annual scale (Martín González *et al.* 2012).

Despite considerable theoretical advances, there are few models available to predict the probability of interaction in plant-pollinator networks and fewer still able to make comparisons between networks. Due to their complexity and variation among years (Chacoff *et al.* 2017), most studies of mutualistic networks have focused on predicting and comparing classic network metrics (nestedness, connectance, modularity, etc.) which are all influenced by network size, *i.e.* the number of plant and insect species (Fortuna *et al.* 2010; Staniczenko *et al.* 2013; Poisot & Gravel 2014; Astegiano *et al.* 2015). Moreover, few studies have compared interaction networks along environmental gradients (Devoto *et al.* 2005; Schleuning *et al.* 2012; Sebastián-

González *et al.* 2015; Pellissier *et al.* 2017). In order to compare networks of different sizes, a better alternative is to switch from network-derived metrics to the comparison of output of regression models, which can consider multiple factors and latent variables and assume that the sampled data are just part of a larger unobserved dataset (Grace *et al.* 2010). Large datasets allowing relevant comparisons of networks are rare; they require parallel investigations in rich communities of plants and insects to favour interactions between them. Calcareous grasslands are characterized by highly diverse plant communities with a high proportion of entomophilous species (Baude *et al.* 2016), thus they are a convenient model for such studies. Most plant-insect pollinator networks involve bee species (Anthophila), but recent studies have also pointed out the importance of hoverflies (Diptera: Syrphidae), which pollinate a large spectrum of wild flowering species (Klecka *et al.* 2018a) and crops (Jauker & Wolters 2008; Rader *et al.* 2011). They usually behave opportunistically, *i.e.* from being pollen generalists as well as pollen or nectar specialists, only limited by morphological constraints (Iler *et al.* 2013; Klecka *et al.* 2018a; Lucas *et al.* 2018). Indeed, their generalisation could be the result of serial specialized diets, since most pollen retrieved on hoverfly individuals usually comes from a single plant taxon (Lucas *et al.* 2018) and depends on flower availability and phenology (Cowgill *et al.* 1993; Colley & Luna 2000). Moreover, some hoverflies have preferences regarding plant colour, morphology and inflorescence height (Branquart & Hemptinne 2000; Colley & Luna 2000; Lunau 2014; Klecka *et al.* 2018b, a).

Here we study the consequences of environmental gradients on plant-pollinator interactions, focusing on how phenology overlap affects interactions between plants and insects in six calcareous grassland sites distributed along a latitudinal gradient. We obtained plant and insect phenologies, abundances, and interactions in all sites from April to October 2016. We modelled plant-pollinator interaction networks following a Bayesian Structural Equation Modelling approach (SEM) using latent variables. The comparison of 16 SEM models and the analysis of latent block models (LBM) of sampled networks evinced that phenology overlap is an important determinant of plant-pollinator interactions, but is less informative than species abundances and performs heterogeneously among sites. Our results suggest that the use of SEMs to compare networks of different sizes along an environmental gradient is an innovative approach which can help understand the structure of plant-pollinator networks.

Materials and methods

Study sites

We sampled plant and pollinator species in six areas (Fig. S2.1) of 1 hectare each in different French regions: two sites in Hauts-de-France (Les Larris de Grouches-Luchuel, thereafter noted LAR, 50°11'22.5"N 2°22'02.9"E and Regional natural reserve Riez de Noeux les Auxi, noted R, 50°14’51.85”N 2°12’05.56”E, in départements Pas-de-Calais and Somme), two sites in Normandie (Château Gaillard – le Bois Dumont, noted CG, 49°14'7.782"N 1°24'16.445"E and les Falaises d’Orival, noted FAL, 49°04'40.08"N 1°33'07.254"E, départements: Eure and Seine Maritime) and two sites in Occitanie (Fourches, noted F, 43°56'07.00"N 3°30'46.1"E and Bois de Fontaret, noted BF, 43°55'17.71"N 3°30'06.06"E, department: Gard). The six sites are included in the European NATURA 2000 network; the four sites in Hauts-de-France and Normandie are managed by the Conservatoire d’espaces naturels of Normandie, Picardie and Nord – Pas-de-Calais and the sites in Occitanie by the CPIE Causses méridionaux. We sampled each site once a month from April to October 2016, except for the site of Riez that was sampled from May to October.

Plant-hoverfly observations and sampling

To collect information at the community level, in each site and at each session we realized: (i) a botanic inventory of the flowering species, recorded their abundances and the total flower covering in the area and (ii) a pollinator sampling using a hand net along a variable transect walk.

Flowering plants were identified at the species level. We recorded the abundances of all flowering species. At first, we estimated the total percentage of surface covered by all flowering species in the selected area. We then estimated the relative abundance of each flowering species. We used Braun-Blanquet coefficients of abundance-dominance to rank flowering species: coefficient ***5*** = 75-100%, coeff ***4*** = 50-75%, coeff ***3***=25-50%, coeff ***2*** = 10-25%, coeff ***1*** = 1-10%, coeff **+** = few individuals less than < 1%, coeff ***i*** = 1 individual. All inventories were realized by the same surveyors to avoid biases.

Pollinator observations were performed by the same team of 3-5 persons each day. The surveyors walked slowly around any potential attractive resource patch included in the selected 1-hectare area for 4h each day. We split the sampling period into 2 hours in the morning (about

10-12h) and 2 hours in the afternoon (about 14-16h) to cover the daily variability of both pollinator (bees and hoverflies, which are more active in the morning than in the afternoon; D'Amen *et al.* 2013) and flower communities. Sampling took place when we had suitable weather conditions for pollinators (following Westphal *et al.* 2008). We sampled all flower-visiting insects and we recorded observed interactions. All sampled insects were immediately put individually in a killing vial with ethyl acetate and were later prepared and pinned in the laboratory and identified at the species level by expert taxonomists. Even if we collected both bees and hoverflies, in this study we focus on syrphids only. Overall, we sampled for 41 days, equivalent to about 164 hours in the field (all the surveyors collected at the same time). For all analyses described here, we only used the list of visited herbaceous plant species and hoverflies which were found visiting a plant. Despite their rarity, we also considered the interactions between hoverflies and plant species of the Fabaceae family because we did not want to exclude data in the absence of the proof of no interaction, even if hoverflies are known to prefer open flowers (Branquart & Hemptinne 2000). However, we observed in the field that they visited Fabaceae species that were already opened by other insects, *e.g.* by large bee species, such as *Eucera* sp. (De Manincor, personal observation).

Plant – hoverfly networks

For each site, we constructed an interaction network consisting of all pairs of interacting plant and insect species, pooling data from all months. A pair of species (*i*,*j*) was connected with intensity *v* when we recorded *v* visits of insect species *i* on plant species *j* in the site. We calculated the network specialization index, *H2'* (Blüthgen *et al.* 2006) using the *H2fun* function implemented in the *bipartite* package (Dormann *et al.* 2009; R Core Team 2018). We also calculated the standardized specialization index *d'* (Blüthgen *et al.* 2006) for each plant and insect species as the ratio of the *d*-value (Kullback-Leibler divergence between the interactions of the focal species and the interactions predicted by the weight of potential partner species in the overall network) to its corresponding *dmax*-value (maximum *d*-value theoretically possible given the observed number of interactions in the network). We obtained these values using the *dfun* function in the bipartite package (Dormann *et al.* 2009), but we did not use the *d'* values provided by this package as they sometimes yielded spurious results based on the computation of the minimal *d* value (e.g. reporting low *d'* for species with only one partner in the network).

We calculated the modularity of the network and the associated partition of species into modules using the *cluster_leading_eigen* method for modularity optimization implemented in

the *igraph* package (Csardi & Nepusz 2006; Newman 2006). Modularity optimization can help gauge strong, simple divisions of a network in relatively independent sub-networks by looking for densest sub-networks. However, modules are not meant to inform about more subtle groupings among the species, *e.g.* particular avoidance of interactions between insects of group A and plants of group 1. In order to detect such groups, we implemented latent block models (LBM) using the *BM_poisson* method for Poisson probability distribution implemented in the *blockmodels* package (Leger *et al.* 2015). Blocks are calculated separately for the two groups (insect and plant) based on the number of visits (*i.e.* a weighted network). The algorithm finds the best divisions of insects and plants through fitting one Poisson parameter in each block of the visit matrix, thus essentially maximizing the ICL (Integrated Completed Likelihood; Biernacki *et al.* 2000; Daudin *et al.* 2008). The LBM script is given in Supplementary Information (Appendix S3). All analyses were performed in R version 3.3.3 (R Core Team 2018).

Plant and hoverfly abundances and phenology overlap

We calculated plant abundance using information about the abundance-dominance recorded in the field following the methodology of Braun-Blanquet presented above. We transformed the coefficients of abundance in percentages (Table S2.1): we used the mean of the percentage which correspond to each class. We then calculated the relative abundance (A_P) of each flowering plant species as the ratio of the focal species cumulated abundance to total flower abundance during its flowering season. We used the recorded number of visiting hoverflies and their presence (recorded months) along the season to calculate their average abundance during months when they were present (A_H).

We refer to plant phenology as their flowering period and insect phenology as the flying period. We considered only flowering plants which had been visited by pollinators. For the pollinators, we considered only hoverflies which were found in interaction. To build the species phenology tables for both plants and hoverflies, we merged the information provided by two sources of data (field data and the literature): we used the observed phenology of both plants and insects during the field session as the only source of information for plants (plants visited by insects and plants found in the botanic inventory in the site at that date), and we complemented the hoverfly phenology with information provided by the Syrph the Net Database (Speight *et al.* 2016). We then built the phenology overlap (PO) matrix based on the species phenology tables

by calculating the number of phenologically active months that are shared by each pair of insect and plant species along the season.

Bayesian Structural Equation Modelling (SEM)

We modelled the hoverfly-plant interaction network using a Bayesian Structural Equation Modelling approach (SEM, Fig. 2.1) with latent variables linking the number of visits per plant-pollinator dyad to abundance and phenology overlap (PO) data through a first latent table representing probabilities of interactions, another latent table representing the possible interactions between plant and pollinators (as a realization of the aforementioned interaction probability matrix), and a third latent table yielding the expected number of visits per plant-pollinator dyad (*i.e.* the intensity of interactions).

In this model, we considered that PO had an effect on possible interactions (I_{ij}) and the number of visits (λ_{ij}) – a longer overlap is intuitively expected to drive a higher probability of interaction and a larger number of visits. Interaction probabilities were also assumed to depend on two random effects (plant and insect species identities), to represent heterogeneity of species degrees in the network. We modelled the probability of interaction I_{ij} between insect species *i* and plant species *j* (*i.e.* $I_{ij} = 1$ when species *i* and *j* can interact) as a Bernoulli random variable of mean μ_{ij} given by:

$$\text{logit}(\mu_{ij}) = \mu_0 + \mu_{PO} PO_{ij} + E_i + E_j$$

where logit is the usual logistic transformation (log(x/(1-x)), μ_0 is the intercept of this relation, μ_{PO} is the coefficient measuring the effect of PO, and E_i and E_j are the random effects associated with insect species i and plant species j respectively.

The number of interactions was assumed to depend on plant and hoverfly abundances, as more abundant species are expected to be more often sampled (and thus more often recorded "in interaction"). The number of visits V_{ij} was modelled as a Poisson random variable to allow for sampling variability, with a conditional mean λ_{ij} (the intensity of visits that can occur) given by:

$$\log(\lambda_{ij}) = \lambda_0 + \lambda_H A_H + \lambda_P A_P + \lambda_{PO} \log(1 + PO)$$

where λ_0 is the intercept of this relation, λ_H is the coefficient measuring the effect of hoverfly abundance A_H, λ_P is that of plant abundance A_P, and λ_{PO} is the coefficient of the effect of PO.

Possible interactions (I_{ij}) and the intensity of visits (λ_{ij}) are multiplied to obtain the unconditional mean number of recorded visits, *i.e.* V_{ij} is then obtained as a Poisson draw of mean $I_{ij}\,\lambda_{ij}$.

Overall we thus estimated four main parameters: the effect of plant abundance on the intensity of interactions ($A_P \rightarrow \lambda_{ij}$, coefficient λ_P), the effect of insect (hoverflies) abundance on the intensity of interactions ($A_H \rightarrow \lambda_{ij}$, λ_H), the effect of phenology overlap on the intensity of interactions ($PO \rightarrow \lambda_{ij}$, λ_{PO}) and the effect of phenology overlap on the probability of interaction ($PO \rightarrow I_{ij}$, μ_{PO}).

We used the *jags* function (*R2jags* package), which provides an interface from R to the JAGS library for Bayesian data analysis, to estimate model parameters. JAGS (Plummer 2003) uses a Markov Chain Monte Carlo algorithm to generate samples from the posterior distribution of the parameters. We ran two Markov chains with 10^6 iterations per chain to check for model convergence. The code of the model is given in Supplementary Material (Appendix S1 and S2).

Model and parameter comparison

We estimated the 16 models that included between 0 and 4 of the above-mentioned effects to understand which effects were more likely to play a role in the structuring of the network. The goodness-of-fit of these models were compared using the leave-one-out cross-validation criterion (LOO) calculated using the R package *loo* (Vehtari *et al.* 2017). Models can thus be ranked according to their LOO scores, with the best model being the one with the lowest LOO value. The LOO criterion is analogous to the classic Akaike and Bayesian Information Criteria, but can be applied to Bayesian models without suffering the same instability issues of the Deviance Information Criterion (Vehtari *et al.* 2017). To rank the models, we then calculated the *ΔLOO* (noted Δ_i) as $\Delta_i = LOO_i - LOO_{min}$ (following Burnham & Anderson 2004), where LOO_{min} is the minimum of the LOO_i values among the 16 models. We used Δ_i to obtain model weights ω_i, following the Akaike weight methodology (Burnham & Anderson 2002):

$$\omega_i = \frac{e^{-\Delta_i/2}}{\sum e^{-\Delta_i/2}}$$

We then summed weights (w_H) over all models that incorporated a given focal parameter to ascertain the plausibility of the effect associated to this parameter. We used this sum to evaluate the null hypothesis (H0) that a given factor has no effect on the plant-pollinator interactions by comparing the sum of weights to null expectations, based on the fact that each tested effect is incorporated in exactly half of the tested models. The effect is considered *plausible* when $w_H >$

0.5, *implausible* otherwise, *likely* when $w_H > 0.73$, and *unlikely* when it corresponds to a value of 0.27 or lower, following Massol *et al.* (2007).

Results

Plant-hoverfly networks and phenology overlap

At the end of the field campaign we had collected 1584 hoverflies and recorded 1668 interactions between 76 hoverfly species and 117 plant species overall (Table 2. 1). The number of sampled hoverfly and plant species varied between sites and among regions. In Normandie we generally sampled a higher number of hoverflies than in the other two regions (Table 2. 1). We observed the highest diversity of both plants and hoverflies in Occitanie and the lowest diversity of hoverflies in Hauts-de-France. Despite the high species diversity in Occitanie, the number of interactions recorded in these sites (BF and F) is not the highest recorded in the field (Table 2. 1).

In spite of differences in diversity and the number of interactions, the overall level of specialization (H2 index) did not show a high variation among the 6 networks (range: 0.32 – 0.37). However, we found that the sites in Occitanie (BF and F) had a higher average degree of specialization (d') for both insect (BF 0.63 and F 0.57) and plant species (BF 0.58 and F 0.48). The sites in Occitanie also had a higher modularity (BF 0.51 and F 0.48) than the ones in Normandie (CG 0.34 and FAL 0.23) and Hauts-de-France (LAR 0.37 and R 0.34; Table 2. 1). Given that these statistics only compare 6 sites, none of these assessments can be properly statistically tested, but the importance of the differences among sites is highly suggestive of a difference in average specialization and modularity. We found that plant phenology is generally shorter in all sites than that of hoverflies (Table 2. 1). The phenology overlap was shorter in Occitanie (BF and F) than in the other sites (Table 2. 1).

Illustrations of the block clustering provided by the LBM analysis (Latent Block Model) are shown in Fig. 2.2 and 3 in the main text and in Fig. S2.2 to S2.5 in Supplementary Information. We found different numbers of blocks in plants and hoverflies among sites: the BF site had 2 insect blocks and 2 plant blocks (Fig. S2.2); the F site had 4 of both (Fig. 2.2); the CG and R sites had 3 blocks for the plants and 4 blocks for the insects in (Fig. 2.3 and S2.5); the FAL site had 4 plant blocks and 3 insect blocks (Fig. S2.3); the LAR site had 3 blocks for the plants and 2 for the insects (Fig. S2.4).

Model ranking and comparison of parameters in each site

For each site we compared the 16 models using the LOO criterion (Table 2. 2, ΔLOO values). We found that models 1, 2 and 4 had consistently better goodness-of-fit than the others. The model incorporating all effects except the effect of phenological overlap on the probability of interaction (Model 4: $\lambda_{ij} \sim A_H + A_P$ + PO, Table 2. 2) was the best model in the sites of CG, FAL and LAR. In the two southern sites (BF and F), we found that the model incorporating all effects except that of phenological overlap on the intensity of visits (Model 1: $\lambda_{ij} \sim A_H + A_P$ / $I_{ij} \sim$ PO, Table 2. 2), was the best one. The model incorporating all effects (Model 0: $\lambda_{ij} \sim A_H + A_P$ + PO / $I_{ij} \sim$ PO, Table 2. 2) was found as the best one only in the site of R, but was a suitable model (ΔLOO <4) in all the other sites (Table 2. 2). We also compared the sum of model weights of the four parameters among sites (Table 2. 2, Evidence ratio). We found that the effect of insect abundance on the intensity of interaction ($A_H \rightarrow \lambda_{ij}$) is always likely (*i.e.* the sum of their weights is always higher than 0.73, Table 2.2) and of large effect size in all sites (standardised coefficient higher than 1, Fig. 2.4). Likewise, we found that the effect of plant abundance on the intensity of interaction ($A_P \rightarrow \lambda_{ij}$) was always likely and had large effect size in most part of sites, except in the site of F (ER = 0.59, Table 2. 2; standardised coefficient = 0.67, Fig. 2.4). The effects of phenological overlap on the probability of interaction (PO $\rightarrow I_{ij}$) and the intensity of visits (PO $\rightarrow \lambda_{ij}$), however, had variable plausibility among sites. The effect of phenological overlap on the probability of interaction was *likely* only in half of the sites (Table 2.2 and Fig. 2.4). The effect of phenological overlap on the intensity of visits was *not plausible* only in the two southern sites (BF and F) and *plausible* in the other four sites (LAR, R CG and FAL, Table 2. 2 and Fig. 2.4). In all sites, the standardised coefficients of PO effects were always less than 1, thus suggesting a low effect size of phenology on interaction probability and intensity (Fig. 2.4).

Discussion

Latitude affects the seasonality, advancing species phenologies at higher latitudes, and thus, can be a limiting factor for the phenological coupling of interacting species (Post *et al.* 2018). In this study we explored the effect of phenology overlap on a large network of species interactions in calcareous grasslands and how this effect could vary along a latitudinal gradient in France using empirical data on six plant-hoverfly networks. We identified plants and insects

at the species level to build detailed interaction networks and hence avoid spurious generalisation levels. In order to better understand the determinants of variation in species interactions in space and time, we used the latitudinal gradient to consider variations linked to environmental cues and the entire flowering period to allow for seasonal variation (Valverde *et al.* 2016; Pellissier *et al.* 2017). One of the main problems of comparing networks along gradients is the dependence of networks metrics on network size (Staniczenko *et al.* 2013; Astegiano *et al.* 2015; Tylianakis & Morris 2017). In this study, we employed Bayesian Structural Equation Models (SEM) to link the numbers of visits to abundance and phenology overlap (PO) through latent probabilities of species interaction and expected numbers of visits per plant-pollinator dyad. We tested different models with variable numbers of effects and compared them in each site. SEM is an emergent approach increasingly used to investigate complex networks of relationship in ecological studies (Grace *et al.* 2010; Eisenhauer *et al.* 2015; Fan *et al.* 2016; Theodorou *et al.* 2017).

We found that in all sites the most important effect affecting pollinator visits was insect abundance (Table 2.2). Likewise we found that plant abundance was also a very important effect in most part of sites, except in the site of F (Table 2. 2). Species abundance often explain the linkage level in pollination network studies (Olesen *et al.* 2008; Bartomeus *et al.* 2016; Chacoff *et al.* 2017; Pellissier *et al.* 2017) but it is often associated with the length of the phenology to better assess the general properties of the interaction network (Vázquez *et al.* 2009; Olito & Fox 2015). In accordance with this verbal prediction, we indeed found that the best models incorporated the effect of PO on either the probability or the intensity of interactions (Table 2.2). Phenology overlap generally cannot predict the probability of interaction on its own (Encinas-Viso *et al.* 2012; CaraDonna *et al.* 2017). Our findings do agree with this general predicament since no site favoured a model that only incorporated PO effects and because these effects always display lower effect sizes than the other variables. In our model, the effect of PO on the probability of interaction and the expected number of visits also vary along the latitudinal gradient (Fig. 2.4).

In general, we observed that southern sites (BF and F) showed shorter plant phenology and phenology overlap (PO) than the other four sites (Table 2.1). In these sites, plant species richness is higher and fewer visits were sampled, probably because the presence of specialist species with short phenophases may increase the number of forbidden or undetected links (Olesen *et al.* 2011; Martín González *et al.* 2012). Conversely, in sites where plant phenology is longer, PO is longer too, as observed in Normandie and Hauts-de-France (CG, FAL, LAR

and R, Table 2. 1). Moreover, when plant richness and specialization are lower, a higher number of visits can be observed (Table 2.1) because generalist species could interact without constraints. Indeed, in Normandie and Hauts-de-France we found that the effect of phenology overlap on the intensity of visits was always likely (PO → λ_{ij}, Table 2.2) and we observed higher numbers of interactions in the first two/three blocks of insects and plants which also corresponded to blocks with longer PO (Fig. 2.3, S3, S4 and S5). A higher phenological overlap is expected to drive a higher probability of interactions and a larger number of visits (Olesen *et al.* 2011). In Occitanie, we did not find any effect of PO on the number of visits because the more densely visited blocks do not correspond to those with longer phenology overlap. Plant phenology can therefore drive the probability and the intensity of interactions in networks in which plant phenology is shorter, thus suggesting that syrphid flies may undergo selection for behavioural flexibility in order to maintain synchrony with their foraging resources (Iler *et al.* 2013; Ogilvie & Forrest 2017).

We also found that modularity decreased along the latitudinal gradient, with richer sites (BF and F) displaying higher modularity (as in Sebastián-González *et al.* 2015). In the two southern sites, higher modularity could be related to shorter phenologies and higher proportions of non-overlapping sets of species, which induce some form of temporal short-term specialisation (Lucas *et al.* 2018). However, modularity also seems to be influenced by species abundances and degrees (Schleuning *et al.* 2014), and is expected to increase with link specificity (Morente-López *et al.* 2018). Indeed, in these sites, species blocks match species degrees (Fig. 2.2 and S2), with generalist and specialist species forming separate blocks among both plants and insects (Martín González *et al.* 2012). With lower modularity and more generalist species, we expect a stronger relationship between phenology and the intensity of interactions because interactions are less influenced by insect preferences and more by seasonal rhythm and flower availability (Dormann *et al.* 2017). Thus, different phenophases might correspond to different compartments (Martín González *et al.* 2012; Morente-López *et al.* 2018), as observed in CG, FAL, LAR and R where higher overlap corresponded to higher numbers of observed visits. Although phenology improved model fit (Table 2. 2), its effect size was modest (Fig. 2.4), which suggests that other types of data such as traits and phylogenies might help predict specific interactions. In our study, we did not consider competition among studied insect species or with other group of insects, such as bees which were present in all sites. Different types of pollinators with different abundances could have context-dependent effects on network topology (Valverde *et al.* 2016).

To conclude, plant phenology here drives the duration of the phenology overlap between plant and insects, which in turn influences either the probability of interaction or the expected number of visits, as well as network compartmentalization. Longer phenologies correspond to less constrained interactions (lower modularity), shorter phenologies to more constrained interactions (higher modularity), which in turn restrict the number of visits. Phenology overlap alone was not sufficient to explain interactions, as suggested elsewhere (CaraDonna *et al.* 2017). Plant and insect abundances played a substantial role to explain the number of visits (as in (Chacoff *et al.* 2017)) since abundances may affect partner choice (Trøjelsgaard *et al.* 2015). Our results, and the ability of the method used here to compare different effects on interaction patterns, suggest that the use of Bayesian SEM to compare networks of different sizes is a valuable tool which can help understand plant-pollinator networks (Eisenhauer *et al.* 2015). The use of latent variables can help predict the probability of interaction and the expected number of visits while avoiding circularity – the introduction of plant and insect specific random effects played the role of an implicit "degree" effect. Our results demonstrate the importance of considering differences in plant and insect phenologies to better predict their interactions in pollination networks at different latitudes. The use of morphological traits (*e.g.* tongue length, inter-tegular distance, …) together with species richness and phylogenies, on top of variables already used, might improve the modelling of interactions and could help better understand some forbidden or missing links in richer communities or considering other pollinators (*e.g.* wild bees).

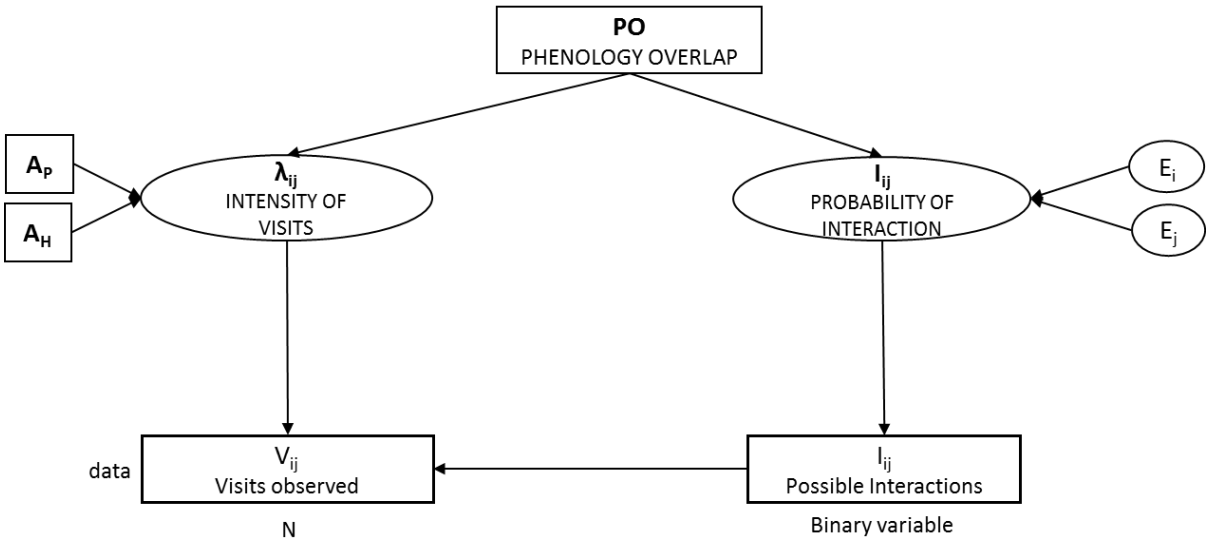

Figure 2. 1. Summary diagram of the SEM model. We estimated 4 effects: the effect of plant abundance (AP → λ_{ij}, coefficient λP), the effect of insect (hoverflies) abundance on the intensity of visits (AH → λ_{ij}, λH), the effect of phenology overlap on the intensity of visits (PO → λ_{ij}, λPO) and the effect of phenology overlap on the probability of interaction (PO → I_{ij}, μPO). The phenology overlap (PO) is the number of phenologically active months that are shared by each pair of insect and plant species along the season. The intensity of visits (λ_{ij}) and the probability of interaction are latent variables in the model. Effect-i and effect-p are random effects calculated by the model which represent the insect and plant degrees. The I_{ij} (Possible interactions) is a binary variable and the V_{ij} (visits observed) follow a Poisson distribution with an expected value given when the probability of interaction is predicted as "true". Rectangles represent observed variables while ovals represent unobserved influences.

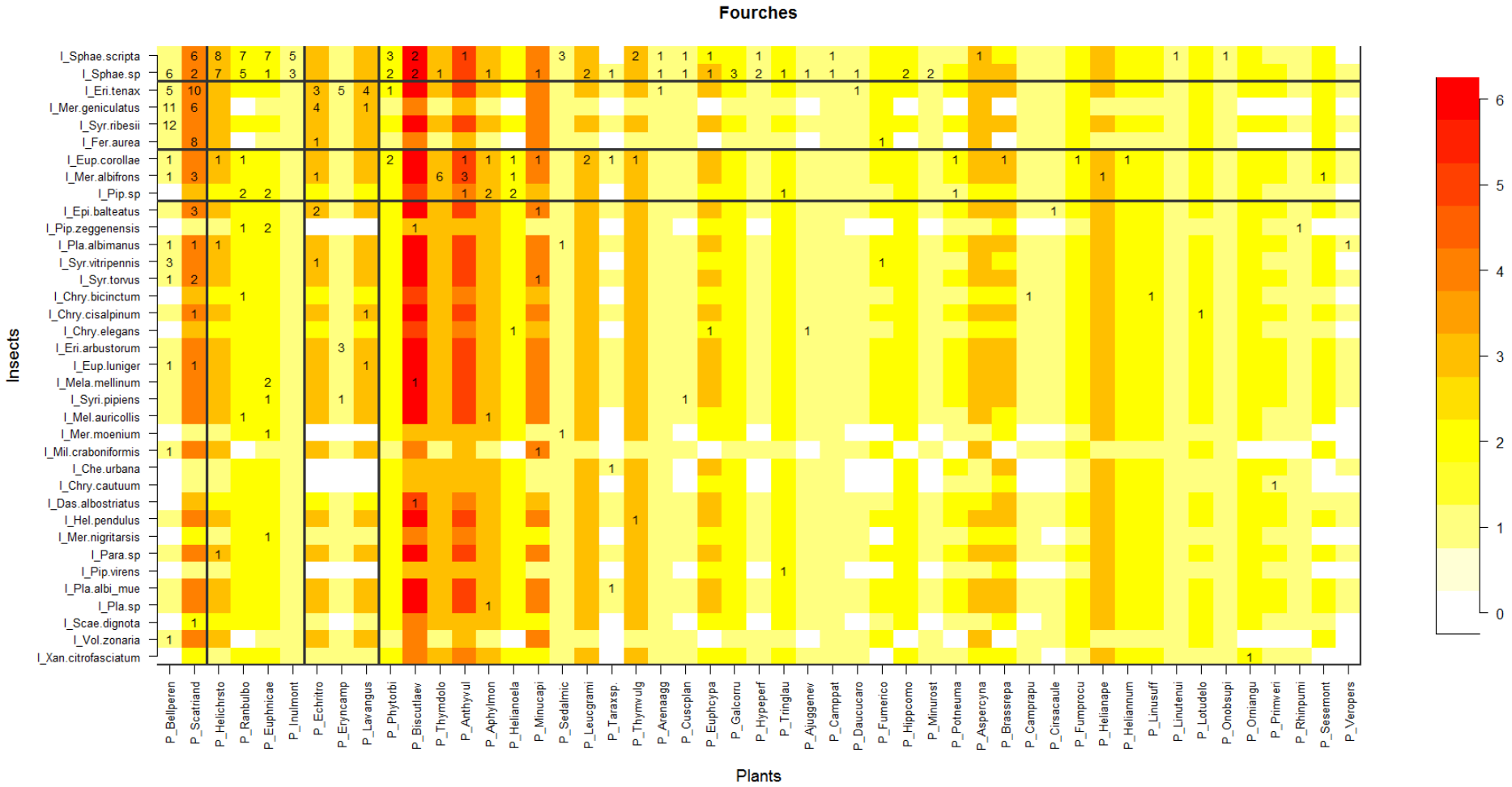

Figure 2. 2. Block clustering provided by LBM in the site of Fourches (F, Occitanie), overlaid on a heatmap of species phenology overlap. The LBM algorithm finds the best division for the group of insects and plants independently through fitting Poisson parameters in each block maximizing the likelihood (ICL). Insect species are displayed in rows and plant species in columns, following their degree (number of partners). The blocks of insects and the blocks of plants are separated by solid black lines. Colours correspond to the number of months that are shared by each pair of plant and insect species (PO, phenology overlap), with higher PO corresponding to darker colours. Numbers are the number of visits observed in the field for a given plant-insect pair.

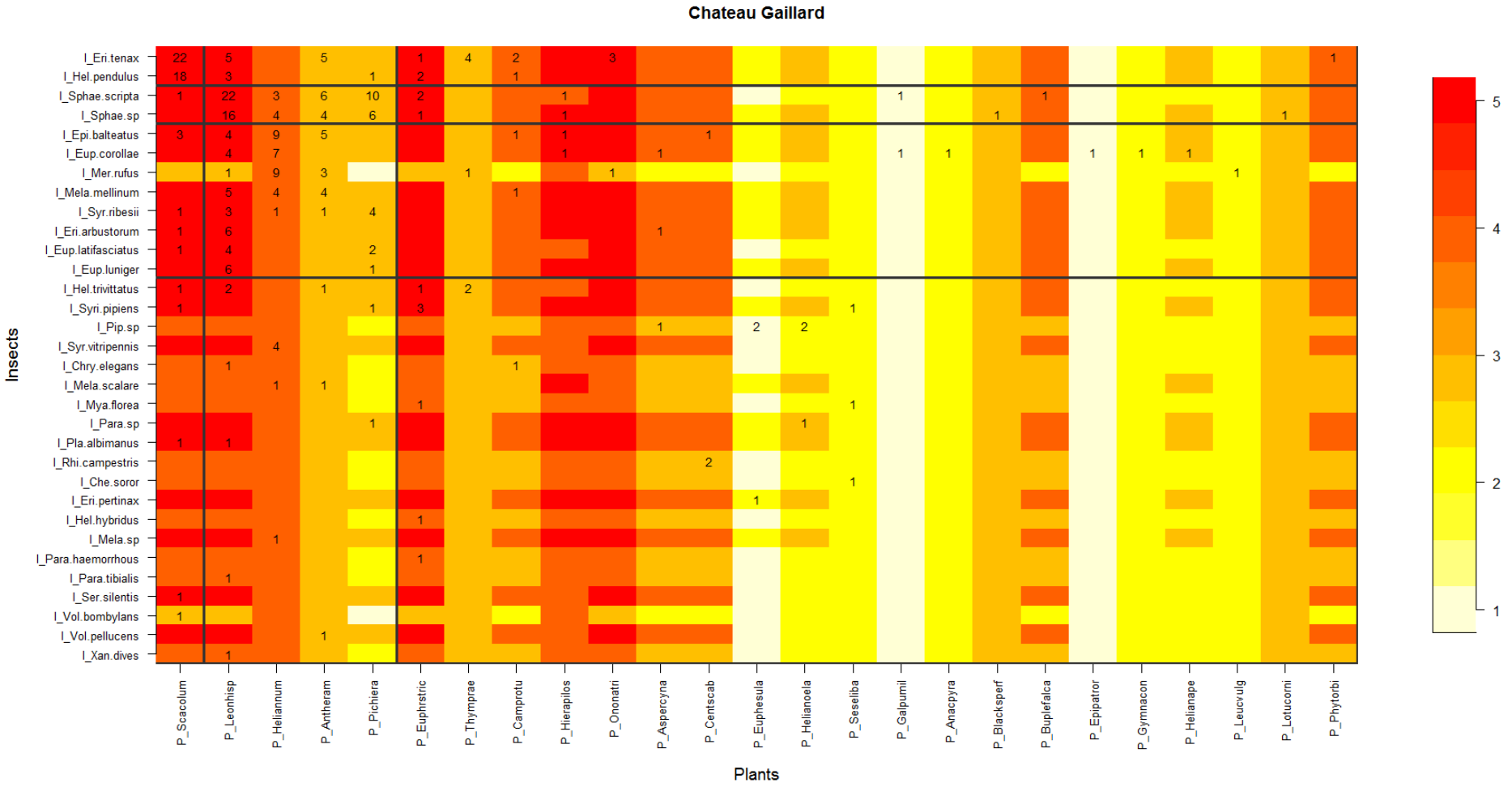

Figure 2. 3. Block clustering provided by LBM in the site of Chateau Gaillard (CG, Normandie) overlaid on a heatmap of species phenology overlap. Insect species are displayed in rows and plant species in columns, following their degree (number of partners). The blocks of insects and the blocks of plants are separated by solid black lines. Colours correspond to the number of months that are shared by each pair of plant and insect species (PO, phenology overlap), with higher PO corresponding to darker colours. Numbers are the number of visits observed in the field for a given plant-insect pair.

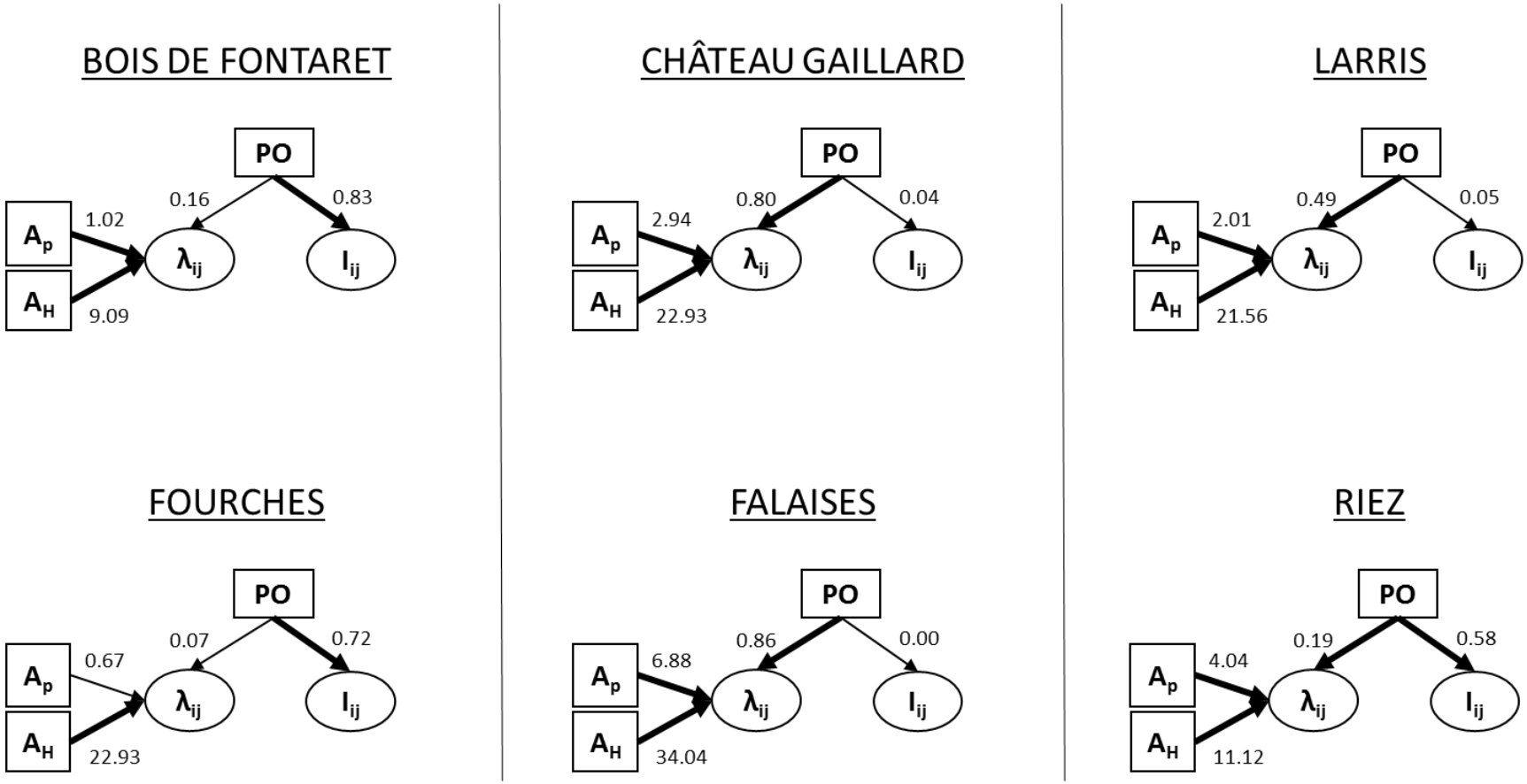

Figure 2. 4. Summary diagram of the best models in all sites. The thickness of the arrows is scaled to Akaike weights (thin ER < 0.73; thick ER > 0.73, cf. Table 2. 2). Standardised coefficients of the model average (computed based on the Akaike weighted model average) are reported next to the arrows. PO is the phenology overlap, I_{ij} is the probability of interaction, λ_{ij} is the intensity of visits, AH and AP are the hoverflies and plant abundances respectively.

Site	Region	Collected data				Specialization index			Species phenology			Modularity analysis	LBM	
		Sampled insects	Insect species	Plant species	Recorded Interactions	H2' index	d' Insects (average + sd)	d' Plants (average + sd)	Insect (average + sd)	Plant (average + sd)	Phenology overlap (PO) (average + sd)	modularity score	blocks I	blocks P
BF	Occitanie	197	40	43	198	0.37	0.63 ± 0.17	0.58 ± 0.17	5.25 ± 1.51	2.14 ± 1.04	1.77 ± 1.03	0.53	2	2
F	Occitanie	223	36	49	286	0.33	0.57 ± 0.18	0.48 ± 0.19	5.61 ± 1.54	2.08 ± 1.13	1.78 ± 1.14	0.48	4	4
CG	Normandie	295	32	25	297	0.34	0.40 ± 0.21	0.47 ± 0.18	6.03 ± 1.00	3.28 ± 1.24	3.02 ± 1.17	0.34	4	3
FAL	Normandie	363	34	30	374	0.32	0.40 ± 0.18	0.41 ± 0.18	6.06 ± 1.13	3.57 ± 1.59	3.23 ± 1.51	0.23	3	4
LAR	Hauts-de-France	220	24	33	220	0.36	0.48 ± 0.19	0.45 ± 0.15	6.38 ± 0.82	3.18 ± 1.38	2.99 ± 1.36	0.37	2	3
R	Hauts-de-France	286	22	29	293	0.32	0.39 ± 0.16	0.40 ± 0.16	5.55 0.74	3.38 ± 1.47	3.11 ± 1.45	0.34	4	3
	Total	**1584**	**76**	**117**	**1668**									

Table 2. 1. Summary table of results obtained in each site (Bois de Fontaret [BF] and Fourches [F] in Occitanie, Château Gaillard [CG] and Falaises [FAL] in Normandie, Larris [LAR] and Riez [R] in Hauts-de-France). H2' and d' indices refer to specialization indices described by Blüthgen et al. (2006) and implemented in the R package bipartite (Dormann *et al.* 2009)*. The modularity score was obtained using the leading-eigenvector method described by Newman (2006) and implemented in the igraph package* (Csardi & Nepusz 2006)*. LBM refers to latent block modelling as implemented in the R package blockmodels* (Leger *et al.* 2015)*.*

			Sites					
			BF	**F**	**CG**	**FAL**	**LAR**	**R**
Model		*Nb of parameters*	**Δ*LOO values***					
0	$\boldsymbol{\lambda_{ij} \sim A_H + A_P + PO / I_{ij} \sim PO}$	4	*2.98*	*2.04*	*3.54*	*2.54*	*2.86*	**0.00**
1	$\boldsymbol{\lambda_{ij} \sim A_H + A_P / I_{ij} \sim PO}$	3	**0.00**	**0.00**	36.75	64.04	10.37	*2.90*
2	$\lambda_{ij} \sim A_P + PO / I_{ij} \sim PO$	3	8.66	78.23	106.46	184.02	44.60	17.00
3	$\lambda_{ij} \sim A_H + PO / I_{ij} \sim PO$	3	6.63	*1.71*	8.09	73.62	11.24	11.42
4	$\boldsymbol{\lambda_{ij} \sim A_H + A_P + PO}$	3	*2.86*	8.06	**0.00**	**0.00**	**0.00**	*2.24*
5	$\lambda_{ij} \sim PO / I_{ij} \sim PO$	2	14.69	73.20	109.85	223.86	55.67	23.09
6	$\lambda_{ij} \sim A_H / I_{ij} \sim PO$	2	*1.45*	*1.31*	33.53	119.04	27.23	19.76
7	$\lambda_{ij} \sim A_P / I_{ij} \sim PO$	2	9.84	72.16	156.61	256.04	47.99	21.53
8	$\lambda_{ij} \sim A_H + PO$	2	11.49	8.18	5.25	71.97	10.28	13.80
9	$\lambda_{ij} \sim A_P + PO$	2	10.71	88.67	103.46	182.14	44.36	17.94
10	$\lambda_{ij} \sim A_H + A_P$	2	24.36	14.04	36.10	66.82	10.51	4.26
11	$I_{ij} \sim PO$	1	11.78	68.52	154.26	272.98	64.12	32.39
12	$\lambda_{ij} \sim PO$	1	19.99	86.20	108.46	219.66	54.64	25.73
13	$\lambda_{ij} \sim A_H$	1	25.58	14.41	36.12	123.30	28.27	22.78
14	$\lambda_{ij} \sim A_P$	1	32.99	87.70	157.74	256.39	48.82	22.87
15	-	0	34.39	83.89	155.68	274.80	64.78	33.52
Model effects			**Evidence ratio (ER)**					
	$\boldsymbol{PO \rightarrow I_{ij}}$		**0.88**	**0.98**	0.15	0.22	0.20	**0.74**
	$\boldsymbol{PO \rightarrow \lambda_{ij}}$		0.26	0.35	**1.00**	**1.00**	**0.99**	**0.79**
	$\boldsymbol{A_H \rightarrow \lambda_{ij}}$		**0.99**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
	$\boldsymbol{A_P \rightarrow \lambda_{ij}}$		**0.74**	0.59	**0.93**	**1.00**	**0.99**	**1.00**

Table 2. 2. (i) Comparison of SEM models using the leave-one-out cross-validation criterion (LOO); (ii) evidence ratios (ER) of model effects in each site. (i) Models are ranked depending on the number of parameters used (from 0 to 4). The best models are the ones with ΔLOO=0 (underlined and bold values). The other suitable models are the ones with ΔLOO <4 (underlined and italic values). λ_{ij} *is the intensity of visits,* I_{ij} *is the probability of interaction,* A_H *is the insect abundance,* A_P *is the plant abundance and PO is the phenology overlap. (ii) We compared 4 model effects: PO →* I_{ij}*, effect of the phenology overlap on the probability of interaction; PO →* λ_{ij} *effect of the phenology overlap on the intensity of visits;* $A_H \rightarrow \lambda_{ij}$ *and* $A_P \rightarrow \lambda_{ij}$ *effects of the hoverflies and plant abundances on the intensity of interaction. The ER limits for unlikelihood is 0.27, plausibility 0.5 and likelihood 0.73. Underlined and bold values represent the likely hypothesis only.*

Supporting Information

The following Supporting Information is available for this article:

Appendix S2.1. Model code.

Appendix S2.2. Model script for the 16 models.

Appendix S2.3. Script modularity and latent block model analysis (LBM).

Figure S2.1. Sites location in France.

Figure S2.2. Block clustering provided by LBM in the site of Bois de Fontaret (BF, Occitanie), overlaid on a heatmap of species phenology overlap.

Figure S2.3. Block clustering provided by LBM in the site of Falaises (FAL, Normandie), overlaid on a heatmap of species phenology overlap.

Figure S2.4. Block clustering provided by LBM in the site of Larris (LAR, Hauts-de-France), overlaid on a heatmap of species phenology overlap.

Figure S2.5. Block clustering provided by LBM in the site of Riez (R, Hauts-de-France), overlaid on a heatmap of species phenology overlap.

Table S2.1. Table of transformed plant abundances.

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Supplementary Information

Does phenology explain plant-pollinator interactions at different latitudes? An assessment of its explanatory power in plant-hoverfly networks in French calcareous grasslands

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Appendix S2.1: Model Code

The model code (in JAGS language) given in this supplementary material refers to the "model Z0" which considers all four parameters (model effects, Table 2. 2 in the main text). Overall, we estimated 16 models that included between 0 and 4 of the above-mentioned effects. To create the code for these other models, parameters should be removed following the order in the Tab. 2. 2. The four parameters tested in the model are: (i) alpha: effect of the phenology overlap (cooc) on the probability of interaction; (ii) epsilon: effect of the phenology overlap on the intensity of visits; (iii) gamma: effect of the insect abundances (ab_I) on the intensity of visits; and (iv) delta: effect of the plant abundances (ab_P) on the intensity of visits.

```
model
{
  for( i in 1 : dim1 ) {
    for( p in 1 : dim2 ) {
      inter[i , p] ~ dbern(mu[i , p])
        logit(mu[i , p]) <- beta + alpha*cooc[i , p] + effet_I[i] + effet_P[p]
        lambda[i,p] <- exp(theta[i,p])
        theta[i,p] <- theta0 + gamma*ab_I[i] + delta*ab_P[p] + epsilon*log(1+cooc[i,p])
        visit[i,p] ~ dpois( inter[i,p]*lambda[i,p] )
        loglik[i,p] <- log(ifelse(visit[i,p]==0,1-mu[i,p]+mu[i ,
p]*dpois(visit[i,p],lambda[i,p]),mu[i , p]*dpois(visit[i,p],lambda[i,p])))
    }
  }

  for( i in 1 : dim1 ) {
    effet_I[i] ~ dnorm( 0.0,tau_I)
  }
```

```
  for( p in 1 : dim2 ) {
    effet_P[p] ~ dnorm( 0.0,tau_P)
  }

        tau_I ~ dexp( 10)
        tau_P ~ dexp( 10)
        alpha ~ dnorm(0,0.01)
        beta ~  dnorm(0,0.01)
        theta0 ~ dnorm(0,0.01)
        gamma ~ dnorm(0,0.01)
        delta ~ dnorm(0,0.01)
        epsilon ~ dnorm(0,0.01)
}
```

Appendix 2.2: Model script for the 16 models – LOO values

The following generic script was applied to all the study sites using all 16 models. The script is separated in three blocks which communicate among them: the script options, the model definitions and the execution (model inference). We defined three options to set (i) the name of the directory (-d), (ii) the site (-s) and (iii) the type of model (-m).

We used, as an example, the information for the site of Bois de Fontaret (BF).

Exemple: Rscript (name) “script-SEMLOO_generique.R” “-d o-BFs-2016” “-s BFs”

In order to calculate the standardised coefficients for each parameters used, at the end of the third block, we added the functions to get the parameter values for each site and each model.

```
############################### BLOCK 1 – SCRIPT OPTION
###############################
library(optparse)
option_list = list(
    make_option(c("-d", "--dir"), type="character", default=NULL, help="directory",
metavar="character"),
    make_option(c("-s", "--site"), type="character", default=NULL, help="site name",
metavar="character"),
      make_option(c("-m", "--modele"), type="character", default="all", help="modele
name", metavar="character"))
opt_parser = OptionParser(option_list=option_list);
opt = parse_args(opt_parser);
site<-opt$site
dossier<-opt$dir
################################## Librairies ##########################
library(bipartite)
library(vegan)
library(igraph)
library(magrittr)
```

```
library(dummies)
library(MuMIn)
library(rjags)
library(boot)
library(R2jags)
library(coda)
library(lattice)
library(ggplot2)
library(loo)
library(matrixStats)
########################## Function to record the LOO values
########################
write_values<-function(x, f, app)
{
        write.table(x, append=app, file=f, sep="\t", row.names=T, col.names=T, quote=F)
}
########################## BLOCK 2 – MODEL FUNCTIONS
############################
#Model function and model initialization: one function for each model from model Z15, with
0 parameters, to Z00 with all the parameters#
### MODEL Z015
mZ015<-function(){
        init.funZ015 <-function(){
          list("tau_I" = rexp(1,10), "tau_P" = rexp(1,10), "beta" = rnorm(1,0,1), "theta0" =
rnorm(1,0,1), "effet_I"=rnorm(dim1,0,1),"effet_P"=rnorm(dim2,0,1), "inter"=inter0)
        }
```

```
        mod.Z015<<-jags(inits=init.funZ015,model.file = "modelZ015_code.txt",data =
list("visit","dim1","dim2"),parameters.to.save =
c("mu","effet_I","effet_P","tau_I","tau_P","beta","theta0", "loglik"),n.chains = 1,
n.iter=1000000, n.burnin = 250000, n.thin = 250)

        mod.Z015.mcmc<-as.mcmc(mod.Z015)

        mZ015<-mod.Z015$BUGSoutput$sims.list

        mZ015.deviance<-mZ015$deviance

        mZ015.loglik<-mZ015$loglik

        dimSEM<-dim(mZ015.loglik)[1]

        list.mZ015<-sapply(1:dimSEM,function(x)
matrix(mZ015.loglik[x,,],nrow=dim1*dim2))

        list.tmZ015<-(t(list.mZ015))

        mZ015.loo<-loo(list.tmZ015)

        loo_file<-paste(dossier, "/", site, "_Z015_loo.txt", sep="")

        write_values("mZ015", app=F, loo_file)

        mZ015_loo_pointwise<-mZ015.loo$pointwise

        mZ015_loo_pareto_k<-mZ015.loo$pareto_k

        mZ015.loo$pareto_k<-NULL

        mZ015.loo$pointwise<-NULL

        write_values(as.matrix(mZ015.loo), app=T, loo_file)

        save.image(paste(dossier, "/", site, "_Z015.RData", sep=""))

}

### MODEL Z014

mZ014<-function(){

        init.funZ014 <-function(){

          list("tau_I" = rexp(1,10), "tau_P" = rexp(1,10), "beta" = rnorm(1,0,1), "delta" =
rnorm(1,0,1), "theta0" = rnorm(1,0,1), "effet_I"=rnorm(dim1,0,1),"effet_P"=rnorm(dim2,0,1),
"inter"=inter0)
```

```
        }
        mod.Z014<<-jags(inits=init.funZ014,model.file = "modelZ014_code.txt",data =
list("visit","ab_P","dim1","dim2"),parameters.to.save =
c("mu","effet_I","effet_P","tau_I","tau_P","delta","beta","theta0","loglik"),n.chains = 1,
n.iter=1000000, n.burnin = 250000, n.thin = 250)
        mod.Z014.mcmc<-as.mcmc(mod.Z014)
        mZ014<-mod.Z014$BUGSoutput$sims.list
        mZ014.deviance<-mZ014$deviance
        mZ014.loglik<-mZ014$loglik
        dimSEM<-dim(mZ014.loglik)[1]
        list.mZ014<-sapply(1:dimSEM,function(x)
matrix(mZ014.loglik[x,,],nrow=dim1*dim2))
        list.tmZ014<-(t(list.mZ014))
        mZ014.loo<-loo(list.tmZ014)
        mZ014.loo
        loo_file<-paste(dossier, "/", site, "_Z014_loo.txt", sep="")
        write_values("mZ014", app=T, loo_file)
        mZ014_loo_pointwise<-mZ014.loo$pointwise
        mZ014_loo_pareto_k<-mZ014.loo$pareto_k
        mZ014.loo$pareto_k<-NULL
        mZ014.loo$pointwise<-NULL
        write_values(as.matrix(mZ014.loo), app=T, loo_file)
        save.image(paste(dossier, "/", site, "_Z014.RData", sep=""))
}
### MODEL Z013
mZ013<-function(){
        init.funZ013 <-function(){
```

```
        list("tau_I" = rexp(1,10), "tau_P" = rexp(1,10), "beta" = rnorm(1,0,1), "gamma" =
rnorm(1,0,1), "theta0" = rnorm(1,0,1), "effet_I"=rnorm(dim1,0,1),"effet_P"=rnorm(dim2,0,1),
"inter"=inter0)

        }

        mod.Z013<<-jags(inits=init.funZ013,model.file = "modelZ013_code.txt",data =
list("visit","ab_I","dim1","dim2"),parameters.to.save =
c("mu","effet_I","effet_P","tau_I","tau_P","gamma","beta","theta0","loglik"),n.chains = 1,
n.iter=1000000, n.burnin = 250000, n.thin = 250)

        mod.Z013.mcmc<-as.mcmc(mod.Z013)

        mZ013<-mod.Z013$BUGSoutput$sims.list

        mZ013.deviance<-mZ013$deviance

        mZ013.loglik<-mZ013$loglik

        dimSEM<-dim(mZ013.loglik)[1]

        list.mZ013<-sapply(1:dimSEM,function(x)
matrix(mZ013.loglik[x,,],nrow=dim1*dim2))

        list.tmZ013<-(t(list.mZ013))

        mZ013.loo<-loo(list.tmZ013)

        mZ013.loo

        loo_file<-paste(dossier, "/", site, "_Z013_loo.txt", sep="")

        write_values("mZ013", app=T, loo_file)

        mZ013_loo_pointwise<-mZ013.loo$pointwise

        mZ013_loo_pareto_k<-mZ013.loo$pareto_k

        mZ013.loo$pareto_k<-NULL

        mZ013.loo$pointwise<-NULL

        write_values(as.matrix(mZ013.loo), app=T, loo_file)

        save.image(paste(dossier, "/", site, "_Z013.RData", sep=""))

}

### MODEL Z012
```

```
mZ012<-function(){
        init.funZ012 <-function(){
          list("tau_I" = rexp(1,10), "tau_P" = rexp(1,10), "beta" = rnorm(1,0,1), "theta0" =
rnorm(1,0,1), "epsilon" = rnorm(1,0,1),
"effet_I"=rnorm(dim1,0,1),"effet_P"=rnorm(dim2,0,1), "inter"=inter0)
        }
        mod.Z012<<-jags(inits=init.funZ012,model.file = "modelZ012_code.txt",data =
list("cooc","visit","dim1","dim2"),parameters.to.save =
c("mu","effet_I","effet_P","tau_I","tau_P","beta","theta0","epsilon","loglik"),n.chains = 1,
n.iter=1000000, n.burnin = 250000, n.thin = 250)
        mod.Z012.mcmc<-as.mcmc(mod.Z012)
        mZ012<-mod.Z012$BUGSoutput$sims.list
        mZ012.deviance<-mZ012$deviance
        mZ012.loglik<-mZ012$loglik
        dimSEM<-dim(mZ012.loglik)[1]
        list.mZ012<-sapply(1:dimSEM,function(x)
matrix(mZ012.loglik[x,,],nrow=dim1*dim2))
        list.tmZ012<-(t(list.mZ012))
        mZ012.loo<-loo(list.tmZ012)
        mZ012.loo
        loo_file<-paste(dossier, "/", site, "_Z012_loo.txt", sep="")
        write_values("mZ012", app=T, loo_file)
        mZ012_loo_pointwise<-mZ012.loo$pointwise
        mZ012_loo_pareto_k<-mZ012.loo$pareto_k
        mZ012.loo$pareto_k<-NULL
        mZ012.loo$pointwise<-NULL
        write_values(as.matrix(mZ012.loo), app=T, loo_file)
        save.image(paste(dossier, "/", site, "_Z012.RData", sep=""))
```

```
}
### MODEL Z011
mZ011<-function(){
        init.funZ011 <-function(){
          list("tau_I" = rexp(1,10), "tau_P" = rexp(1,10), "alpha" = 0.1,"beta" = rnorm(1,0,1),
"theta0" = rnorm(1,0,1), "effet_I"=rnorm(dim1,0,1),"effet_P"=rnorm(dim2,0,1),
"inter"=inter0)
        }
        mod.Z011<<-jags(inits=init.funZ011,model.file = "modelZ011_code.txt",data =
list("cooc","visit","dim1","dim2"),parameters.to.save =
c("mu","effet_I","effet_P","tau_I","tau_P","alpha","beta","theta0","loglik"),n.chains = 1,
n.iter=1000000, n.burnin = 250000, n.thin = 250)
        mod.Z011.mcmc<-as.mcmc(mod.Z011)
        mZ011<-mod.Z011$BUGSoutput$sims.list
        mZ011.deviance<-mZ011$deviance
        mZ011.loglik<-mZ011$loglik
        dimSEM<-dim(mZ011.loglik)[1]
        list.mZ011<-sapply(1:dimSEM,function(x)
matrix(mZ011.loglik[x,,],nrow=dim1*dim2))
        list.tmZ011<-(t(list.mZ011))
        mZ011.loo<-loo(list.tmZ011)
        mZ011.loo
        loo_file<-paste(dossier, "/", site, "_Z011_loo.txt", sep="")
        write_values("mZ011", app=T, loo_file)
        mZ011_loo_pointwise<-mZ011.loo$pointwise
        mZ011_loo_pareto_k<-mZ011.loo$pareto_k
        mZ011.loo$pareto_k<-NULL
        mZ011.loo$pointwise<-NULL
```

```
        write_values(as.matrix(mZ011.loo), app=T, loo_file)
        save.image(paste(dossier, "/", site, "_Z011.RData", sep=""))
}
### MODEL Z010
mZ010<-function(){
        init.funZ010 <-function(){
          list("tau_I" = rexp(1,10), "tau_P" = rexp(1,10), "beta" = rnorm(1,0,1), "gamma" =
rnorm(1,0,1), "delta" = rnorm(1,0,1), "theta0" = rnorm(1,0,1),
"effet_I"=rnorm(dim1,0,1),"effet_P"=rnorm(dim2,0,1), "inter"=inter0)
        }
        mod.Z010<<-jags(inits=init.funZ010,model.file = "modelZ010_code.txt",data =
list("visit","ab_I","ab_P","dim1","dim2"),parameters.to.save =
c("mu","effet_I","effet_P","tau_I","tau_P","gamma","delta","beta","theta0","loglik"),n.chains
= 1, n.iter=1000000, n.burnin = 250000, n.thin = 250)
        mod.Z010.mcmc<-as.mcmc(mod.Z010)
        mZ010<-mod.Z010$BUGSoutput$sims.list
        mZ010.deviance<-mZ010$deviance
        mZ010.loglik<-mZ010$loglik
        dimSEM<-dim(mZ010.loglik)[1]
        list.mZ010<-sapply(1:dimSEM,function(x)
matrix(mZ010.loglik[x,,],nrow=dim1*dim2))
        list.tmZ010<-(t(list.mZ010))
        mZ010.loo<-loo(list.tmZ010)
        mZ010.loo
        loo_file<-paste(dossier, "/", site, "_Z010_loo.txt", sep="")
        write_values("mZ010", app=T, loo_file)
        mZ010_loo_pointwise<-mZ010.loo$pointwise
        mZ010_loo_pareto_k<-mZ010.loo$pareto_k
```

```
        mZ010.loo$pareto_k<-NULL
        mZ010.loo$pointwise<-NULL
        write_values(as.matrix(mZ010.loo), app=T, loo_file)
        save.image(paste(dossier, "/", site, "_Z010.RData", sep=""))
}
### MODEL Z09
mZ09<-function(){
        init.funZ09 <-function(){
          list("tau_I" = rexp(1,10), "tau_P" = rexp(1,10), "beta" = rnorm(1,0,1), "delta" =
rnorm(1,0,1), "theta0" = rnorm(1,0,1), "epsilon" = rnorm(1,0,1),
"effet_I"=rnorm(dim1,0,1),"effet_P"=rnorm(dim2,0,1), "inter"=inter0)
        }
        mod.Z09<<-jags(inits=init.funZ09,model.file = "modelZ09_code.txt",data =
list("cooc","visit","ab_P","dim1","dim2"),parameters.to.save =
c("mu","effet_I","effet_P","tau_I","tau_P","delta","beta","theta0","epsilon","loglik"),n.chains
= 1, n.iter=1000000, n.burnin = 250000, n.thin = 250)
        mod.Z09.mcmc<-as.mcmc(mod.Z09)
        mZ09<-mod.Z09$BUGSoutput$sims.list
        mZ09.deviance<-mZ09$deviance
        mZ09.loglik<-mZ09$loglik
        dimSEM<-dim(mZ09.loglik)[1]
        list.mZ09<-sapply(1:dimSEM,function(x) matrix(mZ09.loglik[x,,],nrow=dim1*dim2))
        list.tmZ09<-(t(list.mZ09))
        mZ09.loo<-loo(list.tmZ09)
        mZ09.loo
        loo_file<-paste(dossier, "/", site, "_Z09_loo.txt", sep="")
        write_values("mZ09", app=T, loo_file)
```

```
	mZ09_loo_pointwise<-mZ09.loo$pointwise
	mZ09_loo_pareto_k<-mZ09.loo$pareto_k
	mZ09.loo$pareto_k<-NULL
	mZ09.loo$pointwise<-NULL
	write_values(as.matrix(mZ09.loo), app=T, loo_file)
	save.image(paste(dossier, "/", site, "_Z09.RData", sep=""))
}
### MODEL Z08
mZ08<-function(){
	init.funZ08 <-function(){
	  list("tau_I" = rexp(1,10), "tau_P" = rexp(1,10), "beta" = rnorm(1,0,1), "gamma" = rnorm(1,0,1), "theta0" = rnorm(1,0,1), "epsilon" = rnorm(1,0,1), "effet_I"=rnorm(dim1,0,1),"effet_P"=rnorm(dim2,0,1), "inter"=inter0)
	}
	mod.Z08<<-jags(inits=init.funZ08,model.file = "modelZ08_code.txt",data = list("cooc","visit","ab_I","dim1","dim2"),parameters.to.save = c("mu","effet_I","effet_P","tau_I","tau_P","gamma","beta","theta0","epsilon","loglik"),n.chains = 1, n.iter=1000000, n.burnin = 250000, n.thin = 250)
	mod.Z08.mcmc<-as.mcmc(mod.Z08)
	mZ08<-mod.Z08$BUGSoutput$sims.list
	mZ08.deviance<-mZ08$deviance
	mZ08.loglik<-mZ08$loglik
	dimSEM<-dim(mZ08.loglik)[1]
	list.mZ08<-sapply(1:dimSEM,function(x) matrix(mZ08.loglik[x,,],nrow=dim1*dim2))
	list.tmZ08<-(t(list.mZ08))
	mZ08.loo<-loo(list.tmZ08)
	mZ08.loo
```

```
	loo_file<-paste(dossier, "/", site, "_Z08_loo.txt", sep="")
	write_values("mZ08", app=T, loo_file)
	mZ08_loo_pointwise<-mZ08.loo$pointwise
	mZ08_loo_pareto_k<-mZ08.loo$pareto_k
	mZ08.loo$pareto_k<-NULL
	mZ08.loo$pointwise<-NULL
	write_values(as.matrix(mZ08.loo), app=T, loo_file)
	save.image(paste(dossier, "/", site, "_Z08.RData", sep=""))
}
### MODEL Z07
mZ07<-function(){
	init.funZ07 <-function(){
	  list("tau_I" = rexp(1,10), "tau_P" = rexp(1,10), "alpha" = 0.1,"beta" = rnorm(1,0,1),
"delta" = rnorm(1,0,1), "theta0" = rnorm(1,0,1),
"effet_I"=rnorm(dim1,0,1),"effet_P"=rnorm(dim2,0,1), "inter"=inter0)
	}
	mod.Z07<<-jags(inits=init.funZ07,model.file = "modelZ07_code.txt",data =
list("cooc","visit","ab_P","dim1","dim2"),parameters.to.save =
c("mu","effet_I","effet_P","tau_I","tau_P","alpha","delta","beta","theta0","loglik"),n.chains =
1, n.iter=1000000, n.burnin = 250000, n.thin = 250)
	mod.Z07.mcmc<-as.mcmc(mod.Z07)
	mZ07<-mod.Z07$BUGSoutput$sims.list
	mZ07.deviance<-mZ07$deviance
	mZ07.loglik<-mZ07$loglik
	dimSEM<-dim(mZ07.loglik)[1]
	list.mZ07<-sapply(1:dimSEM,function(x) matrix(mZ07.loglik[x,,],nrow=dim1*dim2))
	list.tmZ07<-(t(list.mZ07))
```

```
	mZ07.loo<-loo(list.tmZ07)
	mZ07.loo
	loo_file<-paste(dossier, "/", site, "_Z07_loo.txt", sep="")
	write_values("mZ07", app=T, loo_file)
	mZ07_loo_pointwise<-mZ07.loo$pointwise
	mZ07_loo_pareto_k<-mZ07.loo$pareto_k
	mZ07.loo$pareto_k<-NULL
	mZ07.loo$pointwise<-NULL
	write_values(as.matrix(mZ07.loo), app=T, loo_file)
	save.image(paste(dossier, "/", site, "_Z07.RData", sep=""))
}
### MODEL Z06
mZ06<-function(){
	init.funZ06 <-function(){
	  list("tau_I" = rexp(1,10), "tau_P" = rexp(1,10), "alpha" = 0.1,"beta" = rnorm(1,0,1),
"gamma" = rnorm(1,0,1), "theta0" = rnorm(1,0,1),
"effet_I"=rnorm(dim1,0,1),"effet_P"=rnorm(dim2,0,1), "inter"=inter0)
	}
	mod.Z06<<-jags(inits=init.funZ06,model.file = "modelZ06_code.txt",data =
list("cooc","visit","ab_I","dim1","dim2"),parameters.to.save =
c("mu","effet_I","effet_P","tau_I","tau_P","alpha","gamma","beta","theta0","loglik"),n.chains
= 1, n.iter=1000000, n.burnin = 250000, n.thin = 250)
	mod.Z06.mcmc<-as.mcmc(mod.Z06)
	mZ06<-mod.Z06$BUGSoutput$sims.list
	mZ06.deviance<-mZ06$deviance
	mZ06.loglik<-mZ06$loglik
	dimSEM<-dim(mZ06.loglik)[1]
```

```
	list.mZ06<-sapply(1:dimSEM,function(x) matrix(mZ06.loglik[x,,],nrow=dim1*dim2))
	list.tmZ06<-(t(list.mZ06))
	mZ06.loo<-loo(list.tmZ06)
	mZ06.loo
	loo_file<-paste(dossier, "/", site, "_Z06_loo.txt", sep="")
	write_values("mZ06", app=T, loo_file)
	mZ06_loo_pointwise<-mZ06.loo$pointwise
	mZ06_loo_pareto_k<-mZ06.loo$pareto_k
	mZ06.loo$pareto_k<-NULL
	mZ06.loo$pointwise<-NULL
	write_values(as.matrix(mZ06.loo), app=T, loo_file)
	save.image(paste(dossier, "/", site, "_Z06.RData", sep=""))
}
### MODEL Z05
mZ05<-function(){
	init.funZ05 <-function(){
	  list("tau_I" = rexp(1,10), "tau_P" = rexp(1,10), "alpha" = 0.1,"beta" = rnorm(1,0,1),
"theta0" = rnorm(1,0,1), "epsilon" = rnorm(1,0,1),
"effet_I"=rnorm(dim1,0,1),"effet_P"=rnorm(dim2,0,1), "inter"=inter0)
	}
	mod.Z05<<-jags(inits=init.funZ05,model.file = "modelZ05_code.txt",data =
list("cooc","visit","dim1","dim2"),parameters.to.save =
c("mu","effet_I","effet_P","tau_I","tau_P","alpha","beta","theta0","epsilon","loglik"),n.chains
= 1, n.iter=1000000, n.burnin = 250000, n.thin = 250)
	mod.Z05.mcmc<-as.mcmc(mod.Z05)
	mZ05<-mod.Z05$BUGSoutput$sims.list
	mZ05.deviance<-mZ05$deviance
```

```
	mZ05.loglik<-mZ05$loglik
	dimSEM<-dim(mZ05.loglik)[1]
	list.mZ05<-sapply(1:dimSEM,function(x) matrix(mZ05.loglik[x,,],nrow=dim1*dim2))
	list.tmZ05<-(t(list.mZ05))
	mZ05.loo<-loo(list.tmZ05)
	mZ05.loo
	loo_file<-paste(dossier, "/", site, "_Z05_loo.txt", sep="")
	write_values("mZ05", app=T, loo_file)
	mZ05_loo_pointwise<-mZ05.loo$pointwise
	mZ05_loo_pareto_k<-mZ05.loo$pareto_k
	mZ05.loo$pareto_k<-NULL
	mZ05.loo$pointwise<-NULL
	write_values(as.matrix(mZ05.loo), app=T, loo_file)
	save.image(paste(dossier, "/", site, "_Z05.RData", sep=""))
}
### MODEL Z04
mZ04<-function(){
	init.funZ04 <-function(){
	  list("tau_I" = rexp(1,10), "tau_P" = rexp(1,10), "beta" = rnorm(1,0,1), "gamma" = rnorm(1,0,1), "delta" = rnorm(1,0,1), "theta0" = rnorm(1,0,1), "epsilon" = rnorm(1,0,1), "effet_I"=rnorm(dim1,0,1),"effet_P"=rnorm(dim2,0,1), "inter"=inter0)
	}
	mod.Z04<<-jags(inits=init.funZ04,model.file = "modelZ04_code.txt",data = list("cooc","visit","ab_I","ab_P","dim1","dim2"),parameters.to.save = c("mu","effet_I","effet_P","tau_I","tau_P","gamma","delta","beta","theta0","epsilon","loglik"),n.chains = 1, n.iter=1000000, n.burnin = 250000, n.thin = 250)
	mod.Z04.mcmc<-as.mcmc(mod.Z04)
```

```
	mZ04<-mod.Z04$BUGSoutput$sims.list
	mZ04.deviance<-mZ04$deviance
	mZ04.loglik<-mZ04$loglik
	dimSEM<-dim(mZ04.loglik)[1]
	list.mZ04<-sapply(1:dimSEM,function(x) matrix(mZ04.loglik[x,,],nrow=dim1*dim2))
	list.tmZ04<-(t(list.mZ04))
	mZ04.loo<-loo(list.tmZ04)
	mZ04.loo
	loo_file<-paste(dossier, "/", site, "_Z04_loo.txt", sep="")
	write_values("mZ04", app=T, loo_file)
	mZ04_loo_pointwise<-mZ04.loo$pointwise
	mZ04_loo_pareto_k<-mZ04.loo$pareto_k
	mZ04.loo$pareto_k<-NULL
	mZ04.loo$pointwise<-NULL
	write_values(as.matrix(mZ04.loo), app=T, loo_file)
	save.image(paste(dossier, "/", site, "_Z04.RData", sep=""))
}
### MODEL Z03
mZ03<-function(){
	init.funZ03 <-function(){
	  list("tau_I" = rexp(1,10), "tau_P" = rexp(1,10), "alpha" = 0.1,"beta" = rnorm(1,0,1),
"gamma" = rnorm(1,0,1), "theta0" = rnorm(1,0,1), "epsilon" = rnorm(1,0,1),
"effet_I"=rnorm(dim1,0,1),"effet_P"=rnorm(dim2,0,1), "inter"=inter0)
	}
	mod.Z03<<-jags(inits=init.funZ03,model.file = "modelZ03_code.txt",data =
list("cooc","visit","ab_I","dim1","dim2"),parameters.to.save =
```

```
c("mu","effet_I","effet_P","tau_I","tau_P","alpha","gamma","beta","theta0","epsilon","loglik
"),n.chains = 1, n.iter=1000000, n.burnin = 250000, n.thin = 250)
        mod.Z03.mcmc<-as.mcmc(mod.Z03)
        mZ03<-mod.Z03$BUGSoutput$sims.list
        mZ03.deviance<-mZ03$deviance
        mZ03.loglik<-mZ03$loglik
        dimSEM<-dim(mZ03.loglik)[1]
        list.mZ03<-sapply(1:dimSEM,function(x) matrix(mZ03.loglik[x,,],nrow=dim1*dim2))
        list.tmZ03<-(t(list.mZ03))
        mZ03.loo<-loo(list.tmZ03)
        mZ03.loo
        loo_file<-paste(dossier, "/", site, "_Z03_loo.txt", sep="")
        write_values("mZ03", app=T, loo_file)
        mZ03_loo_pointwise<-mZ03.loo$pointwise
        mZ03_loo_pareto_k<-mZ03.loo$pareto_k
        mZ03.loo$pareto_k<-NULL
        mZ03.loo$pointwise<-NULL
        write_values(as.matrix(mZ03.loo), app=T, loo_file)
        save.image(paste(dossier, "/", site, "_Z03.RData", sep=""))
}
### MODEL Z02
mZ02<-function(){
        init.funZ02 <-function(){
          list("tau_I" = rexp(1,10), "tau_P" = rexp(1,10), "alpha" = 0.1, "beta" = rnorm(1,0,1),
"delta" = rnorm(1,0,1), "theta0" = rnorm(1,0,1), "epsilon" = rnorm(1,0,1),
"effet_I"=rnorm(dim1,0,1),"effet_P"=rnorm(dim2,0,1), "inter"=inter0)
        }
```

```
	mod.Z02<<-jags(inits=init.funZ02,model.file = "modelZ02_code.txt",data =
list("cooc","visit","ab_P","dim1","dim2"),parameters.to.save =
c("mu","effet_I","effet_P","tau_I","tau_P","alpha","delta","beta","theta0","epsilon","loglik"),
n.chains = 1, n.iter=1000000, n.burnin = 250000, n.thin = 250)
	mod.Z02.mcmc<-as.mcmc(mod.Z02)
	mZ02<-mod.Z02$BUGSoutput$sims.list
	mZ02.deviance<-mZ02$deviance
	mZ02.loglik<-mZ02$loglik
	dimSEM<-dim(mZ02.loglik)[1]
	list.mZ02<-sapply(1:dimSEM,function(x) matrix(mZ02.loglik[x,,],nrow=dim1*dim2))
	list.tmZ02<-(t(list.mZ02))
	mZ02.loo<-loo(list.tmZ02)
	mZ02.loo
	loo_file<-paste(dossier, "/", site, "_Z02_loo.txt", sep="")
	write_values("mZ02", app=T, loo_file)
	mZ02_loo_pointwise<-mZ02.loo$pointwise
	mZ02_loo_pareto_k<-mZ02.loo$pareto_k
	mZ02.loo$pareto_k<-NULL
	mZ02.loo$pointwise<-NULL
	write_values(as.matrix(mZ02.loo), app=T, loo_file)
	save.image(paste(dossier, "/", site, "_Z02.RData", sep=""))
}
### MODEL Z01
mZ01<-function(){
	init.funZ01 <-function(){
```

```
    list("tau_I" = rexp(1,10), "tau_P" = rexp(1,10), "alpha" = 0.1,"beta" = rnorm(1,0,1),
"gamma" = rnorm(1,0,1), "delta" = rnorm(1,0,1), "theta0" = rnorm(1,0,1),
"effet_I"=rnorm(dim1,0,1),"effet_P"=rnorm(dim2,0,1), "inter"=inter0)

    }

    mod.Z01<<-jags(inits=init.funZ01,model.file = "modelZ01_code.txt",data =
list("cooc","visit","ab_I","ab_P", "dim1", "dim2"),parameters.to.save =
c("mu","effet_I","effet_P","tau_I","tau_P","alpha","gamma","delta","beta","theta0","loglik"),
n.chains = 1, n.iter=1000000, n.burnin = 250000, n.thin = 250)

    mod.Z01.mcmc<-as.mcmc(mod.Z01)

    mZ01<-mod.Z01$BUGSoutput$sims.list

    mZ01.deviance<-mZ01$deviance

    mZ01.loglik<-mZ01$loglik

    dimSEM<-dim(mZ01.loglik)[1]

    list.mZ01<-sapply(1:dimSEM,function(x) matrix(mZ01.loglik[x,,],nrow=dim1*dim2))

    list.tmZ01<-(t(list.mZ01))

    mZ01.loo<-loo(list.tmZ01)

    mZ01.loo

    loo_file<-paste(dossier, "/", site, "_Z01_loo.txt", sep="")

    write_values("mZ01", app=T, loo_file)

    mZ01_loo_pointwise<-mZ01.loo$pointwise

    mZ01_loo_pareto_k<-mZ01.loo$pareto_k

    mZ01.loo$pareto_k<-NULL

    mZ01.loo$pointwise<-NULL

    write_values(as.matrix(mZ01.loo), app=T, loo_file)

    save.image(paste(dossier, "/", site, "_Z01.RData", sep=""))

}

### MODEL Z00
```

```
mZ00<-function(){
    init.funZ00 <-function(){
     list("tau_I" = rexp(1,10), "tau_P" = rexp(1,10), "alpha" = 0.1,"beta" = rnorm(1,0,1), "gamma" = rnorm(1,0,1), "delta" = rnorm(1,0,1), "theta0" = rnorm(1,0,1), "epsilon" = rnorm(1,0,1), "effet_I"=rnorm(dim1,0,1),"effet_P"=rnorm(dim2,0,1), "inter"=inter0)
    }
    mod.Z00<<-jags(inits=init.funZ00,model.file = "modelZ00_code.txt",data = list("cooc","visit","ab_I","ab_P","dim1","dim2"),parameters.to.save = c("mu","effet_I","effet_P","tau_I","tau_P","alpha","gamma","delta","beta","theta0","epsilon", "loglik"),n.chains = 1, n.iter=1000000, n.burnin = 250000, n.thin = 250)
    mod.Z00.mcmc<-as.mcmc(mod.Z00)
    mZ00<-mod.Z00$BUGSoutput$sims.list
    mZ00.deviance<-mZ00$deviance
    mZ00.loglik<-mZ00$loglik
    dimSEM<-dim(mZ00.loglik)[1]
    list.mZ00<-sapply(1:dimSEM,function(x) matrix(mZ00.loglik[x,,],nrow=dim1*dim2))
    list.tmZ00<-(t(list.mZ00))
    mZ00.loo<-loo(list.tmZ00)
    mZ00.loo
    loo_file<-paste(dossier, "/", site, "_Z00_loo.txt", sep="")
    write_values("mZ00", app=T, loo_file)
    mZ00_loo_pointwise<-mZ00.loo$pointwise
    mZ00_loo_pareto_k<-mZ00.loo$pareto_k
    mZ00.loo$pareto_k<-NULL
    mZ00.loo$pointwise<-NULL
    write_values(as.matrix(mZ00.loo), app=T, loo_file)
    save.image(paste(dossier, "/", site, "_Z00.RData", sep=""))
```

```
}
###### end model functions
print("JOB DONE")
#####################################################
###     Network information (do not change)         ###
#####################################################
################################BLOCK 3 – MODEL EXECUTION
########################
#launch_modele<-function(){
        ntw<-read.table(paste(dossier, "/", site, "_ntw.txt", sep=""),
sep="\t",header=T,row.names=1)
        dim1<-dim(ntw)[1]
        dim2<-dim(ntw)[2]
        web<-as.matrix(ntw,dim1,dim2)
        inter0<-dget(paste(dossier, "/", site, "_web_i.txt", sep=""))
        cooc<-dget(paste(dossier, "/", site, "_co.txt", sep=""))
        visit<-read.table(paste(dossier, "/", site, "_ntw.txt", sep=""),sep="\t",header=T)
        visit<-as.matrix(visit)
        abundanceI<-read.table(paste(dossier, "/", site, "_abI.txt", sep=""), sep="\t", header=T)
        ab_I <- log(abundanceI[,2])
        abundanceP<-read.table(paste(dossier, "/", site, "_abP.txt", sep=""), sep="\t",
header=T)
        ab_P <- log(abundanceP[,2])
        if(opt$modele == "all")
        {
                print("modele: all")
                for(i in 0:15)
```

```
        {
            print(paste("COMPUTING MODELE ", i, "\n", sep=""))
            mod<-eval(parse(text=paste("mZ0", i, sep="")))
            mod()

        }
    }else{
        print(paste("modele: ", opt$modele), sep="")
        mod<-eval(parse(text=paste("m", opt$modele, sep="")))    #recupération de la
fonction du modele
        mod()
    }
#### end model execution
#launch_modele()

################################PARAMETER
VALUES################################
library(optparse)
option_list = list(
    make_option(c("-d", "--dir"), type="character", default=NULL, help="model
directory", metavar="character"),
    make_option(c("-s", "--site"), type="character", default=NULL, help="site name",
metavar="character"))
opt_parser = OptionParser(option_list=option_list);
opt = parse_args(opt_parser);
rdata<-list.files(opt$dir, pattern="*_Z015.RData")
```

```
load(paste(opt$dir, "/", rdata, sep=""))        #chargement du RData qui contient tous les modèles pour un site donné
print(paste("RData ", rdata, " loaded", sep=""))
for(mod in ls(pattern="mod.Z0*"))
{
        print(paste("getting values from ", mod, sep=""))
        model<-eval(parse(text=mod))
        if(is.null(model$BUGSoutput$mean$alpha)){model$BUGSoutput$mean$alpha<-NA}
        if(is.null(model$BUGSoutput$mean$beta)){model$BUGSoutput$mean$beta<-NA}
        if(is.null(model$BUGSoutput$mean$delta)){model$BUGSoutput$mean$delta<-NA}
        if(is.null(model$BUGSoutput$mean$epsilon)){model$BUGSoutput$mean$epsilon<-NA}
        if(is.null(model$BUGSoutput$mean$gamma)){model$BUGSoutput$mean$gamma<-NA}
        val<-matrix(c(model$BUGSoutput$mean$alpha, model$BUGSoutput$mean$beta, model$BUGSoutput$mean$delta, model$BUGSoutput$mean$epsilon, model$BUGSoutput$mean$gamma), 1, 5, dimnames=list("values", c("alpha", "beta", "delta", "epsilon", "gamma")))
        write.table(val, file=paste(opt$dir, "/", opt$site, "_", mod, "_values.txt", sep=""), quote=F, sep="\t", row.names=F, col.names=T)
}
```

Appendix S2.3: Modularity and latent block model analysis

We calculated the modularity of the network using the cluster_leading_eigen method for modularity optimization implemented in the igraph package (Csardi and Nepusz 2006, Newman 2006). We then performed latent block models (LBM) using the BM_poisson method for quantitative network data implemented in the blockmodels package (Leger et al. 2015). Blocks are calculated separately for the two groups (insect and plant) based on the number of visits (*i.e.* a weighted network). The algorithm finds the best divisions of insects and plants through fitting one Poisson parameter in each block of the visit matrix, thus essentially maximizing the ICL (Integrated Completed Likelihood; Biernacki et al. 2000, Daudin et al. 2007).

```
library(bipartite)
library(vegan)
library(igraph)
library(dummies)
library(blockmodels)
library(ade4)
library(fields)

#site data (ex: Bois de Fontaret, BFs)
BFs<-read.table("ntwBFs.txt",header=T,sep="\t")
webBFs <- as.matrix(BFs)
############################ Modularity analysis, binary data
#########################
BFs.graph.bin<-graph_from_incidence_matrix(webBFs,multiple=F) #binary
BFs.bin.cle<-cluster_leading_eigen(BFs.graph.bin)
BFs.bin.cle
#get phenology overlap matrix
```

```
coBF<-dget("coBFs.txt")
########################## LBM code: LBM analysis following Poisson
###################
bmi_BFs<-BM_poisson('LBM', webBFs)
bmi_BFs$estimate()
numi_BFs<-which.max(bmi_BFs$ICL)
densi_BFs<-sum(webBFs)/(nrow(webBFs)*ncol(webBFs))
probi_BFs<-bmi_BFs$model_parameters[[numi_BFs]]$lambda
row.nb.gpi<-nrow(probi_BFs)
col.nb.gpi<-ncol(probi_BFs)
prob.rowi<-bmi_BFs$memberships[[numi_BFs]]$Z1
hh.namei<-rownames(webBFs)
mbrshp.hhi<-apply(prob.rowi,1,which.max)
ls.freq.rowi<-rowSums(webBFs)
res.hhi<-cbind.data.frame(hh.namei=hh.namei, mbrshp.hhi=mbrshp.hhi,
freq.hhi=ls.freq.rowi)
res.hh.ordi<-res.hhi[order(res.hhi$freq.hhi),]
cpt=0
for(k in 1: (nrow(res.hh.ordi)-1))
{
  if (res.hh.ordi$mbrshp.hhi[k] !=res.hh.ordi$mbrshp.hhi[k+1]) cpt=cpt+1
}
nb.diff.hhi=cpt-(length(levels(as.factor(res.hh.ordi$mbrshp.hhi)))-1)
#write tables
write.table(res.hh.ordi,sep="\t",row.names=FALSE)
prob.coli<-bmi_BFs$memberships[[numi_BFs]]$Z2
sp.namei<-colnames(webBFs)
```

```
mbrshp.spi<-apply(prob.coli,1,which.max)
ls.freq.coli<-colSums(webBFs)
res.spi<-cbind.data.frame(sp.namei=sp.namei, mbrshp.spi=mbrshp.spi, freq.spi=ls.freq.coli)
res.sp.ordi<-res.spi[order(res.spi$freq.spi),]
cpt=0
for (k in 1: (nrow(res.sp.ordi)-1))
{
  if(res.sp.ordi$mbrshp.spi[k] !=res.sp.ordi$mbrshp.spi[k+1]) cpt=cpt+1
}
nb.diff.spi=cpt-(length(levels(as.factor(res.sp.ordi$mbrshp.spi)))-1)
res.sp.ord2i=res.spi[order(res.spi$mbrshp.spi),]
write.table(res.sp.ordi,sep="\t",row.names=FALSE)
write.table(probi_BFs,file="_prob_BFs",sep="\t",row.names=FALSE)

################################ Matrix organization ###############
par(mfrow=c(1,1))
webBFs2<-webBFs
webBFs[which(webBFs>1)]=1
nb.row=nrow(webBFs)
nb.col=ncol(webBFs)
nds=webBFs
nps=coBF
res.prob=read.table("_prob_BFs",sep="\t",h=TRUE)
ls.ord.col.prob=order(colSums(res.prob),decreasing=TRUE)
ls.ord.row.prob=order(rowSums(res.prob),decreasing=TRUE)
ls.ord.hhi=sapply(res.hhi$mbrshp.hhi,function(x) which (x==ls.ord.row.prob))
```

```
res.hh.ord2i=res.hhi[order(ls.ord.hhi),]
row.nb.gpi=length(levels(as.factor(res.hhi$mbrshp.hhi)))
res.hh.ord3i=NULL
for (h in ls.ord.row.prob)
{
 part=res.hh.ord2i[res.hh.ord2i$mbrshp.hhi==h,]
 part.ord=part[order(part$freq.hhi,decreasing=TRUE),]
 res.hh.ord3i=rbind.data.frame(res.hh.ord3i,part.ord)
}
ls.ord.sp=sapply(res.spi$mbrshp.spi,function(x) which (x==ls.ord.col.prob))
res.sp.ord2i=res.spi[order(ls.ord.sp),]
col.nb.gb=length(levels(as.factor(res.spi$mbrshp.spi)))
res.sp.ord3i=NULL
for (h in ls.ord.col.prob)
{
 part=res.sp.ord2i[res.sp.ord2i$mbrshp.spi==h,]
 part.ord=part[order(part$freq.spi,decreasing=TRUE),]
 res.sp.ord3i=rbind.data.frame(res.sp.ord3i,part.ord)
}
nds=nds[as.character(res.hh.ord3i$hh.namei),as.character(res.sp.ord3i$sp.namei)]
nps=nps[as.character(res.hh.ord3i$hh.namei),as.character(res.sp.ord3i$sp.namei)]
webBFs2=webBFs2[as.character(res.hh.ord3i$hh.namei),as.character(res.sp.ord3i$sp.namei)]

######## Plot matrix with heatcolours and the number of visits #######
visits<-matrix(webBFs2,nrow=dim(webBFs2)[1]*dim(webBFs2)[2],ncol=1)
visits<-visits[which(visits>0)] #without the zeros
```

```
coord.function<-function(x,nI,nP){
  c(((x-1)%%nI)+1,((x-1)%/%nI)+1)
}
func.plot.matrix<-function(x,y){
  indices<-which(x==1)
  min<-min(y)
  max<-max(y)
  yLabels<-rownames(x)
  xLabels<-colnames(x)
  title<-c("Bois de Fontaret")
  if(is.null(xLabels)){
    xLabels<-c(1:ncol(x))
  }
  if(is.null(yLabels)){
    yLabels<-c(1:nrow(x))
  }
  reverse<-nrow(x):1
  yLabels<-yLabels[reverse]
  y<-y[reverse,]
  image.plot(1:length(xLabels),1:length(yLabels),t(y),col=c("white",heat.colors(12)[12:1]),
xlab="", ylab="",axes=FALSE,zlim=c(min,max))
  if(!is.null(title)){
    title(ylab="Insects", line=8, cex.lab=1)
    title(xlab="Plants", line=6, cex.lab=1.2)
    title("Bois de Fontaret")
  }
```

```
  axis(BELOW<-1,at=1:length(xLabels),labels=as.factor(as.character(xLabels)),las =2,
cex.axis=0.6)
  axis(LEFT<-2,at=1:length(yLabels), labels=as.factor(as.character(yLabels)),las=
2,cex.axis=0.6)
  axis(BELOW<-1,at=1:length(xLabels),labels=rep("",length(xLabels)),las =2,cex.axis=0.6)
  axis(LEFT<-2,at=1:length(yLabels),labels=rep("",length(yLabels)),las=2,cex.axis<-0.6)
  coo<-t(rbind(sapply(indices,function(xx) coord.function(xx,nrow(x),ncol(x)))))
  text(coo[,2],nrow(webBFs)+1-coo[,1],labels=visits, cex=0.6)
}
func.plot.matrix(nds,nps)
####### Black lines to delimit blocks in the plot ######
if (row.nb.gpi>1)
{
  ls.class=as.numeric(as.data.frame(table(res.hh.ord2i$mbrshp.hhi))[ls.ord.row.prob,2])
  ls.cum=sum(ls.class)-cumsum(ls.class)
  abline(h=ls.cum+0.5,col="grey20", lwd=3)
}
if (col.nb.gpi>1)
{
  ls.class=as.numeric(as.data.frame(table(res.sp.ord2i$mbrshp.spi))[ls.ord.col.prob,2])
  ls.cum=cumsum(ls.class)
  abline(v=ls.cum+0.5,col="grey20", lwd=3)
}
```

Figures and Tables

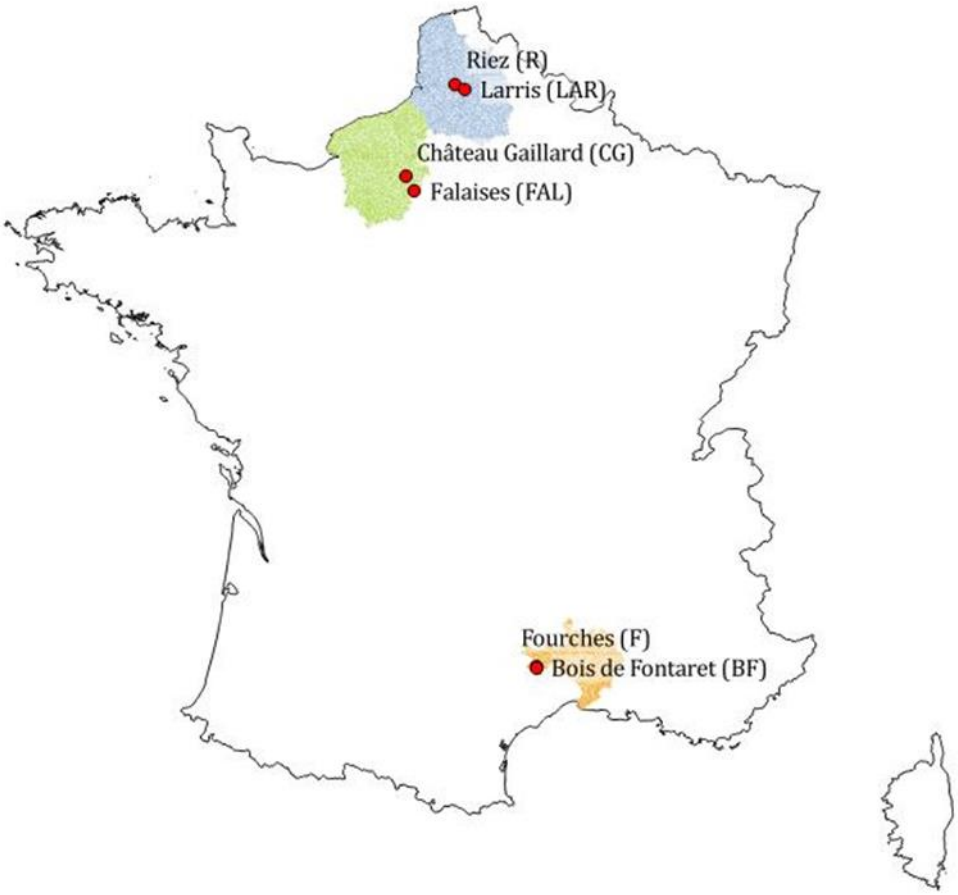

Figure S2. 1. Site location in France: in blue the French départements Pas-de-Calais and Somme (Hauts-de-France region), in green the départements Eure and Seine Maritime (Normandie region), in orange the department Gard (Occitanie region). The six sites correspond to the red dots.

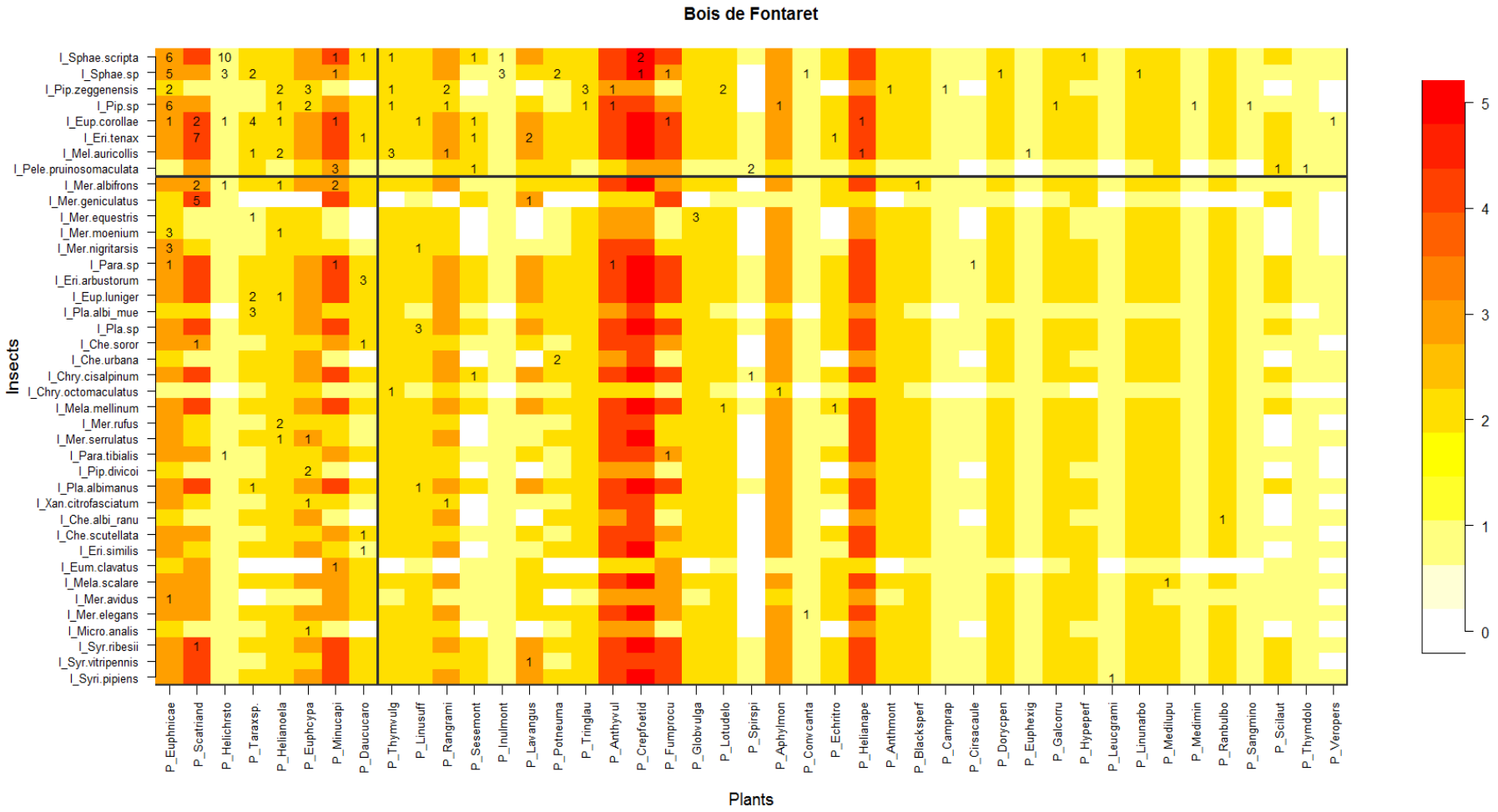

Figure S2. 2. Block clustering provided by LBM in the site of Bois de Fontaret (BF, Occitanie), overlaid on a heatmap of species phenology overlap. Insect species are displayed in rows and plant species in columns, following their degree (number of partners). The blocks of insects and the blocks of plants are separated by solid black lines. Colours correspond to the number of months that are shared by each pair of plant and insect species (PO, phenology overlap), with higher PO corresponding to darker colours. Numbers are the number of visits observed in the field for a given plant-insect pair.

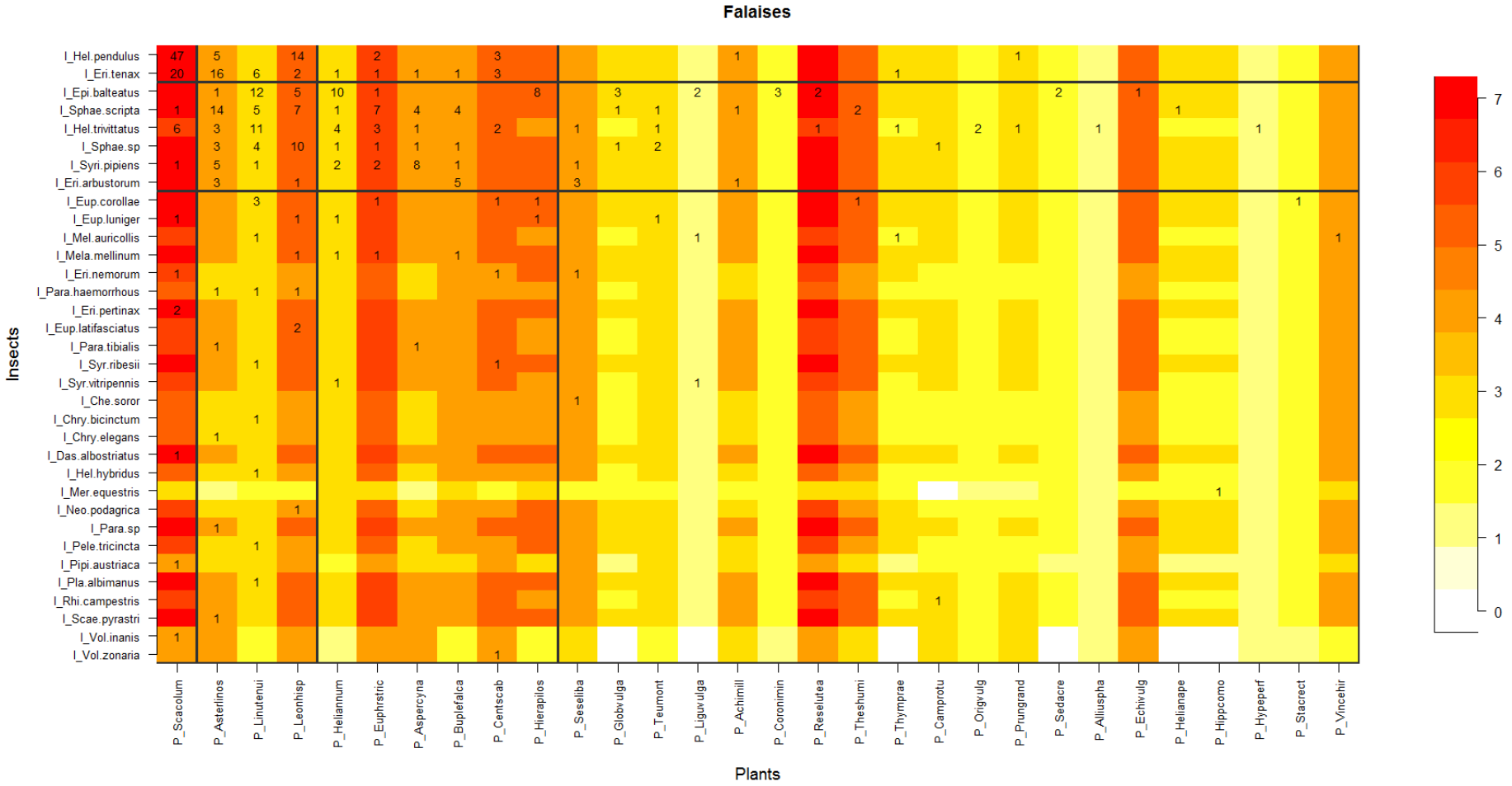

Figure S2. 3. Block clustering provided by LBM in the site of Falaises (FAL, Normandie), overlaid on a heatmap of species phenology overlap. Insect species are displayed in rows and plant species in columns, following their degree (number of partners). The blocks of insects and the blocks of plants are separated by solid black lines. Colours correspond to the number of months that are shared by each pair of plant and insect species (PO, phenology overlap), with higher PO corresponding to darker colours. Numbers are the number of visits observed in the field for a given plant-insect pair.

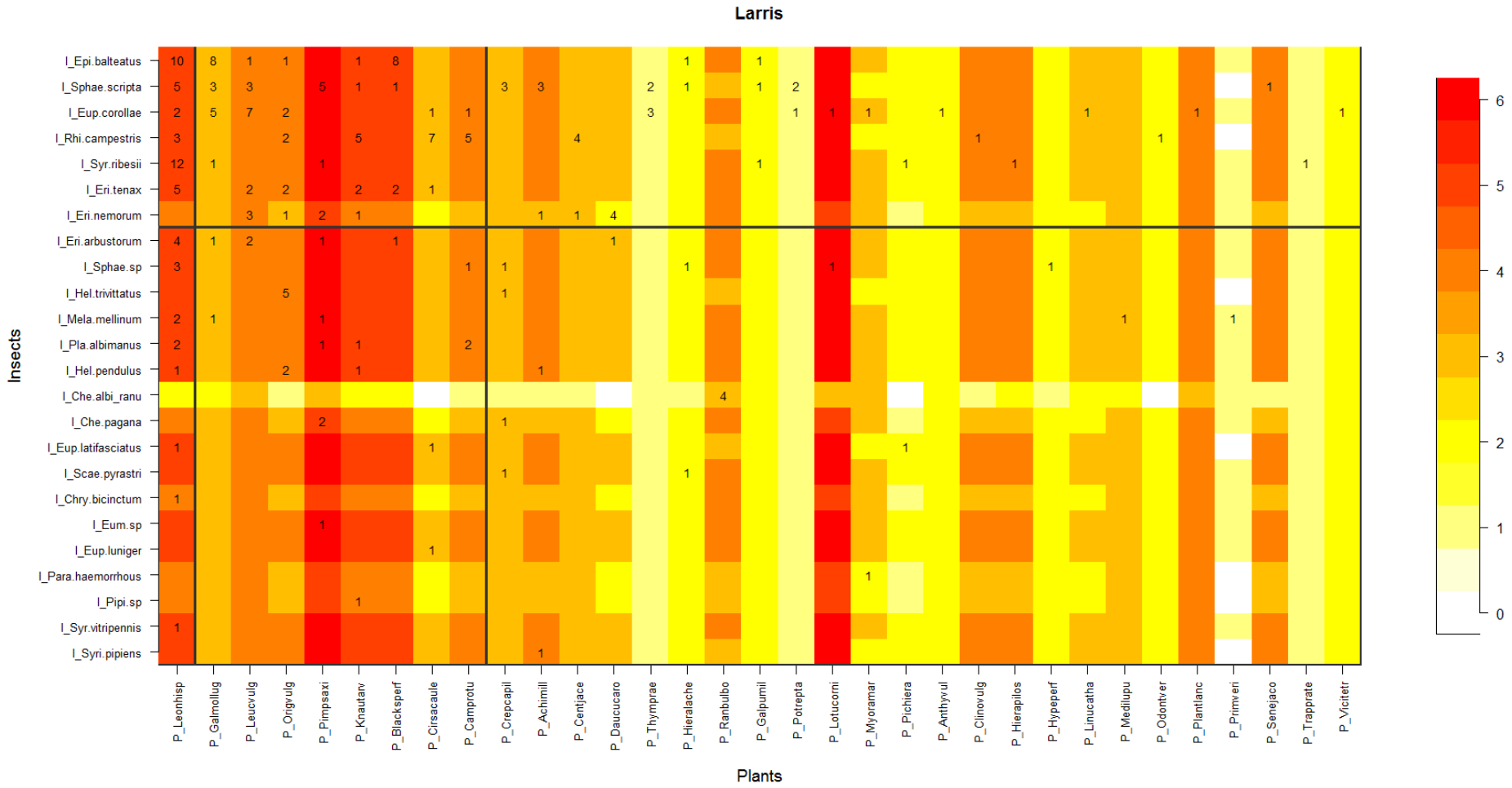

Figure S2. 4. Block clustering provided by LBM in the site of Larris (LAR, Hauts-de-France), overlaid on a heatmap of species phenology overlap. Insect species are displayed in rows and plant species in columns, following their degree (number of partners). The blocks of insects and the blocks of plants are separated by solid black lines. Colours correspond to the number of months that are shared by each pair of plant and insect species (PO, phenology overlap), with higher PO corresponding to darker colours. Numbers are the number of visits observed in the field for a given plant-insect pair.

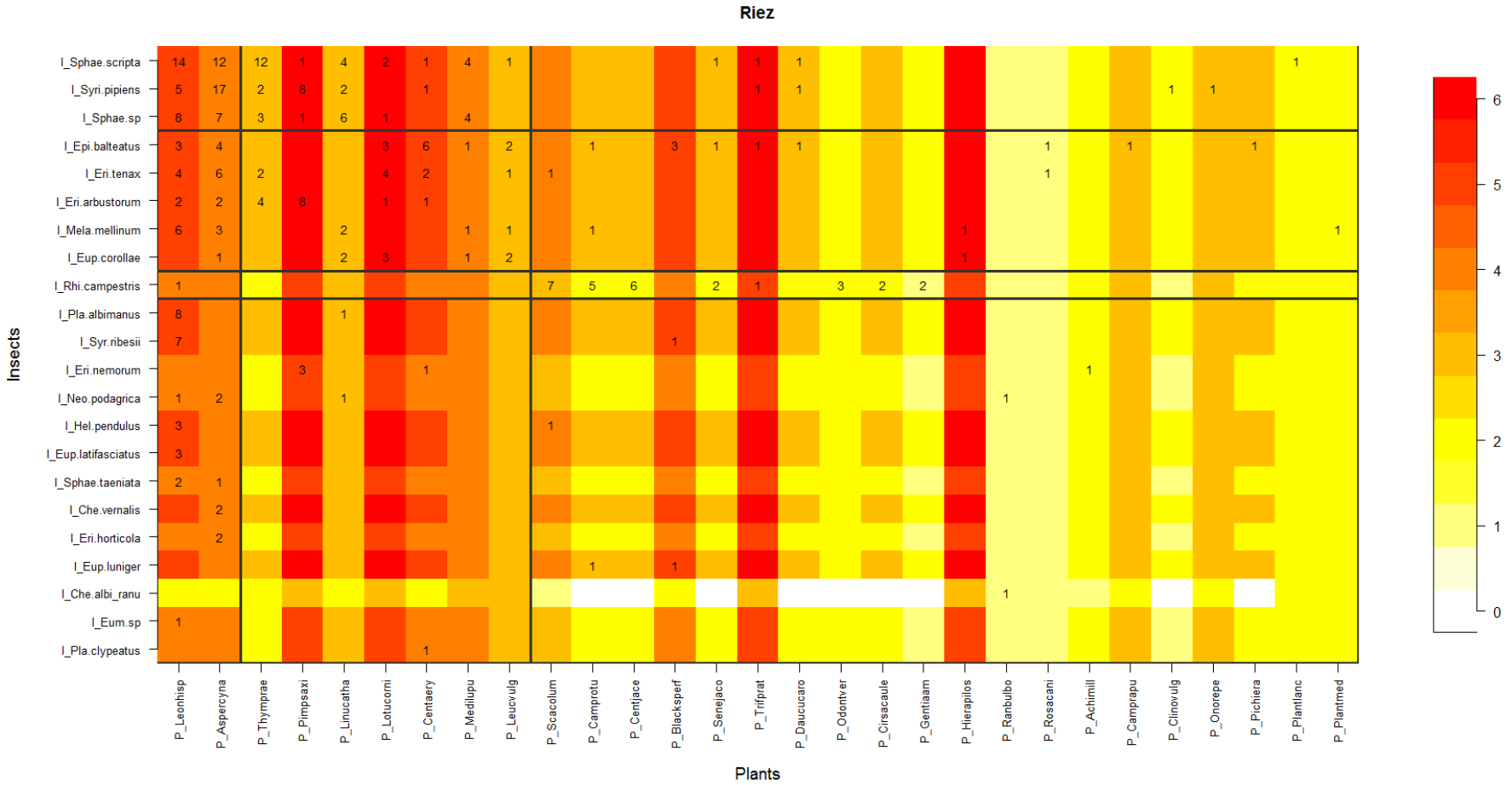

Figure S2. 5. Block clustering provided by LBM in the site of Riez (R, Hauts-de-France), overlaid on a heatmap of species phenology overlap. Insect species are displayed in rows and plant species in columns, following their degree (number of partners). The blocks of insects and the blocks of plants are separated by solid black lines. Colours correspond to the number of months that are shared by each pair of plant and insect species (PO, phenology overlap), with higher PO corresponding to darker colours. Numbers are the number of visits observed in the field for a given plant-insect pair.

Coefficient Braun-Blanquet	Abundance percentage interval	Abundance percentage
i	1 individual	0.1%
+	< 1 %	0.5%
1	1-10 %	5%
2	10-25 %	15%
3	25-50 %	35%
4	50-75 %	65%
5	75-100 %	85%

Table S2. 1. Table of transformed plant abundances. The first column shows the Braun-Blanquet coefficients of, the second column, their percentages, and the third column, the transformed abundances used as the plant abundances in the model.

Chapter III

How biased is our perception of plant-pollinator networks? A comparison of visit- and pollen-based representations of the same networks

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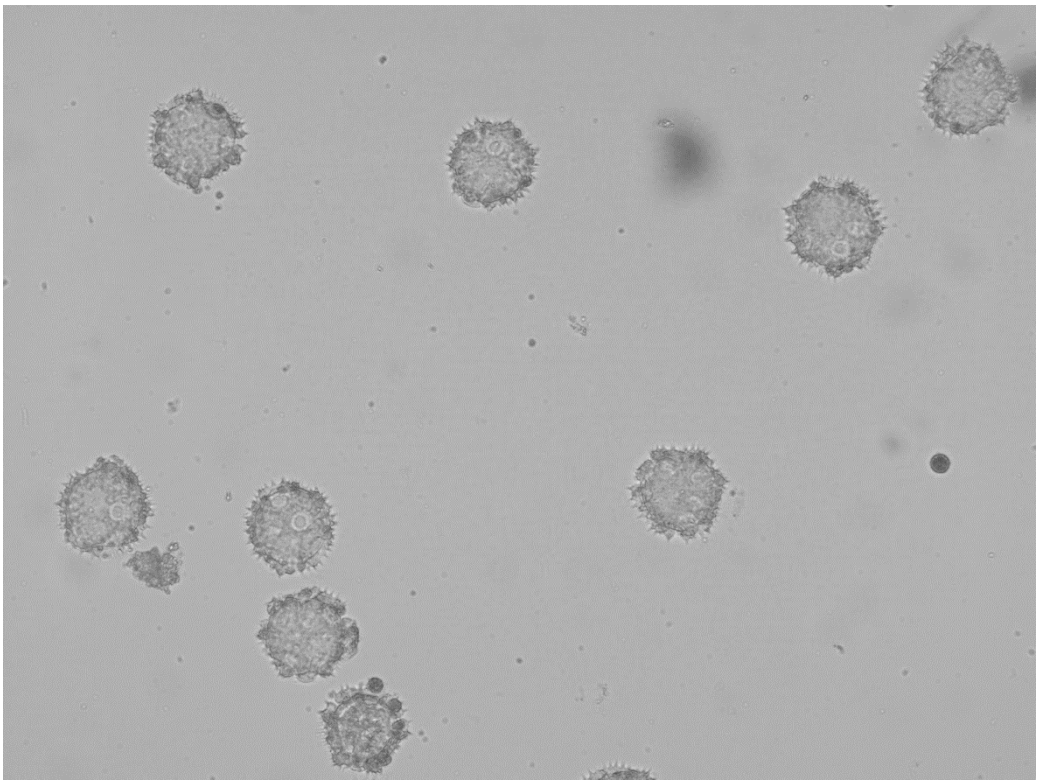


(Article submitted in Acta Oecologica, Special Issue "Pollination in the Anthropocene")

Abstract

Most plant-pollinator networks are based on observations of contact between an insect and a flower in the field. Despite significant sampling efforts, some links are easier to report, while others remain unobserved. Therefore, visit-based networks represent a subsample of possible interactions in which the ignored part is variable. Pollen is a natural marker of the plants visited by a given insect. The identification of pollen found on insect bodies can be used as an alternative method to study plant-pollinator interactions, with a potentially lower risk of bias than the observation of visits, since it increases the number of interactions in the network. Here we compare plant-pollinator networks constructed (i) from direct observation of pollinator visits and (ii) from identification of pollen found on the same insects. We focused on three calcareous grasslands along an environmental gradient in France, with different plant and pollinator species diversities. Since pollen identification always yields richer, more connected networks, we focused our comparisons on sampling bias at equal network connectance. To do so, we first compared network structures with an analysis of latent blocks and motifs. We then compared species roles between both types of networks with an analysis of specialization and species positions within motifs. Our results suggest that the sampling from observations of insect visits does not lead to the construction of a network intrinsically different from the one obtained using pollen found on insect bodies, at least when field sampling strives to be exhaustive. Most of the significant differences are found at the species level, not at the network structure level, with singleton species accounting for a respectable fraction of these differences. Overall, this suggests that recording plant-pollinator interactions from pollinator visit observation does not provide a biased picture of the network structure, regardless of species richness; however, it provided less information on species roles than the pollen-based network.

Introduction

Plant-pollinator interaction networks are critical to ecological systems and agriculture (Ashworth *et al.* 2009; Memmott 2009). Pollinators indeed provide an invaluable service, on which much of current agriculture depends (Klein *et al.* 2007; Gallai *et al.* 2009). Reciprocally, wild plants provide various resources to pollinators, hence maintaining their populations through provisioning services (Deguines *et al.* 2014). Understanding the structure and functioning of these networks and obtaining more accurate information on plant-pollinator networks are among the current important goals of ecology. For these reasons it is essential to manage these ecosystem services because disruption of interactions can affect the diversity, abundance and distribution of both plants and pollinators, with cascading consequences affecting the whole network (Gill *et al.* 2016). Most plant-pollinator networks are based on direct observations of contact between an insect and a flower in the field. However, some links are biologically (*i.e.* morphologically) or temporally (*i.e.* phenologically) forbidden, while other links can remain unobserved (Olesen *et al.* 2011). Thus, such visit-based networks can only represent a subsample of all possible interactions. Alternative methodologies or more intense sampling can reduce the probability of missing some existing interactions. One such alternative method is the identification of pollen found on pollinator bodies.

Pollen is a major attractant for many pollinators since it is an important part of their diet (Kearns & Inouye 1993). Moreover, it can favour long-term associative learning in wild bees (Muth *et al.* 2016), influencing the floral choice of pollinators and their foraging strategy (Somme *et al.* 2015). As a result of this "visitation activity", *i.e.* when pollinators visit a plant, pollen becomes attached to their bodies. Thus, it becomes a natural marker indicating the recent history of pollinator visits (Jones 2012) since a significant part of the pollen grains generally stay on the pollinator's body. The identification of this pollen provides valuable information on the spectrum of pollen resources and it is an important method to elucidate the foraging behaviour and the floral preferences of wild pollinators, such as solitary and social bees (Carvell *et al.* 2006; Beil *et al.* 2008; Marchand *et al.* 2015; Fisogni *et al.* 2018), hoverflies (Lucas et al., 2018a; 2018b, Rader et al., 2011), butterflies and other pollinators (Stewart & Dudash 2016; Macgregor *et al.* 2019). Pollen is also often used to assess pollinator effectiveness both at the community level (King *et al.* 2013; Ballantyne *et al.* 2015; Willmer *et al.* 2017) and at the individual level (Marchand *et al.* 2015; Tur *et al.* 2015). Indeed, not all the visits recorded in the field correspond to actual pollination (King *et al.* 2013; Popic *et al.* 2013) and not all the pollinators are equally efficient. For example, not all pollen grains transported by corbiculated-bees are available for the pollination event, since the moistening (using nectar) may cause physiological changes in the pollen grain (Parker *et al.* 2015).

The identification of pollen found on insect bodies can be used as an alternative method to study plant-pollinator interactions, with potentially less bias than the observation of visits. However, few studies have compared visit-based networks to pollen-based ones (Alarcón, 2010; Bosch et al., 2009; Olesen et al., 2011; Pornon et al., 2017, 2016), mostly because the identification of pollen grains is time-consuming and depends on the availability of experts with skills in palynology. The precision of pollen identification depends on knowledge of the floral community in the study sites (Westrich & Schmidt 1990), thus suggesting that the use of a complete pollen atlas of the co-flowering species of the study site, as we used in the present study, may enhance the precision of identification. An alternative method to microscopic identification that recently garnered interest is the use of DNA barcoding (Richardson *et al.* 2015; Pornon *et al.* 2016, 2017; Bell *et al.* 2017, 2019; Macgregor *et al.* 2019). It is, however, a recent methodology not widely used in the study of plant-pollinator networks and it can have some limits (Bell *et al.* 2017; Macgregor *et al.* 2019).

Various studies have pointed out that when pollen information is used to build networks, the number of links between plant and insect species significantly increases, revealing changes in the network structure (Bosch *et al.* 2009; Pornon *et al.* 2017). However, all these studies compared network structure using classic network metrics, such as connectance, nestedness and modularity, which are strongly affected by network dimensions (*i.e.* the number of species and realised links among them; Rivera-Hutinel et al., 2012; Staniczenko et al., 2013). Thus, differences obtained in the network structure when visit- and pollen-based networks are compared are essentially due to the higher number of species and new links recorded in the latter. To our knowledge, only one study (Popic *et al.* 2013) used a null model approach to take into account differences in network size when comparing pollen- and visit-based networks. They found that network structure does not significantly change between the two methods but did not investigate changes at the species level.

The aim of this study is to understand to what extent networks obtained using pollinator visit records can introduce biases in the representation of the "true" network when compared to those obtained through pollen identification. For a constant sampling effort, we could expect that richer communities are more likely to be undersampled than poorer communities. Then, the addition of pollen information can lead to changes in the network structure and species roles, since apparent specialised species may be more generalist than observed and thus separate groups of species may be more connected, revealing a biased picture of the "real" network. To test these hypotheses, we used simulated networks mimicking the ones obtained through observation of insect visits but based on the pool of possible interactions given by the pollen-based network. In a sense, these randomized networks can be considered as different "virtual observers" sampling from all the possible interactions detected using

the pollen on insect bodies, but with a sampling effort equal to that used in the field. This technique allowed us to compare two networks of the same size and to check for congruence between networks. Armed with this methodological framework, we studied the plant-pollinator networks encountered in three different calcareous grasslands distributed along an environmental and diversity gradient in France. We compared the two types of networks (visit- and pollen-based networks) using a new methodological approach combining different analyses. First, we compared the network structure using latent block models (LBM) and motif analyses (Leger *et al.* 2015; Simmons *et al.* 2019a). Second, we compared species specialisation level (Blüthgen *et al.* 2006) and species roles, based on the frequency of species positions within motifs (Simmons *et al.* 2019a).

Materials and methods

Study sites and plant inventories

In this study we focus on a part of a larger survey, in which fieldwork was performed from April to October 2016, once a month, in six calcareous grasslands located in three different French regions. We chose calcareous grasslands since they are characterised by highly diverse plant communities with a high proportion of entomophilous species (WallisDeVries *et al.* 2002; Butaye *et al.* 2005; Baude *et al.* 2016). The six sites, of 1 hectare each, are included in the European NATURA 2000 network, which aims to protect a number of habitats and species representative of European biodiversity. We sampled both wild bees and hoverflies and we recorded their interactions with flowering species, at each session. Flowering plants were identified at the species level in the field and their abundances recorded. All plant inventories were performed by the same two surveyors to avoid biases. In this study we focused on wild bees in three of these protected areas (one per region choosing the most diversified one, Fig. S3.1): one in Hauts-de-France (Regional natural reserve Riez de Noeux les Auxi, noted R, 50°14’51.85”N 2°12’05.56”E), one in Normandie (Château Gaillard – le Bois Dumont, noted CG, 49°14'7.782"N 1°24'16.445"E) and one in Occitanie (Fourches, noted F, 43°56'07.00"N 3°30'46.1"E).

Pollen atlas

During each field session we sampled plant anthers of all species flowering within the study site. We put the anthers in individual Eppendorf tubes filled with 70% ethanol to preserve them. From this collection, we prepared a pollen atlas representative of the pollen diversity present in the three areas.

In the laboratory, we extracted and transferred the pollen released by anthers in the Eppendorf tube on a microscopic slide mounted with a cube of glycerine jelly (Kaiser's Glycerol Gelatine for microscopy) to maintain the natural colour of the pollen grains, and we sealed the cover slip with nail varnish. For each slide we recorded the plant species and the site and date of collection, and we took a photograph of the pollen grain as reference.

Direct observations of plant-pollinator interactions in the field

For this study, we focused on the session of July 2016. Surveys of plant-pollinator interactions were performed under suitable weather conditions for pollinators (following Westphal *et al.* 2008). The surveyors (from 4 to 5 at each session) walked slowly and randomly within the site and sampled by hand-net all pollinators that visited open flowers, recording the observed plant-pollinator interaction. The sampling period consisted of 4 hours split into 2 hours in the morning (about 10am-12am) and 2 hours in the afternoon (about 2pm-4pm), to cover the daily variability of both pollinator groups and flower communities. All sampled insects were immediately put into individual killing vials with ethyl acetate and were later prepared and pinned in the laboratory for identification at the species level by expert taxonomists. Some individuals were observed visiting more than one plant species before we were able to collect them.

Bee pollen load analysis

We focused on wild bees (superfamily: Apoidea, clade Anthophila) because they are very efficient pollinators with specialized structures for the pollen collection. Pollen was collected from the bodies of female bees and prepared on two different microscope slides as follows: one slide with the pollen passively transported on the body (scattered pollen, PS), and the other slide with pollen actively collected in specialised structures (*i.e.* curbiculae or scopae, PC). We only used female bees because males lack adapted structure to carry pollen, such as corbiculae or scopae. We collected PS from insect bodies using a small cube of glycerine jelly (volume 2 mm^3) following Kearns and Inouye (1993). PC was removed by brushing the specialised structures with a small needle or a small spoon and put in an Eppendorf tube filled with 70% ethanol for conservation. Only a fraction of PC (10 µl) was used to prepare pollen slides. We prepared a total of 782 pollen slides, considering both types of pollen and with information on sampling date, hour and site (Fourches 346 slides, Chateau Gaillard 256 slides and Riez 180 slides). Pollen identification was performed at the lowest taxonomic level (mostly at species level) by an expert (K. Bieri at the *Biologishes Institut für Pollen analyses*, Kehrsatz, Switzerland) using a combination of diagnostic keys and comparison with the pollen atlas

described above. When it was not possible to discriminate between two closely related species, we aggregated them in higher categories (family, genus or morphotype). Microscope slides were observed at 400x magnification by random transects until we counted 100 pollen grains, then the rest of the slide was searched for undetected pollen types. However, for the statistical analyses we did not consider plant species for which we detected ≤ 5 pollen grains per bee individual, which we considered as infrequent or accidentally collected (Bosch *et al.* 2009; Fisogni *et al.* 2018).

Characterisation of plant-pollinator interactions

To understand whether and how pollen added new links to the fieldwork observations, we separated recorded plant-pollinator interactions in five categories: (i) interactions observed as visits that were confirmed by both pollen types (PS and PC); (ii) interactions detected only by observing both types of pollen but not as visits (PS+PC); (iii) interactions found only with PS; (iv) interactions found only with PC; and (v) interactions only observed as visits but not confirmed by pollen.

We divided plant species in three groups: (a) plant species which were present in the study area (and included in the botanic inventory) and that were visited by pollinators; (b) plant species which were present in the study but whose interaction with pollinators was detected only by pollen analysis; (c) plant species present only in the surroundings of the study sites but not within them (and whose interaction with pollinators was detected only by pollen analysis). Plant species which were present in the study area but were never visited by pollinators and whose pollen we did not find on the insect bodies (*i.e.* plant species with no interactions) were excluded from the analysis altogether, and group (c) was not used for the purpose of comparing networks obtained from direct observation of visits and pollen identification. Results of this classification were represented using a heat map (function *quilt.plot* in R, see Supplementary Information Fig. S3.2, S3.3, S3.4).

Prior to conducting the rest of the analyses, we tested whether the information provided by the two types of pollen (PS and PC) was different. To do so, we compared the two interaction-based rarefaction curves using a Wilcoxon test. We found that there was not significant difference in the number of observed links between PS and PC, even if the percentage of unique links was higher for PS than for PC (results not shown). Thus, we decided to merge the information given by the two pollen types and we further refer to them as “pollen-based network” in the following analysis.

Plant – pollinator network analysis

For each site, we constructed two weighted (*i.e.* quantitative) bipartite networks including all pairs of interacting plant and insect species (i) directly observed as visits in the field ("visit-based" network) or (ii) retrieved from the pollen found on insect bodies ("pollen-based" network). Raw networks were weighted networks accounting for the intensity of interactions between species pairs – in the case of visits, intensity equals the number of recorded visits of the focal pollinator species on the focal plant species; in the case of pollen identifications, intensity equals the number of insects of the focal pollinator species found with at least 5 pollen grains from the focal plant species. For some analyses (connectance, motifs and position analyses) we transformed weighted networks into binary ones.

For both binary "visit-based" and "pollen-based" networks, we calculated its *connectance* as the proportion of observed links divided by the number of all possible links. We also calculated the specialization index H2' of the weighted networks (Blüthgen *et al.* 2006), using the *H2fun* function implemented in the bipartite package (Dormann *et al.* 2009; R Core Team 2018).

To model compartmental structure within networks, we applied latent block models (LBM) to each network, visit-based, simulated or pollen-based. We used the *BM_poisson* method for Poisson probability distribution implemented in the *blockmodels* package (Leger *et al.* 2015) to calculate blocks on the weighted networks. The algorithm finds the best groupings of insects and plants that maximize the Integrated Completed Likelihood (ICL; Biernacki *et al.* 2000; Daudin *et al.* 2008) of a model that fits the intensity of interactions between each pair of species as a Poisson draw with a parameter defined by the blocks each species belong to.

While the LBM approach reveals consistent species groups in complex networks, it neither informs on how species interact within each block, nor does it seek regularities in the arrangement of links within a small group of species. Such information can be obtained by counting the number of motifs observed within networks (Simmons *et al.* 2019b). To calculate how frequently different motifs occurred in our networks, we used the function *mcount* implemented in the new package *bmotif* in R (Simmons *et al.* 2019b) and normalized these values using the maximum number of times each motif could have occurred given the number of species in the network (correction "*normalise_nodesets*").

Insect roles and specialization index

Within motifs, species (nodes) can be found at different positions. Each position reflects a particular ecological role (*e.g.* pollinator species linked to at least two plant species with one of these connected to another pollinator species) and the same species can appear at different positions in different motifs. We calculated the sum-normalised frequencies of each position for each species using the *node_position* function implemented in the *bmotif* package in R (Simmons *et al.* 2019b).

We also calculated the standardized specialization index *d'* (Blüthgen *et al.* 2006), but we did not use the *d'* values provided by the *dfun* function in the bipartite package (Dormann *et al.* 2009) as they sometimes yielded spurious results based on the computation of the minimal *d* value (*e.g.* reporting low *d'* for species with only one partner in the network). However, we used the *d* and *dmax* values, obtained from the *dfun* function, and we calculated the *d'* index, for each plant and insect species, as the ratio of the *d*-value (Kullback-Leibler divergence between the interactions of the focal species and the interactions predicted by the weight of potential partner species in the overall network) to its corresponding *dmax*-value (maximum *d*-value theoretically possible given the observed number of interactions in the network).

Comparing network structure and species' roles using a null model

To understand to what extent the networks obtained using pollinator visit records did not bias the representation of the network when compared to those obtained through pollen identification, we compared species roles and specialization (node-level statistics), motif counts and the congruence between latent blocks (network-level statistics) using a null model accounting for the difference in sample size between visit- and pollen-based networks. We thus constructed null model networks (hereafter called "simulated" networks) in which we fixed the number of interactions per pollinator species as found in the visit-based network, but with randomized interactions pairs obtained from the interactions recorded in the pollen-based network. In other words, we can consider a simulated network as the result of a virtual observer that samples the same insects visiting plants, but the plants on which the insects are virtually observed are drawn from the distribution given by the pollen-based network. We performed 10,000 randomizations using the function *rmultinom* (package *stat* in R) to generate multinomial distribution drawings following the interaction frequencies reported in the pollen-based network.

To gauge if the network structure changed between the two networks, we compared the results of LBM and motif analyses between visit-based networks and simulated ones. Then, to detect whether species roles changed between networks, we compared results on specialization and node positions. Overall, we performed 197 tests in the site of F, 117 tests in the site of CG and 107 tests in the site of R and we adjusted all p-values in each site using the function *p.adjust* (package *stat*) and the false discovery rate correction method of Benjamini-Hochberg ("*BH*" or "*fdr*", Benjamini and Hochberg, 1995).

Latent Block Model – We performed LBM on weighted versions of the visit-based, pollen-based and simulated networks (10,000 simulations). To show the species rearrangement among groups between the visit- and pollen-based networks, we used alluvial diagrams (package *alluvial* in R). We then computed the congruence between the classifications given by node memberships of, first, the visit- and pollen-based networks and, then, the pollen-based network and each of the simulated networks, using the normalized mutual information index (NMI), implemented as method "*nmi*" of the function *compare* in the R package *igraph* (Danon *et al.* 2005; Astegiano *et al.* 2017). NMI values range between 0 (no congruence between classifications) and 1 (perfect congruence). The distribution of NMIs obtained when comparing the blocks of the pollen-based networks and those of the simulated networks allowed us to compute the probability (p-value) that the NMI between the visit-based and pollen-based blocks was significantly less than expected from the null model. Corrected p-values less than 5% were deemed significantly inferior to the null model expectation.

Motifs – The motif analysis was performed on binary networks (Simmons *et al.* 2019b) and explored all motifs with up to 6 species. The frequency of each motif in the visit-based network was compared to the corresponding frequencies in the ensemble of randomized networks using a two-tailed test for the purpose of significance (*i.e.* the difference in frequency was deemed significantly different if it fell below 2.5% or above 97.5% of the simulated cumulated frequencies for the same motif).

Positions –To explore if insect and plant species had different roles in the networks based on visits vs. pollen, we calculated the frequency with which species occurred at different positions within all possible motifs of 2 to 6 species. This vector of position frequencies represented the species' "role" in the network. We then calculated the distance of each species' role to the centroid of all the simulated roles for the same species, and compared this distance to the distribution of distances between simulated roles and their centroid, with observed distances greater than 95% of the simulated distances deemed as significantly different from the null expectation. To account for heterogeneous variances and correlations between position frequencies (*i.e.* coordinates in species' role vectors), we

used Mahalanobis distance on modified coordinates obtained by first running a principal component analysis (PCA) on the set of all roles of all species in all simulated networks. The covariance matrix used in the Mahalanobis distance was simply the diagonal matrix of singular values associated with the principal components of the PCA. The modified coordinates of the centroid and the observed role of a given species were obtained by projecting their position frequencies into the PCA space.

All analyses were performed in R version 3.5.2 (R Core Team 2018).

Results

Characterisation of plant-pollinator interactions

We recorded a total of 96 flowering species, 62 in the site of Fourches (F), 33 in the site of Château Gaillard (CG) and 30 in the site of Riez (R). However, these species were not all visited by pollinators, and those without any visits were not considered in the analysis.

We sampled 574 visiting insects overall, but for the statistical analysis we only used female insects with the information on both types of pollen (collected and scattered). For the following analyses, we used 391 insects overall, 173 in the site of F, 128 in the site of CG and 90 in the site of R.

Based on visits recorded in the field, we built three visit-based networks; based on pollen (PC and PS) found on insects, we built three pollen-based networks, one for each site. Visit- and pollen-based networks in the same site have comparable number of species (the number of insect species is fixed, but the number of plant species can vary depending on the sampling, *i.e.* visit or pollen), except in the site of Fourches: for the site of Fourches 50 insect species x 44 potential plant species (29 species in the visit-based network and 40 in the pollen-based one); for the site of Château Gaillard 22 insect species x 18 potential plant species (13 in the visit- and 18 in the pollen-based networks) and for the site of Riez 19 insect species x 16 potential plant species (12 in the visit- and 15 in the pollen-based networks). For three insect species (2 species in the site of CG, *Lasioglossum laticeps* and *Lasioglossum politum*, and 1 species in the site of R, *Halictus rubicundus*) which were sampled once in the visit-based network, we did not record any interaction in the pollen-based network due to the low number of pollen grains (< 5) or to interactions with plant species not included in the botanic inventory (Fig. S3.3, S3.4). These species were thus excluded from the analyses in the problematic sites. In the site of Fourches we recorded 179 visit-based interactions and 340 pollen-based interactions; in the site of Château Gaillard we recorded 130 visit-based interactions and 228 pollen-based interactions and in the site of Riez we recorded 93 and 173 interactions in the visit- and the

pollen-based networks, respectively. Overall, with the pollen information we doubled the number of interactions in all sites.

Plant – pollinator networks analysis

When we compared network connectances, *i.e.* the proportion of realized links over all possible ones, we found that the pollen-based network connectance was always higher than the visit-based network connectance in the three sites (Table 3.1). We observed the opposite pattern for the network specialization index, since the H_2 values were higher in the visit-based network than in the pollen-based one in all sites (Table 3.1; Fig. S3.5). However, when we compared the visit-based network and the simulated networks, we did not find any significant difference both for the connectance and the H_2 index (Table 3.1).

Analysis	Region	Site	Visit-based network	Pollen-based network	Simulated network	p-value adjusted	
Connectance	Occitanie	Fourches (F)	0.07	0.09	0.07	1	n.s
	Normandie	Château Gaillard (CG)	0.17	0.20	0.14	1	n.s
	Hauts-de-France	Riez (R)	0.18	0.22	0.16	1	n.s
H2	Occitanie	Fourches (F)	0.52	0.33	0.47	0.64	n.s
	Normandie	Château Gaillard (CG)	0.32	0.29	0.37	0.35	n.s
	Hauts-de-France	Riez (R)	0.36	0.30	0.44	0.13	n.s

Table 3. 1. Results for the analyses of networks connectance and H2 (network specialisation index) in the visit-based and pollen-based networks and for the simulated networks in the three sites.

In order to compare network structures between visit- and pollen-based networks, we performed LBMs and compared the classification induced by latent blocks using NMI (Table 3.2). In the site of Fourches, we found a total of 5 blocks (2 insect blocks and 3 plant blocks) in the visit-based network and a total of 7 blocks (4 blocks for insects and 3 for plants) in the pollen-based network. In the site of Château Gaillard, we found 7 blocks (4 for insects and 3 for plants) in both networks, and a similar pattern for the site of Riez, but with 5 blocks (2 for insects and 3 for plants) in both networks. Block clustering largely followed species degrees (high, medium and low degree, Fig. S3.6). We observed plant species rearrangements in all sites (green lines in the alluvial diagrams), but insect block rearrangements only in the sites of CG (in two insect species, *Andrena flavipes* and *Seladonia tumulorum*) and R (for one insect species, *Lasioglossum pauxillum*). Block rearrangements are mainly due to the higher number of links in the pollen-based network but not to substantial changes in the network structure. The higher number of blocks found in the pollen-based network in the site of Fourches (Fig. 3.1) is due to the occurrence of two new blocks in the group of insects: the first block in the visit-based network (constituted by three species with the highest degrees, Fig. 3.1 and Fig.

S3.6 Fourches visits) split in two blocks in the pollen-based network (blocks 1 and 3, Fig. 3.1 and Fig. S3.6 Fourches pollen); and the fourth block in the visit-based network (Fig. 3.1 and Fig. S3.6 Fourches visits) also split in two other blocks of species (respectively with species with medium and low degree in the pollen network, Fig. 3.1 and Fig. S3.6 Fourches pollen). Even if we found species rearrangements among groups between the visit- and pollen-based networks in all three sites (Fig. 3.1), the network structures were not intrinsically different. When we compared the congruence between the memberships of species in the visit- and pollen-based networks using the NMI, we obtained NMI values close to 1 (perfect congruence) in all sites (Table 3.2). Moreover, we did not find any significant difference when we compared these NMIs with those obtained from comparisons of the pollen-based network and each of the simulated networks.

Site	NMI Visit-based network	NMI Simulated networks (quantile)				p-value	
		2.5%	50%	95%	97.5%		
Fourches (F)	0.76	0.68	0.76	0.82	0.82	0.98	n.s.
Ch. Gaillard (CG)	0.80	0.76	0.84	0.89	0.90	0.31	n.s.
Riez (R)	0.84	0.73	0.83	0.88	0.94	0.75	n.s.

Table 3. 2. Normalized mutual information (NMI) values obtained in the three sites when we compared the congruence between the classifications given by node memberships of, first, the visit- and pollen-based networks (NMI visit-based network) and, second, the pollen-based and each of the simulated networks (NMI simulated networks). The p-value corresponds to the probability that the NMI between the visit-based and pollen-based blocks was inferior to what would be expected from the null model.

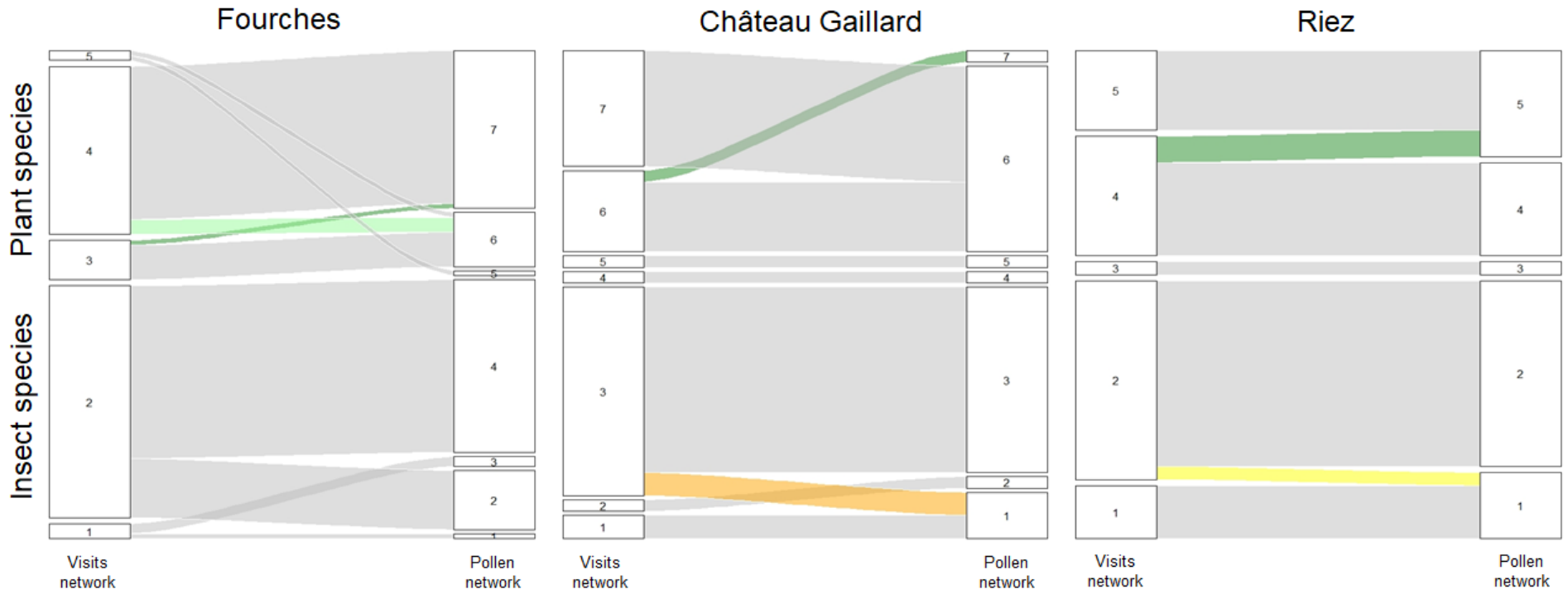


Figure 3. 1. Alluvial diagrams showing the species rearrangement among blocks between the visit- and pollen-based networks. Green lines show the species rearrangement for plant species and orange and yellow lines for insect species. The plant species that changed modules are Linum sp. from block 3 to block 7 (dark green line) and Lotus delortii, Ononis striata and Sedum sp. from block 4 to block 6 (pale green line), in the site of F. In the site of CG the plant species that changed from block to block 7 is Ononis natrix and the insects species that changed from block 3 to block 1 are Andrena flavipes and Seladonia tumulorum (orange line). Plant species that changed block in the pollen-based network, in the site of R, are Rubus plicatus and Trifolium repens and the insect species that changed from block 2 to block 1 is Lasioglossum pauxillum (yellow line).

In general, when we compared the network structure using the motifs, we did not find important differences between the visit-based network and the simulated networks. We did not find any significant difference when we compared the frequency of each motif in the visit-based network to the corresponding frequencies in the simulated networks in the site of Fourches and Riez. However, we found significant differences for three motifs (motifs 16, 33 and 43; see Fig. 3 in Simmons *et al.* 2019a) in the site of Château Gaillard. All three motifs were less represented in the simulated networks than in the visit-based network (Fig. 3.2). Motif 16 is constituted by 5 nodes and 6 links, while motifs 33 and 43 are constituted by 6 nodes and 7 links. In motifs 16 and 43, all species of one group interact with all species in the other group, while in motif 33 there are some "specialist" species, *i.e.* species in one group that interact with one "generalist" species in the other group.

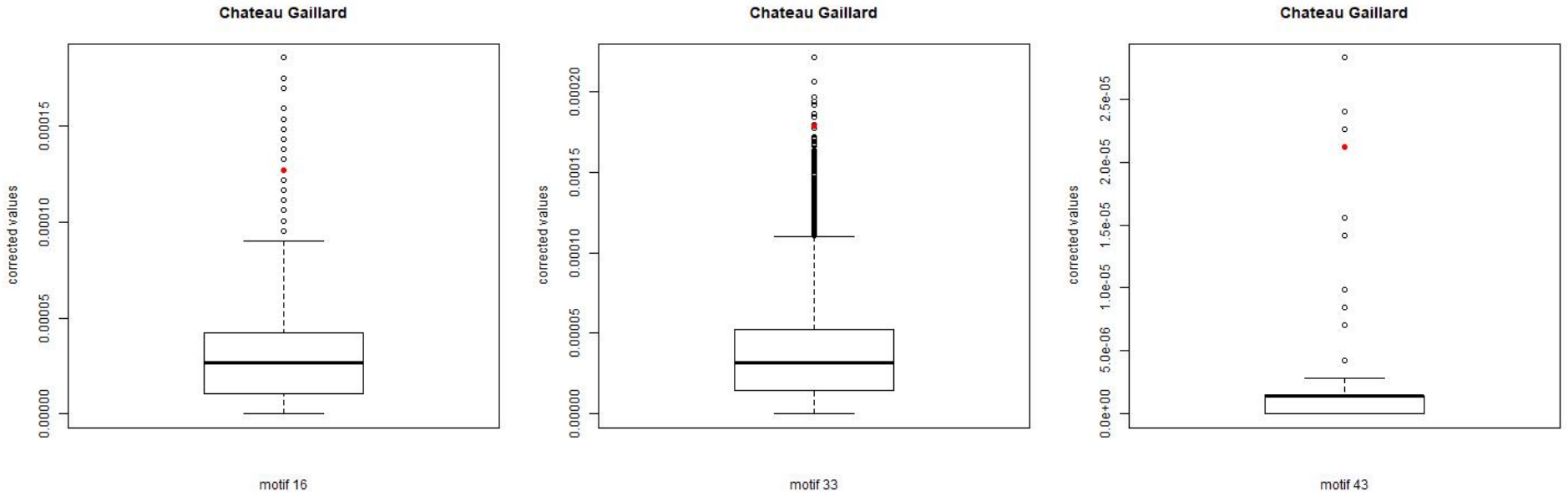


Figure 3. 2. Motifs 16, 33 and 43 in the site of Château Gaillard. Red dots correspond to the frequency value (corrected value) in the visit-based network and the boxplot and outliner dots correspond to all the frequency values in the simulated network

Insect roles and specialization index

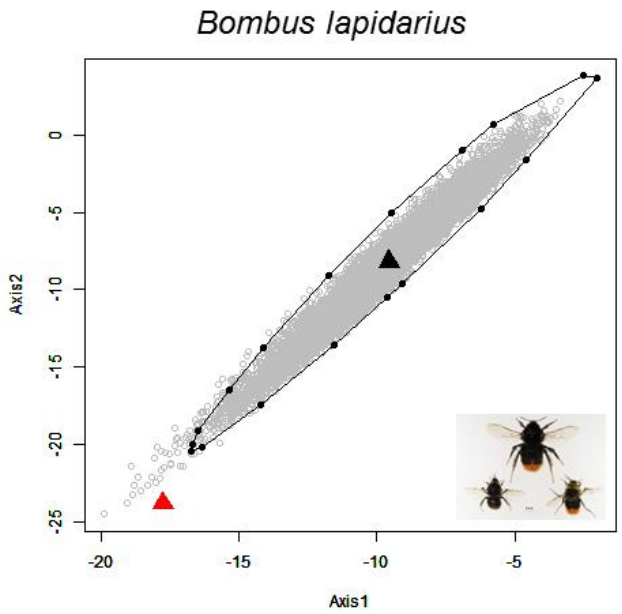
We found that several species in the site of Château Gaillard and Riez had significantly different roles (adjusted p-value < 0.05, Tables S3.2, S3.3, Fig. 3.3) when we compared the simulated distances to their visit-based distances, but we did not find any role change at all in the site of Fourches (Table S3.1).

In the site of Château Gaillard, we found 13 species significantly more distant from the simulated centroid (seven insect species, *Anthidiellum strigatum, Bombus lapidarius* (Fig. 3.3a), *Ceratina cucurbitina, Lasioglossum interruptum, Megachile willughbiella, Osmia rufohirta and Trachusa byssina*, and six plant species *Allium sphaerocephalon, Centaurea scabiosa* (Fig. 3.3b), *Echium vulgare, Origanum vulgare, Scabiosa columbaria* and *Teucrium* sp.; Table S3.2). In the site of Riez we found three species (one insect species, *Osmia bicolor* (Fig. 3.3c), and two plant species, *Achillea millefolium* (Fig. 3.3d), and *Prunella vulgaris*) that had significantly different roles between the visit-based and the simulated distances (Table S3.3).

We also compared for each species the specialization index *d'* calculated in the visit-based network to the average *d'* of the simulated networks. We found that the specialization of most species was not significantly different in the two networks in all sites (Tables S3.1, S3.2, and S3.3). We recorded significant differences in the specialization level for 8 species in the site of F (5 insect and 3 plant species), for 2 species in the site of CG (1 insect and 1 plant species) and for 5 species in the site of R (4 insect and 1 plant species). Overall, nearly half of the species for which we found significant differences in their node positions or/and in the specialization level were singletons (13 species out of 27) in the visit-based or pollen-based network, *i.e.* species that had only one observed interaction (Tables S3.1, S3.2 and S3.3). Only four species out of the 27 (one insect and one plant species both in the sites of CG and R, Tables S3.2 and S3.3) showed significant differences in both their role and specialization level.

Figure 3. 3. The PCA plot shows the significant distance (adjusted p-value < 0.05) along principal axes 1 and 2, between the visit-based position (red triangle), the simulated centroid (black triangle) and the convex hull (black lines and dots) obtained on the 95% of the simulated positions which were close to the centroid (grey dots) in the randomized network, in (a) Bombus lapidarius and (b) Centaurea scabiosa in the site of Château Gaillard (Normandie) and in (c) Osmia ruforhirta and (d) Achillea millefolium in the site of Riez (Hauts-de-France). The visit-based distance was greater than 95% of all the simulated distances. Photo credits: Atlas Hymenoptera and Acta plantarum.

a.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Axis1
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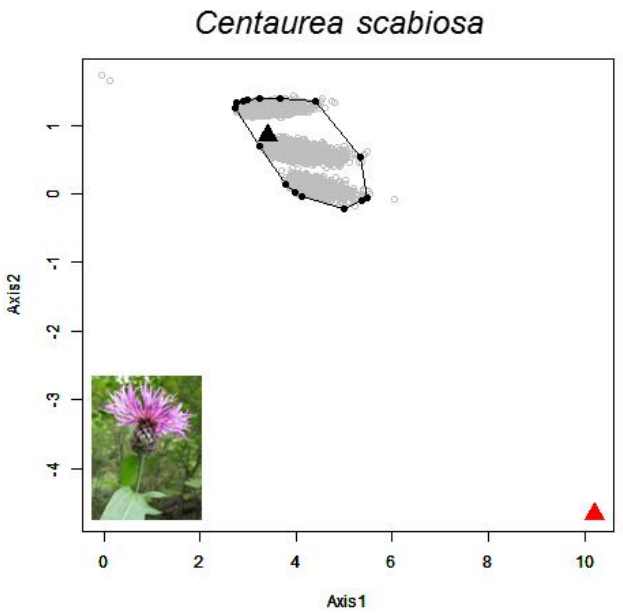
-15
-10
-5
0
-25

b.


Centaurea scabiosa
Axis2
Axis1
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2
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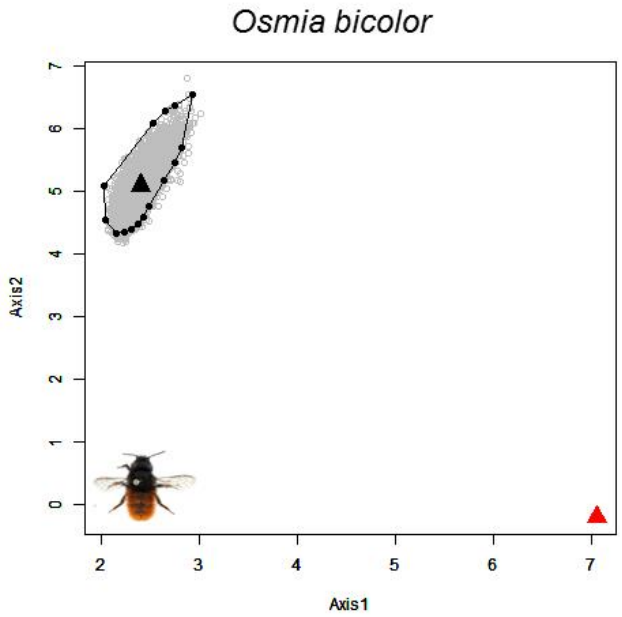
6
8
10
1
-1
-2
-3
-4

c.


Osmia bicolor
Axis2
Axis1
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3
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7
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3
4
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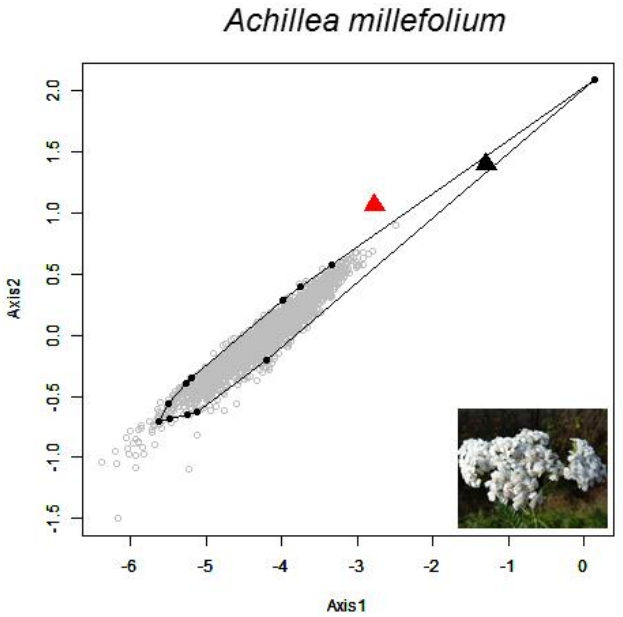
7

d.


Achillea millefolium
Axis2
Axis1
-6
-5
-4
-3
-2
-1
0
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5

Discussion

Plant-pollinator networks are mainly constructed using direct observations of plant-pollinator interactions in the field, a method subject to undersampling (Vázquez *et al.* 2009; Blüthgen 2010; Olesen *et al.* 2011). The problem of undersampling is much higher in richer communities where some flower visits are scarcer and hence more difficult to detect (Sørensen *et al.* 2011). The use of pollen found on insect bodies is an alternative method that might help reconstruct the insect visitation history and give a better image of the whole network. Few studies have compared the visit- and pollen-based networks (Bosch *et al.* 2009; Pornon *et al.* 2017), and all of these comparisons have used classic networks metrics, which are known to be influenced by network dimensions (Blüthgen *et al.* 2008; Staniczenko *et al.* 2013; Astegiano *et al.* 2015).

In our study, we compared plant-pollinator networks constructed (i) from direct observation of pollinator visits and (ii) from identification of pollen found on these same insects in three different calcareous grasslands. The three plant-pollinator networks used in this study are distributed along an environmental and diversity gradient, showing differences in the identity and number of species in both plants and insects. We used a null model approach (*i.e.* simulated networks), accounting for differences in network size, to understand how differences in sampling method, not intensity, can contribute to changes in observed network structure.

Pollen identification increases the number of observed links and always yields richer and more connected networks (Bosch *et al.* 2009), independently from the site richness and diversity (Table 3.1). Nevertheless, the pollen-based links often coincide with the links observed in the field (Alarcón 2010; Popic *et al.* 2013). We did not find any significant change in any of the study sites when we compared network structures between visit-based and simulated networks. However, we found changes in the species roles for some insect and plant species.

Although we observed that the use of pollen data increased the number of interactions (we doubled the number of interactions in all sites), pollen information mostly increased the number of links for abundant and already highly connected species (number of links > 5 in the visit-based networks, Tables S3.1, S3.2 and S3.3), while for rare (singletons) and not abundant species we recorded few interactions even in the pollen-based network. Since visit-based network construction is essentially pollinator-based (and not plant-based), the information given by pollen found on insects is especially useful to add links to plant species that were not observed in the visit-based network (plant species with no links in the visit-based network, Tables S3.1, S3.2 and S3.3). Indeed, block rearrangements

are observed more often in plants than in insects (Fig. 3.1 and S3.6). However, block changes in the LBM representation did not correspond to changes in species position.

Block rearrangements are influenced by the number of links and the species degree, *i.e.* the number of partners with which a species interacts, but it neither informs on species role nor specialization. For example, the singleton species *Prunella vulgaris* in the site of Riez clustered in the same block (block 4) in both visit- and pollen-based networks (Fig. 3.1). Nevertheless, it was the only plant species for which we observed a significant change in its role and specialization degree in this site. In the visit-based network, this species was found in interaction with only one insect species (Fig. S3.6, Riez visits), *Ceratina cyanea*, in a one-to-one interaction ("direct interaction"), and thus only in position 1 (in motif 1). Conversely, in the pollen-based network, even if *P. vulgaris* always interacted only with *C. cyanea* (Fig. S3.6 Riez pollen), *C. cyanea* interacted with two new plant species (*Trifolium repens* and *Centaurium erythraea*). Thus, the specialization for *C. cyanea* changed significantly. Moreover, the specialization level and the role of *P. vulgaris* also changed significantly since in the pollen-based network its interaction with *C. cyanea* was affected indirectly by two other plant species, which may be potential competitors. Moreover, all the new positions of *P. vulgaris* in the pollen-based network, were "unique" in more complex motifs, *i.e. P. vulgaris* interacted with one generalist insect species that had other interactions with other plants (Simmons *et al.* 2019a). Similarly to *P. vulgaris*, *Achillea millefolium*, in the same site, was a singleton in the visit-based network while it gains one link in the pollen-based network. This new interaction was observed with *Lasioglossum pauxillum* which was a "super-generalist" species (visiting 11 plant species). Therefore, the role of *A. millefolium* changed significantly (Fig. 3.3d) through possible indirect interactions with new potential competitors. These examples show that indirect interactions, *i.e.* the impact of one species on another mediated by other intermediary species, are important to give a more complete picture of the species' role when comparing networks, especially when accounting for singleton species in the visit-based network.

We also found changes in species roles for 6 species which were more connected in the visit-based network, *i.e.* with more than 5 observed interactions, such as *Bombus lapidarius* and *Centaurea scabiosa* in the site of Château Gaillard (with 20 and 8 interactions, respectively; Fig. 3.3a, b, Table S3.2). In "complex" motifs where all species are generalists in both groups and all interact together, changes in species roles through indirect interactions are expected to be stronger than in "simple" motifs which are composed of specialist species that affect each other indirectly via their effect on one generalist species (Simmons *et al.* 2019a).

In most species for which we observed a significant change in their positions or specialisation degree, we recorded a slightly higher number of links or the same number of links in the pollen-based networks than in the visit-based ones, and only 3 species out of 27 nearly doubled their interactions (Tables S3.1, S3.2 and S3.3). However, for four species, *Anthyllis vulneraria* in the site of F, *Origanum vulgare* and *Centaurea scabiosa* in the site of CG and *Lasioglossum fulvicorne* in the site of R (Tables S3.1, S3.2 and S3.3), we recorded a lower number of links in the pollen-based network than in the visit-based one, since some interactions were only observed in the field but they were not confirmed with pollen identification (blue squares in the Fig. S3.2, S3.3 and S3.4), which might explain the significant difference in their specialisation level or species role obtained when we compared the visit-based network to the simulated networks. For both *A. vulneraria* and *L. fulvicorne*, the specialization level recorded in the visit-based network was always lower than the one recorded in the simulated networks, which means that both species were more specialized than expected by chance. For *C. scabiosa* the number of links recorded in the pollen-based network was lower than in the visit-based one since the interactions with two insect species, *Bombus lapidarius* and *Osmia leaiana*, were not confirmed by pollen identification (Fig. S3.3), probably because the two visitors were not carrying enough pollen grains (less than 5) of this plant species. Therefore, in the pollen-based network the loss of partners and their interactions influenced *C. scabiosa*'s role, especially in highly connected motifs (*i.e.* motifs where all species in one group interact with all species in the other group) such as motifs 16 and 43, which were less represented in the simulated networks than in the visit-based network. Consequently, the loss of the interaction with *C. scabiosa* indirectly induced a change in the position of *B. lapidarius* (Fig. 3.3a).

Thus, even if we did not find any significant changes in the network structures, we observed important changes at the species level when we integrated the pollen information in all the three sites. Indeed, we found differences both in species role and specialization in a few species, as also evidenced by other studies (Ballantyne *et al.* 2015; Lucas *et al.* 2018b). We showed that non-significant change in network structure can mask more subtle changes of species roles and specialisation level. However, these changes are in part observed in singleton species such as *P. vulgaris* in the site of Riez, which showed a low number of links both in the visit- and in the pollen-based networks. Singleton species are expected even in well-sampled communities, since they are often considered as rare species accounting for rare interactions (Novotný & Basset 2000; Bascompte & Jordano 2013).

To conclude, our results suggest that more detailed sampling, obtained from pollen found on insect bodies, does not lead to the construction of an intrinsically different network, independently from the site richness and diversity. Almost all of the significant differences are found at the species level, not

at the network structure level, with singleton species accounting for half of these species-level differences. Overall, this suggests that recording plant-pollinator interactions from pollinator visit observation is enough to provide a satisfactory representation of the network structure. However, the use of pollen can provide a more exhaustive image at the species level, highlighting important changes in species role and specialization, especially for studies investigating pollinator effectiveness and/or dealing with scarce pollinators. Since pollen identification is a time-consuming endeavour, new methods such as DNA-barcoding might simplify and accelerate pollen identification in the future if improved with new specific (regional or local) botanic databases.

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Supplementary Information

How biased is our perception of plant-pollinator networks? A comparison of visit- and pollen-based representations of the same networks

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Supplementary Figures

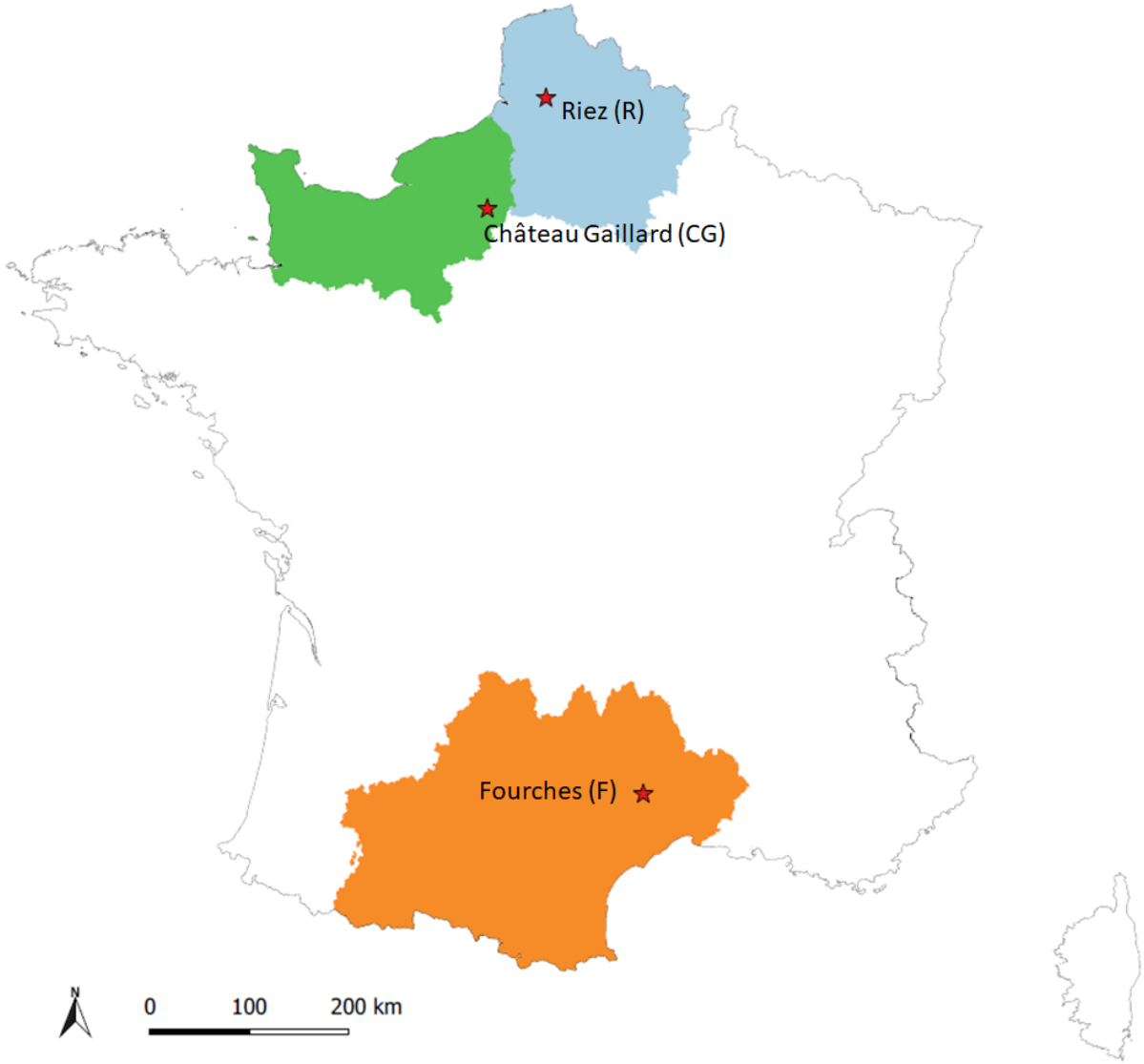


Figure S3. 1. Site location in France: in blue the French region Hauts-de-France (Regional natural reserve Riez de Noeux les Auxi, noted R, 50°14'51.85"N 2°12'05.56"E), in green the Normandie region (Château Gaillard – le Bois Dumont, noted CG, 49°14'7.782"N 1°24'16.445"E), in orange the Occitanie region (Fourches, noted F, 43°56'07.00"N 3°30'46.1"E). The three sites correspond to the red stars.

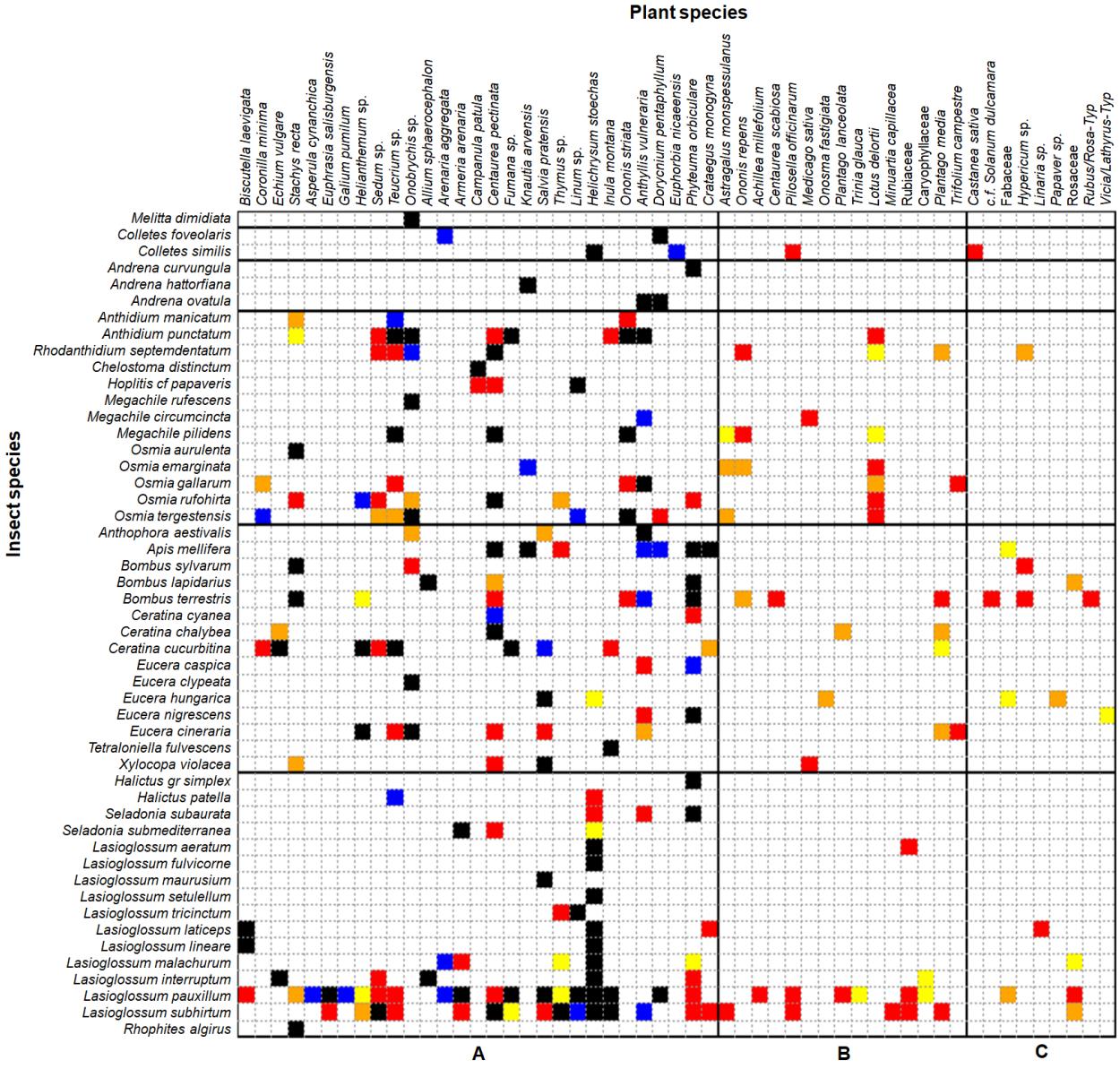


Figure S3. 2. Heat map of the plant-pollinator interaction in the site of Fourches, with both visit- and pollen-based interactions. We separated the types of interaction in five categories: (i) interactions that were observed as visits and confirmed by both pollen types (PS and PC) in black; (ii) interactions detected only by observing both types of pollen but not as visits (PS+PC) in red; (iii) interactions found only with PS in yellow; (iv) interactions found only with PC in orange; and (v) interactions only observed as visits but not confirmed by pollen in blue. We divided plant species in three groups: (A) plants species which were present in the study area (and included in the botanic inventory) and that that were visited by pollinators in the visit-based network; (B) plants species which were present in the study but whose interactions with pollinators were detected only by pollen analysis; (C) plant species present only in the surroundings of the study sites but not within them (and whose interaction with pollinators was detected only by pollen analysis). Insect species are presented in line and plant species in column. Insect species are ranged by family (from the less abundant on the top to the most abundant on the bottom; "M." stands for Melittidae, "C." for Colletidae and "A." for Andrenidae families) separated by horizontal black lines. Within family insect species are ranged by genus and abundance (from the less abundant on the top to the most abundant on the bottom). Plants species in the first two groups (A and B, separated by vertical black lines) are ranged following their abundances (from the less to the most abundant species, from the left to the right).

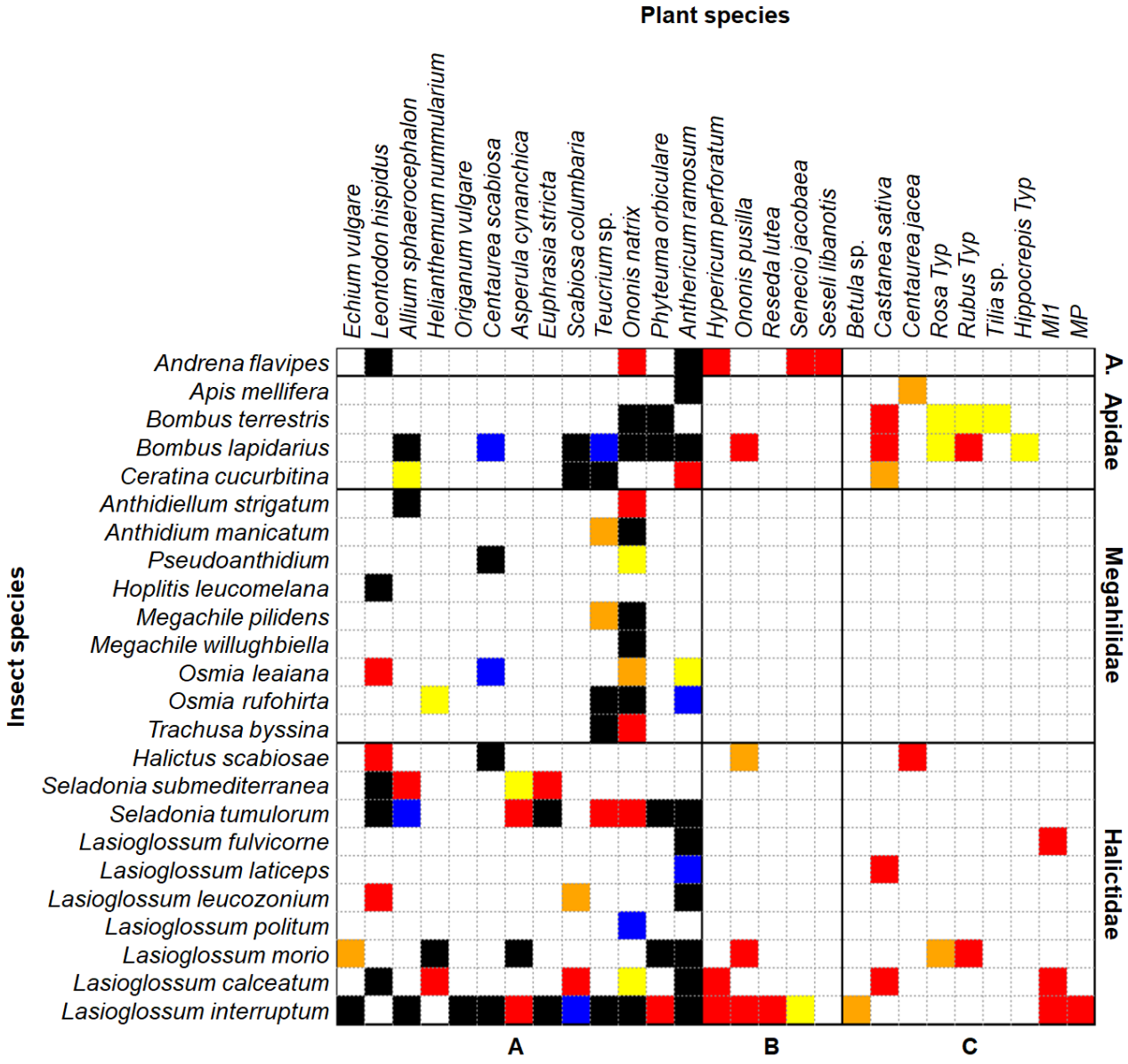


Figure S3. 3. Heat map of the plant-pollinator interaction in the site of Château Gaillard, with both visit- and pollen-based interactions. We separated the types of interaction in five categories: (i) interactions that were observed as visits and confirmed by both pollen types (PS and PC) in black; (ii) interactions detected only by observing both types of pollen but not as visits (PS+PC) in red; (iii) interactions found only with PS in yellow; (iv) interactions found only with PC in orange; and (v) interactions only observed as visits but not confirmed by pollen in blue. We divided plant species in three groups: (A) plants species which were present in the study area (and included in the botanic inventory) and that that were visited by pollinators in the visit-based network; (B) plants species which were present in the study but whose interactions with pollinators were detected only by pollen analysis; (C) plant species present only in the surroundings of the study sites but not within them (and whose interaction with pollinators was detected only by pollen analysis). Insect species are presented in line and plant species in column. Insect species are ranged by family (from the less abundant on the top to the most abundant on the bottom; "A." correspond to the family Andrenidae) separated by horizontal black lines. Within family insect species are ranged by genus and abundance (from the less abundant on the top to the most abundant on the bottom). Plants species in the first two groups (A and B, separated by vertical black lines) are ranged following their abundances (from the less to the most abundant species, from the left to the right).

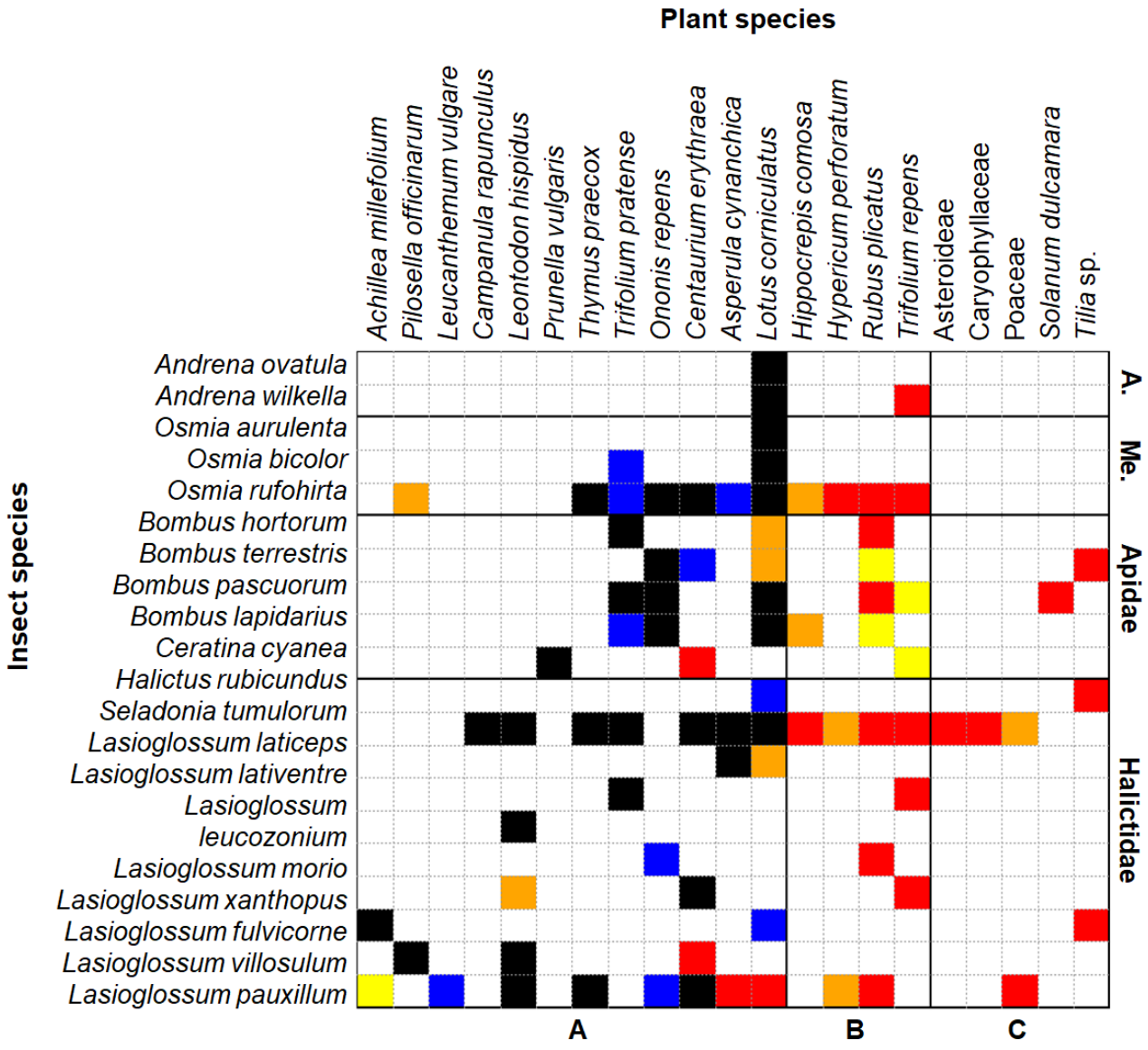


Figure S3. 4. Heat map of the plant-pollinator interaction in the site of Riez, with both visit- and pollen-based interactions. We separated the types of interaction in five categories: (i) interactions that were observed as visits and confirmed by both pollen types (PS and PC) in black; (ii) interactions detected only by observing both types of pollen but not as visits (PS+PC) in red; (iii) interactions found only with PS in yellow; (iv) interactions found only with PC in orange; and (v) interactions only observed as visits but not confirmed by pollen in blue. We divided plant species in three groups: (A) plants species which were present in the study area (and included in the botanic inventory) and that that were visited by pollinators in the visit-based network; (B) plants species which were present in the study but whose interactions with pollinators were detected only by pollen analysis; (C) plant species present only in the surroundings of the study sites but not within them (and whose interaction with pollinators was detected only by pollen analysis). Insect species are presented in line and plant species in column. Insect species are ranged by family (from the less abundant on the top to the most abundant on the bottom; "A." stands for Andrenidae and "Me." For Megachilidae families) separated by horizontal black lines. Within family insect species are ranged by genus and abundance (from the less abundant on the top to the most abundant on the bottom). Plants species in the first two groups (A and B, separated by vertical black lines) are ranged following their abundances (from the less to the most abundant species, from the left to the right).

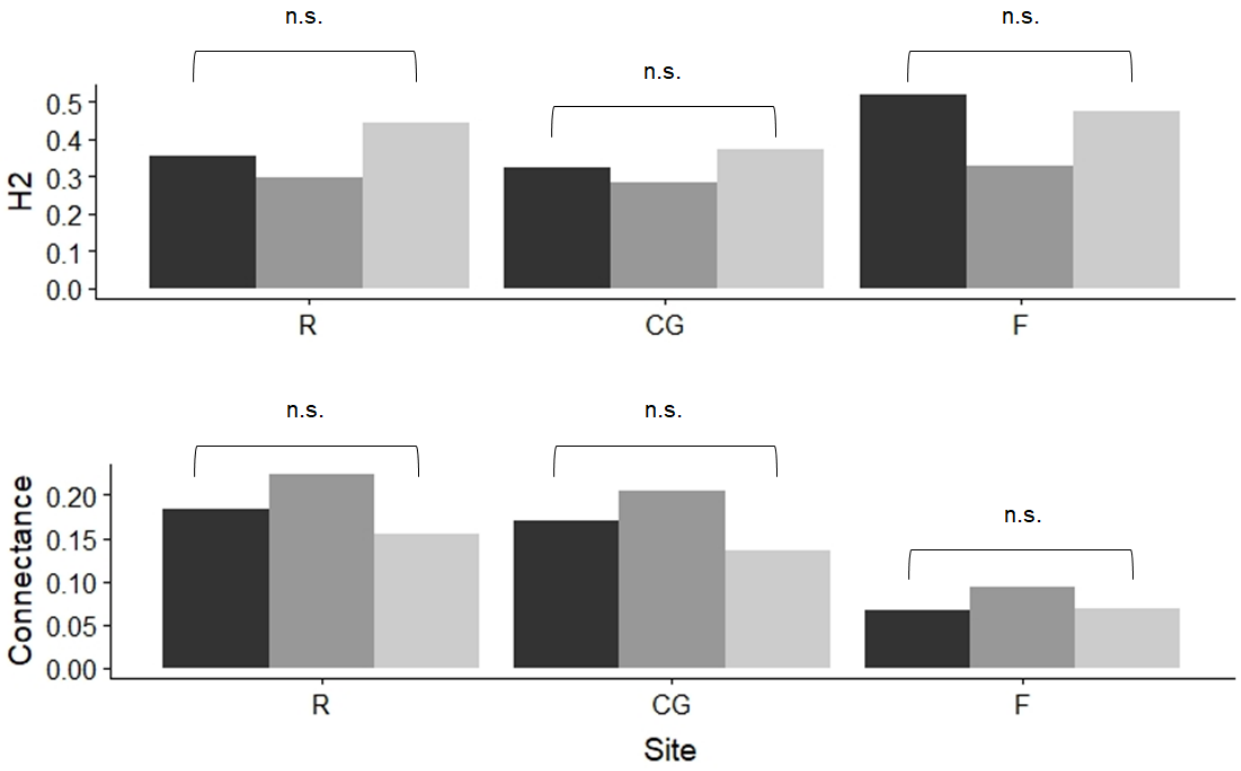
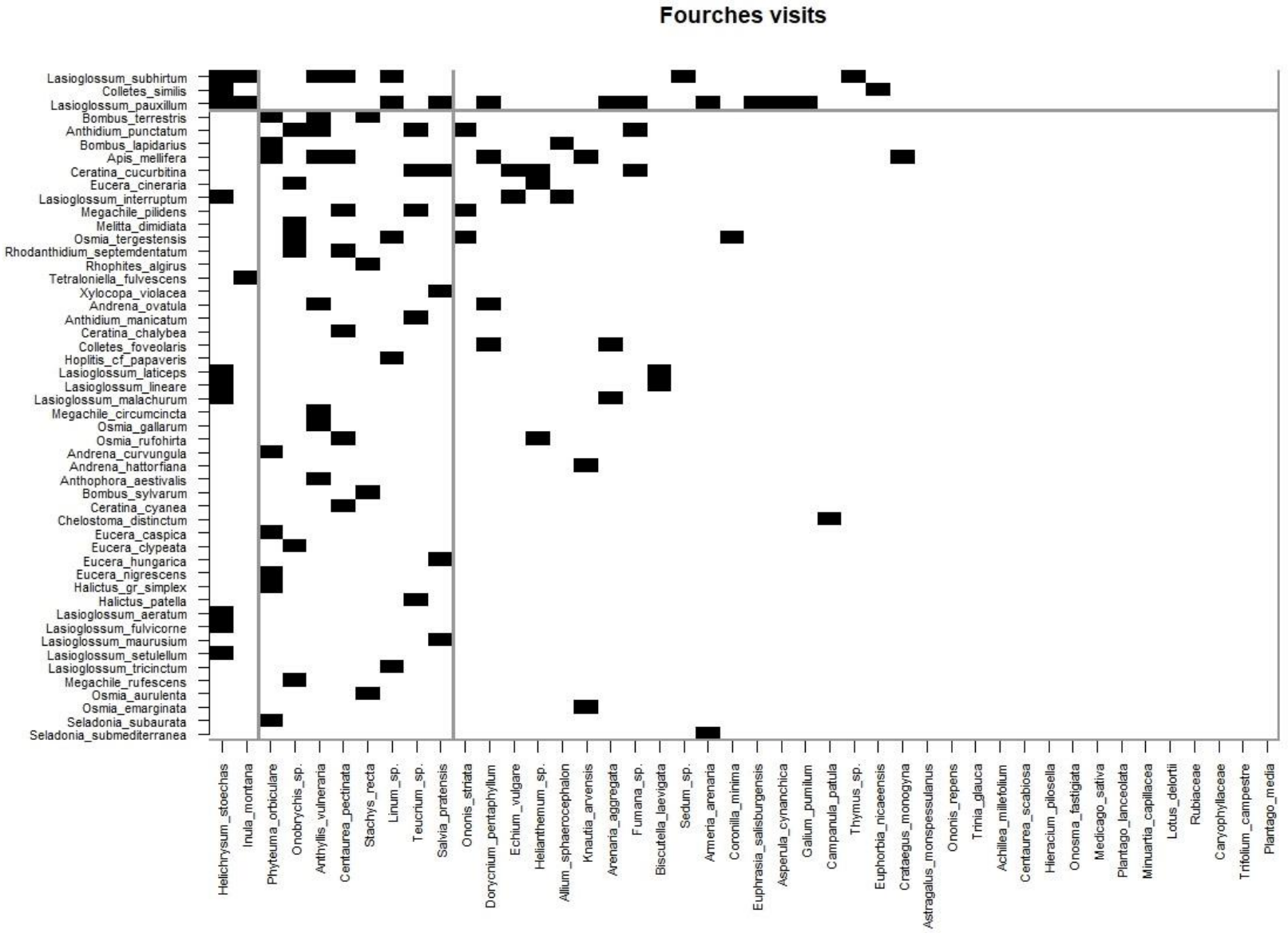


Figure S3. 5. Network connectance and specialization index H2 in the visit-based network (black columns), the pollen-based one (dark gray columns) and in the simulated-network (light gray colums) in the three sites (R, Riez; CG, Chateau Gaillard and F, Fourches).


Fourches visits
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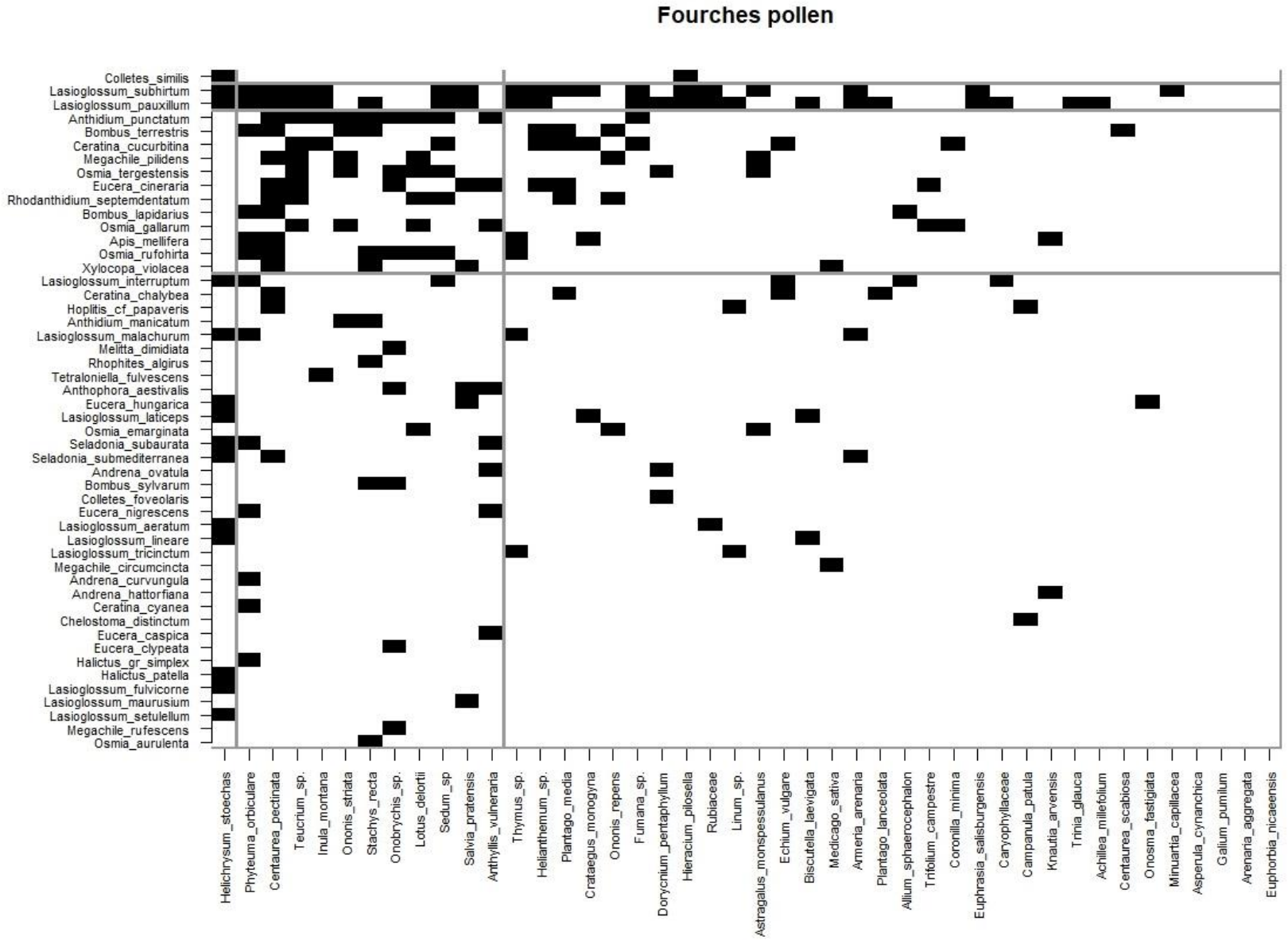
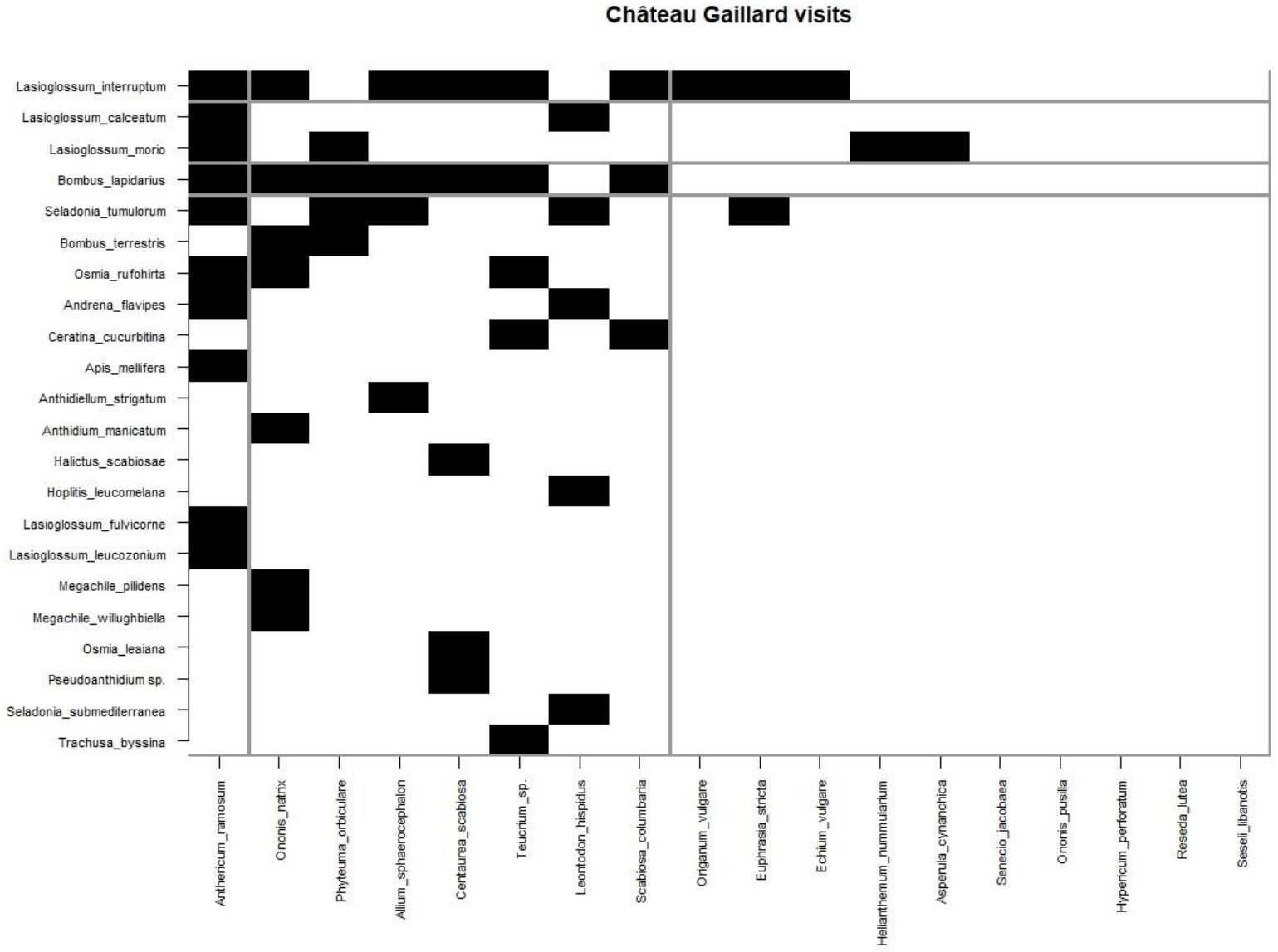
Colletes_similis
Lasioglossum_pauxillum
Bombus_terrestris
Anthidium_punctatum
Bombus_lapidarius
Apis_mellifera
Ceratina_cucurbitina
Eucera_cineraria
Lasioglossum_interruptum
Megachile_pilidens
Melitta_dimidiata
Osmia_tergestensis
Rhodanthidium_septemdentatum
Rhophites_algirus
Tetraloniella_fulvescens
Xylocopa_violacea
Andrena_ovatula
Anthidium_manicatum
Ceratina_chalybea
Colletes_foveolaris
Hoplitis_cf_papaveris
Lasioglossum_laticeps
Lasioglossum_lineare
Lasioglossum_malachurum
Megachile_circumcincta
Osmia_gallarum
Osmia_rufohirta
Andrena_curvungula
Andrena_hattorfiana
Anthophora_aestivalis
Bombus_sylvarum
Ceratina_cyanea
Chelostoma_distinctum
Eucera_caspica
Eucera_clypeata
Eucera_hungarica
Eucera_nigrescens
Halictus_gr_simplex
Halictus_patella
Lasioglossum_aeratum
Lasioglossum_fulvicorne
Lasioglossum_maurusium
Lasioglossum_setulellum
Lasioglossum_tricinctum
Megachile_rufescens
Osmia_aurulenta
Osmia_emarginata
Seladonia_subaurata
Seladonia_submediterranea
Helichrysum_stoechas
Inula_montana
Phyteuma_orbiculare
Onobrychis_sp.
Anthyllis_vulneraria
Centaurea_pectinata
Stachys_recta
Linum_sp.
Teucrium_sp.
Salvia_pratensis
Ononis_striata
Dorycnium_pentaphyllum
Echium_vulgare
Helianthemum_sp.
Allium_sphaerocephalon
Knautia_arvensis
Arenaria_aggregata
Fumana_sp.
Biscutella_laevigata
Sedum_sp.
Armeria_arenaria
Coronilla_minima
Euphrasia_salisburgensis
Asperula_cynanchica
Galium_pumilum
Campanula_patula
Thymus_sp.
Euphorbia_nicaeensis
Crataegus_monogyna
Astragalus_monspessulanus
Ononis_repens
Trinia_glauca
Achillea_millefolium
Centaurea_scabiosa
Hieracium_pilosella
Onosma_fastigiata
Medicago_sativa
Plantago_lanceolata
Minuartia_capillacea
Lotus_delortii
Rubiaceae
Caryophyllaceae
Trifolium_campestre
Plantago_media


Fourches pollen
Colletes_similis
Lasioglossum_subhirtum
Lasioglossum_pauxillum
Anthidium_punctatum
Bombus_terrestris
Ceratina_cucurbitina
Megachile_pilidens
Osmia_tergestensis
Eucera_cineraria
Rhodanthidium_septemdentatum
Bombus_lapidarius
Osmia_gallarum
Apis_mellifera
Osmia_rufohirta
Xylocopa_violacea
Lasioglossum_interruptum
Ceratina_chalybea
Hoplitis_cf_papaveris
Anthidium_manicatum
Lasioglossum_malachurum
Melitta_dimidiata
Rhophites_algirus
Tetraloniella_fulvescens
Anthophora_aestivalis
Eucera_hungarica
Lasioglossum_laticeps
Osmia_emarginata
Seladonia_subaurata
Seladonia_submediterranea
Andrena_ovatula
Bombus_sylvarum
Colletes_foveolaris
Eucera_nigrescens
Lasioglossum_aeratum
Lasioglossum_lineare
Lasioglossum_tricinctum
Megachile_circumcincta
Andrena_curvungula
Andrena_hattorfiana
Ceratina_cyanea
Chelostoma_distinctum
Eucera_caspica
Eucera_clypeata
Halictus_gr_simplex
Halictus_patella
Lasioglossum_fulvicorne
Lasioglossum_maurusium
Lasioglossum_setulellum
Megachile_rufescens
Osmia_aurulenta
Helichrysum_stoechas
Phyteuma_orbiculare
Centaurea_pectinata
Teucrium_sp.
Inula_montana
Ononis_striata
Stachys_recta
Onobrychis_sp.
Lotus_delortii
Sedum_sp
Salvia_pratensis
Anthyllis_vulneraria
Thymus_sp.
Helianthemum_sp.
Plantago_media
Crataegus_monogyna
Ononis_repens
Fumana_sp.
Dorycnium_pentaphyllum
Hieracium_pilosella
Rubiaceae
Linum_sp.
Astragalus_monspessulanus
Echium_vulgare
Biscutella_laevigata
Medicago_sativa
Armeria_arenaria
Plantago_lanceolata
Allium_sphaerocephalon
Trifolium_campestre
Coronilla_minima
Euphrasia_salisburgensis
Caryophyllaceae
Campanula_patula
Knautia_arvensis
Trinia_glauca
Achillea_millefolium
Centaurea_scabiosa
Onosma_fastigiata
Minuartia_capillacea
Asperula_cynanchica
Galium_pumilum
Arenaria_aggregata
Euphorbia_nicaeensis


Château Gaillard visits
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Lasioglossum_calceatum
Lasioglossum_morio
Bombus_lapidarius
Seladonia_tumulorum
Bombus_terrestris
Osmia_rufohirta
Andrena_flavipes
Ceratina_cucurbitina
Apis_mellifera
Anthidiellum_strigatum
Anthidium_manicatum
Halictus_scabiosae
Hoplitis_leucomelana
Lasioglossum_fulvicorne
Lasioglossum_leucozonium
Megachile_pilidens
Megachile_willughbiella
Osmia_leaiana
Pseudoanthidium sp.
Seladonia_submediterranea
Trachusa_byssina
Anthericum_ramosum
Ononis_natrix
Phyteuma_orbiculare
Allium_sphaerocephalon
Centaurea_scabiosa
Teucrium_sp.
Leontodon_hispidus
Scabiosa_columbaria
Origanum_vulgare
Euphrasia_stricta
Echium_vulgare
Helianthemum_nummularium
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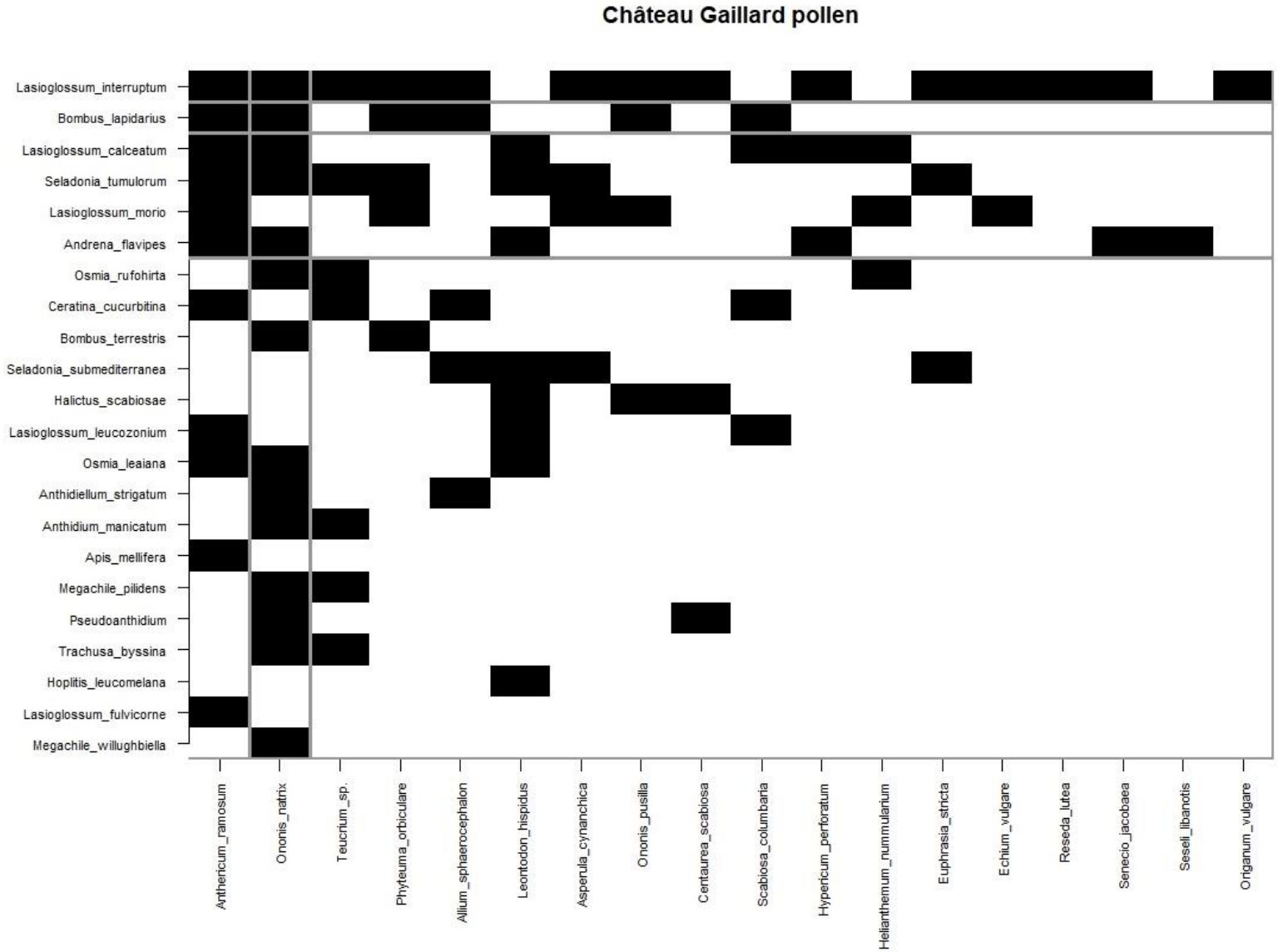
Senecio_jacobaea
Ononis_pusilla
Hypericum_perforatum
Reseda_lutea
Seseli_libanotis


Château Gaillard pollen
Lasioglossum_interruptum
Bombus_lapidarius
Lasioglossum_calceatum
Seladonia_tumulorum
Lasioglossum_morio
Andrena_flavipes
Osmia_rufohirta
Ceratina_cucurbitina
Bombus_terrestris
Seladonia_submediterranea
Halictus_scabiosae
Lasioglossum_leucozonium
Osmia_leaiana
Anthidiellum_strigatum
Anthidium_manicatum
Apis_mellifera
Megachile_pilidens
Pseudoanthidium
Trachusa_byssina
Hoplitis_leucomelana
Lasioglossum_fulvicorne
Megachile_willughbiella
Anthericum_ramosum
Ononis_natrix
Teucrium_sp.
Phyteuma_orbiculare
Allium_sphaerocephalon
Leontodon_hispidus
Asperula_cynanchica
Ononis_pusilla
Centaurea_scabiosa
Scabiosa_columbaria
Hypericum_perforatum
Helianthemum_nummularium
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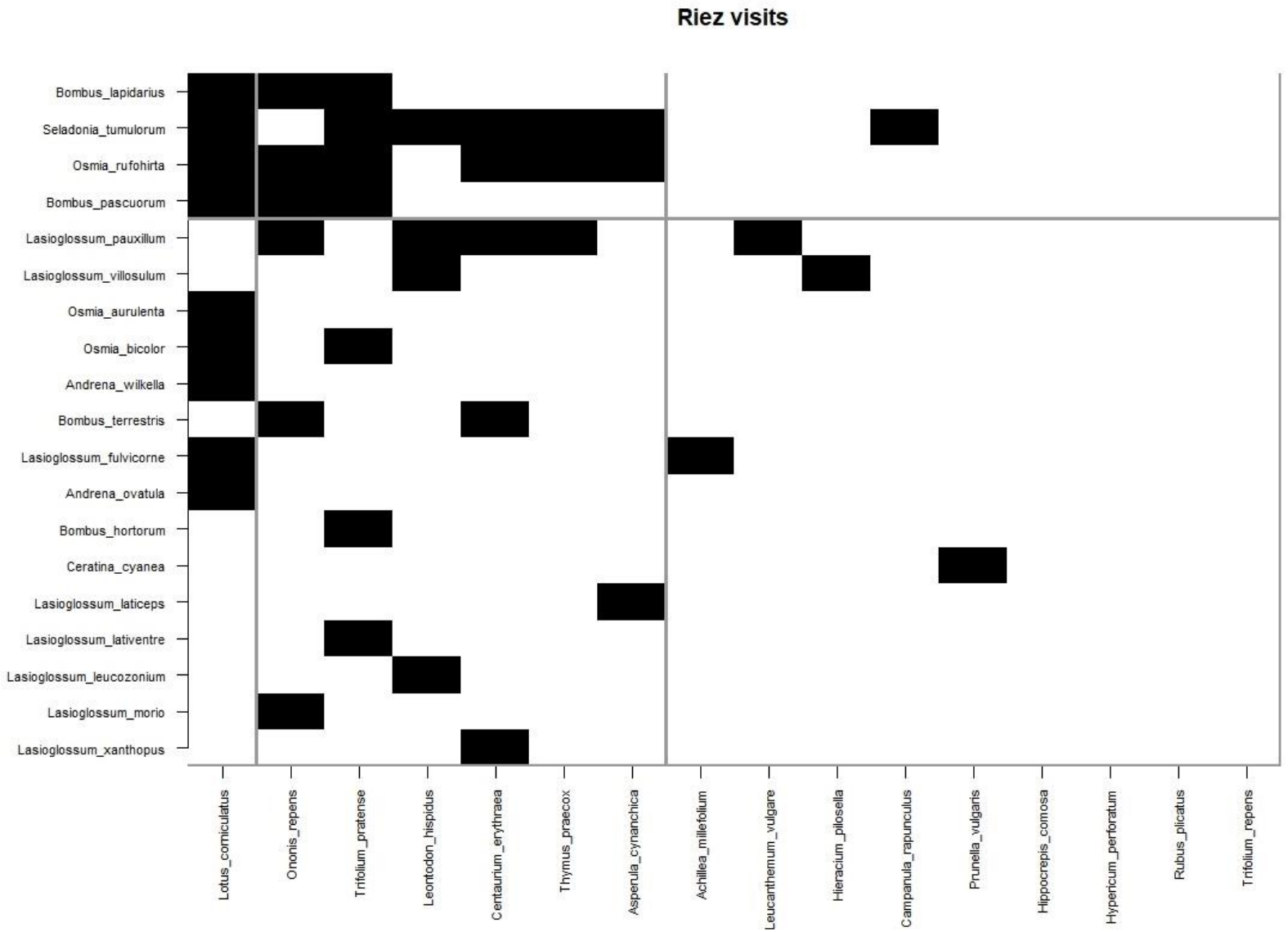
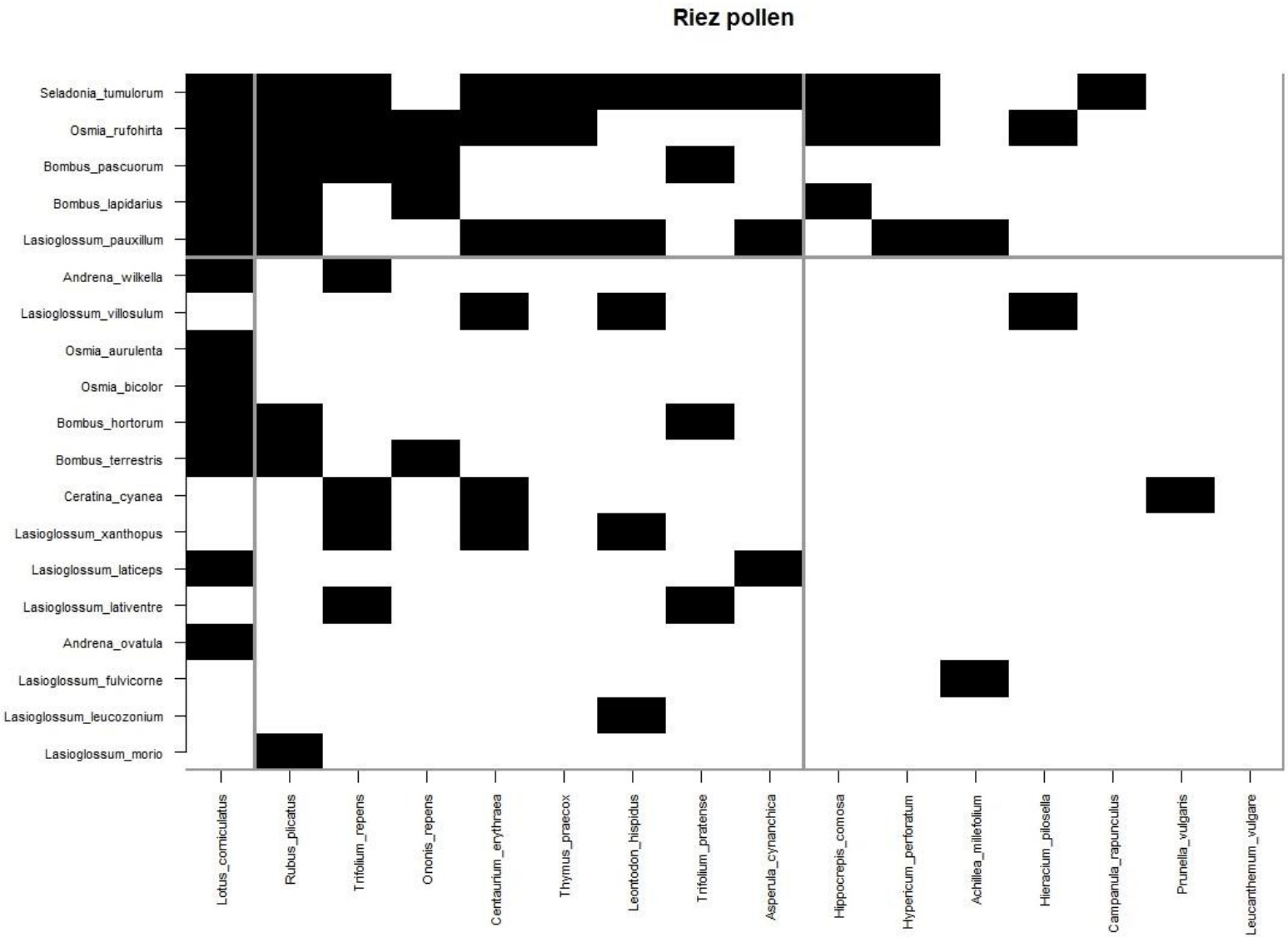
Echium_vulgare
Reseda_lutea
Senecio_jacobaea
Seseli_libanotis
Origanum_vulgare


Riez visits
Bombus_lapidarius
Seladonia_tumulorum
Osmia_rufohirta
Bombus_pascuorum
Lasioglossum_pauxillum
Lasioglossum_villosulum
Osmia_aurulenta
Osmia_bicolor
Andrena_wilkella
Bombus_terrestris
Lasioglossum_fulvicorne
Andrena_ovatula
Bombus_hortorum
Ceratina_cyanea
Lasioglossum_laticeps
Lasioglossum_lativentre
Lasioglossum_leucozonium
Lasioglossum_morio
Lasioglossum_xanthopus
Lotus_corniculatus
Ononis_repens
Trifolium_pratense
Leontodon_hispidus
Centaurium_erythraea
Thymus_praecox
Asperula_cynanchica
Achillea_millefolium
Leucanthemum_vulgare
Hieracium_pilosella
Campanula_rapunculus
Prunella_vulgaris
Hippocrepis_comosa
Hypericum_perforatum
Rubus_plicatus
Trifolium_repens


Riez pollen
Seladonia_tumulorum
Osmia_rufohirta
Bombus_pascuorum
Bombus_lapidarius
Lasioglossum_pauxillum
Andrena_wilkella
Lasioglossum_villosulum
Osmia_aurulenta
Osmia_bicolor
Bombus_hortorum
Bombus_terrestris
Ceratina_cyanea
Lasioglossum_xanthopus
Lasioglossum_laticeps
Lasioglossum_lativentre
Andrena_ovatula
Lasioglossum_fulvicorne
Lasioglossum_leucozonium
Lasioglossum_morio
Lotus_corniculatus
Rubus_plicatus
Trifolium_repens
Ononis_repens
Centaurium_erythraea
Thymus_praecox
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Trifolium_pratense
Asperula_cynanchica
Hippocrepis_comosa
Hypericum_perforatum
Achillea_millefolium
Hieracium_pilosella
Campanula_rapunculus
Prunella_vulgaris
Leucanthemum_vulgare

Figure S3. 6. Block clustering provided by LBM in the site of Fourches, Château Gaillard and Riez for the visits-matrix and the pollen-matrix. In the site of Fourches, we found a total of 5 blocks (2 insect blocks and 3 plant blocks) in the visits-matrix and a total of 7 blocks (4 blocks for insects and 3 for plants) in the pollen-matrix. In the site of Château Gaillard, we found 7 blocks (4 for insects and 3 for plants) in both matrices, and a similar pattern for the site of Riez, but with 5 blocks (2 for insects and 3 for plants) in both matrices. Blocks clustering followed the species degrees (high, medium and low degree). Insects are displayed in lines and plant in columns.

Supplementary Tables

Insect species	nb links Visit-based network	nb links Pollen-based network	Distance Visit-based network	Distance Simulated network (mean)	2.5% quantile	97.5% quantile	p-value adjusted positions	d' Visit-based network	d' Simulated network (mean)	p-value adjusted d'
Andrena curvungula	1	1	4.67	4.69	2.48	9.84	0.98	0.50	0.51	0.98
Andrena hattorfiana	1	1	7.09	5.55	4.41	10.17	0.78	0.81	0.92	0.73
Anthophora aestivalis	1	3	5.82	6.84	3.55	19.47	0.98	0.59	0.68	0.72
Bombus sylvarum	1	2	6.81	5.48	2.86	13.11	0.78	0.65	0.61	0.59
Ceratina cyanea	**1**	**1**	4.39	4.69	2.48	9.84	0.98	0.61	0.51	**0.04**
Chelostoma distinctum	**1**	**1**	3.45	7.05	3.45	34.72	0.98	1.00	0.96	**0.00**
Eucera caspica	**1**	**1**	5.56	7.98	3.67	20.06	0.98	0.50	0.76	**0.00**
Eucera clypeata	1	1	4.15	5.93	2.87	14.32	0.98	0.55	0.62	0.16
Eucera hungarica	1	3	5.16	5.25	3.87	11.36	0.98	0.67	0.70	0.95
Eucera nigrescens	1	2	4.34	6.50	3.33	15.31	0.98	0.50	0.62	0.79
Halictus gr simplex	1	1	4.67	4.69	2.48	9.84	0.98	0.50	0.51	0.98
Halictus patella	**1**	**1**	8.02	4.13	2.01	8.21	0.38	0.67	0.40	**0.00**
Lasioglossum aeratum	1	2	4.57	6.23	3.63	11.65	0.98	0.38	0.63	0.65
Lasioglossum fulvicorne	1	1	4.08	4.13	2.01	8.21	0.98	0.38	0.40	0.72
Lasioglossum maurusium	1	1	4.35	6.10	3.14	15.42	0.98	0.67	0.73	0.73
Lasioglossum setulellum	1	1	4.08	4.13	2.01	8.21	0.98	0.38	0.40	0.72
Lasioglossum tricinctum	1	2	5.75	7.28	3.73	18.60	0.98	0.65	0.80	0.57
Megachile rufescens	1	1	4.15	5.93	2.87	14.32	0.98	0.55	0.62	0.16
Osmia aurulenta	1	1	6.45	5.10	2.78	12.21	0.78	0.65	0.62	0.72
Osmia emarginata	1	3	7.15	8.46	4.86	21.75	0.98	0.81	0.83	0.93
Seladonia subaurata	1	3	4.26	5.91	2.92	13.90	0.98	0.50	0.54	0.98

Seladonia submediterranea	1	3	7.96	6.44	3.36	13.07	0.83	0.88	0.62	0.68
Andrena ovatula	2	2	7.28	9.76	5.11	31.16	0.98	0.64	0.77	0.07
Anthidium manicatum	2	4	7.23	9.75	4.82	24.08	0.98	0.76	0.66	0.65
Ceratina chalybea	2	5	5.27	8.67	4.60	20.70	0.98	0.69	0.74	0.94
Colletes foveolaris	2	2	14.66	7.93	4.11	17.76	0.52	0.76	0.87	0.51
Hoplitis cf papaveris	2	5	6.17	9.29	4.80	36.00	0.98	0.73	0.76	0.95
Lasioglossum laticeps	2	3	10.03	7.94	4.60	14.28	0.78	0.58	0.65	0.95
Lasioglossum lineare	2	2	9.72	7.13	3.91	13.38	0.78	0.58	0.63	0.98
Lasioglossum malachurum	2	4	6.93	9.10	4.70	20.03	0.98	0.54	0.61	0.86
Megachile circumcincta	**2**	**2**	7.21	6.66	4.45	18.55	0.88	0.67	0.94	**0.00**
Osmia gallarum	2	9	6.98	14.66	6.45	48.27	0.98	0.67	0.78	0.73
Osmia rufohirta	2	7	5.43	9.51	4.99	21.67	0.98	0.67	0.61	0.73
Xylocopa violacea	3	7	5.93	8.15	4.31	29.69	0.98	0.82	0.71	0.73
Megachile pilidens	4	12	8.79	13.89	6.56	39.36	0.98	0.66	0.70	0.77
Melitta dimidiata	4	4	4.15	5.93	2.87	14.32	0.98	0.72	0.82	0.16
Osmia tergestensis	4	12	6.95	15.10	6.90	44.06	0.98	0.66	0.69	0.88
Rhodanthidium septemdentatum	4	11	11.81	10.46	5.65	23.48	0.88	0.65	0.67	0.93
Rhophites algirus	4	4	6.45	5.10	2.78	12.21	0.78	0.85	0.81	0.72
Tetraloniella fulvescens	4	4	5.06	6.69	3.39	12.26	0.98	0.69	0.80	0.38
Eucera cineraria	5	11	9.04	11.65	6.35	26.21	0.98	0.71	0.67	0.79
Lasioglossum interruptum	5	8	6.96	10.87	5.59	22.46	0.98	0.63	0.67	0.94
Apis mellifera	6	8	16.49	10.51	5.98	22.55	0.61	0.59	0.69	0.71
Ceratina cucurbitina	6	12	12.51	14.37	7.72	33.99	0.98	0.72	0.73	0.94
Bombus lapidarius	7	9	9.18	8.74	4.81	19.26	0.98	0.71	0.69	0.88
Anthidium punctatum	8	21	9.83	14.26	7.90	31.71	0.98	0.61	0.67	0.73
Bombus terrestris	10	17	11.85	10.63	6.33	19.65	0.91	0.66	0.64	0.86
Lasioglossum pauxillum	15	41	14.62	17.69	9.54	39.83	0.98	0.56	0.61	0.73
Colletes similis	20	21	7.33	7.17	4.20	15.17	0.98	0.76	0.80	0.73
Lasioglossum subhirtum	26	57	14.85	17.50	9.96	35.14	0.98	0.64	0.59	0.72

Plant species	nb links Visit-based network	nb links Pollen-based network	Distance Visit-based network	Distance Simulated network (mean)	2.5% quantile	97.5% quantile	p-value adjusted positions	d' Visit-based network	d' Simulated network (mean)	p-value adjusted d'
Campanula patula	**1**	**2**	3.30	7.61	3.30	38.15	0.98	1.00	0.98	**0.00**
Coronilla minima	1	2	8.10	13.09	6.04	34.08	0.98	0.76	0.77	0.94
Crataegus monogyna	1	7	12.18	9.24	5.66	16.15	0.76	0.70	0.65	0.82
Euphrasia salisburgensis	1	2	6.27	9.23	4.41	20.53	0.98	0.54	0.49	0.65
Thymus sp.	1	9	9.60	9.85	5.53	20.19	0.98	0.45	0.64	0.52
Armeria arenaria	2	4	6.34	9.59	5.61	19.55	0.98	0.74	0.71	0.92
Biscutella laevigata	2	4	25.91	10.90	6.22	27.76	0.43	0.87	0.80	0.86
Sedum sp.	2	11	9.83	12.01	6.83	25.42	0.98	0.51	0.57	0.73
Allium sphaerocephalon	3	3	8.89	10.46	6.02	22.94	0.98	0.74	0.74	0.88
Echium vulgare	3	4	7.55	10.95	5.71	26.53	0.98	0.73	0.76	0.97
Fumana sp.	3	6	9.33	11.45	6.30	23.98	0.98	0.54	0.56	0.98
Helianthemum sp.	3	8	9.84	11.35	6.54	23.44	0.98	0.71	0.56	0.65
Knautia arvensis	3	2	9.32	6.30	4.18	12.93	0.76	0.88	0.91	0.98
Dorycnium pentaphyllum	**4**	**6**	11.00	10.54	5.50	24.17	0.98	0.67	0.88	**0.00**
Ononis striata	4	18	12.96	15.41	7.43	40.86	0.98	0.69	0.76	0.73
Salvia pratensis	7	11	7.98	9.61	5.27	25.33	0.98	0.84	0.74	0.73
Teucrium sp.	7	20	13.20	14.12	7.56	31.90	0.98	0.81	0.64	0.28
Linum sp.	8	5	8.64	10.09	5.35	37.81	0.98	0.65	0.81	0.72
Stachys recta	8	15	12.98	10.24	6.03	21.96	0.78	0.89	0.86	0.73
Centaurea pectinata	10	25	10.51	12.32	7.19	25.55	0.98	0.78	0.64	0.51
Anthyllis vulneraria	**11**	**9**	12.62	15.15	7.11	48.35	0.98	0.72	0.91	**0.00**
Onobrychis sp.	14	14	11.02	13.76	6.62	36.57	0.98	0.86	0.92	0.51
Inula montana	16	18	7.98	9.22	5.11	19.27	0.98	0.76	0.72	0.73
Phyteuma orbiculare	19	30	12.91	12.56	7.19	25.88	0.98	0.91	0.79	0.28
Helichrysum stoechas	39	49	11.02	12.30	6.78	24.58	0.98	0.77	0.82	0.55
Achillea millefolium	0	1	NA	NA	NA	NA	NA	NA	0.55	NA
Astragalus monspessulanus	0	4	NA	NA	NA	NA	NA	NA	0.72	NA
Caryophyllaceae	0	2	NA	NA	NA	NA	NA	NA	0.67	NA

Centaurea scabiosa	0	1	NA	NA	NA	NA	NA	NA	0.63	NA
Hieracium pilosella	0	5	NA	NA	NA	NA	NA	NA	0.49	NA
Lotus delortii	0	11	NA	NA	NA	NA	NA	NA	0.77	NA
Medicago sativa	0	4	NA	NA	NA	NA	NA	NA	0.96	NA
Minuartia capillacea	0	1	NA	NA	NA	NA	NA	NA	0.46	NA
Ononis repens	0	6	NA	NA	NA	NA	NA	NA	0.76	NA
Onosma fastigiata	0	1	NA	NA	NA	NA	NA	NA	1.00	NA
Plantago lanceolata	0	3	NA	NA	NA	NA	NA	NA	0.68	NA
Plantago media	0	8	NA	NA	NA	NA	NA	NA	0.59	NA
Rubiaceae	0	5	NA	NA	NA	NA	NA	NA	0.62	NA
Trifolium campestre	0	3	NA	NA	NA	NA	NA	NA	0.82	NA
Trinia glauca	0	1	NA	NA	NA	NA	NA	NA	0.55	NA
Asperula cynanchica	1	0	NA	NA	NA	NA	NA	NA	NA	NA
Euphorbia nicaeensis	1	0	NA	NA	NA	NA	NA	NA	NA	NA
Galium pumilum	1	0	NA	NA	NA	NA	NA	NA	NA	NA
Arenaria aggregata	3	0	NA	NA	NA	NA	NA	NA	NA	NA

Table S3. 1 Insect and plant species in the site of Fourches. Species are ranged following the number of links in the visits-network (first column). Species underlined and in bold showed a significative change both in their position (column p-value adjusted position) OR in the specialization degree (column p-value adjusted d'). Species in bold showed a significative change in their position (column p-value adjusted position) OR in the specialization degree (column p-value adjusted d'). Plant species which show NA values were not observed in interaction in the visits-network (nb of links Visits-network, first column) or in the pollen network (nb of links Pollen-network, second column). For these species were not possible to calculate the species positions and the specialisation degree.

Insect species	nb links Visit-based network	nb links Pollen-based network	Distance Visit-based network	Distance Simulated network (mean)	2.5% quantile	97.5% quantile	p-value adjusted positions	d' Visit-based network	d' Simulated network (mean)	p-value adjusted d'
Anthidiellum strigatum	**1**	**2**	14.88	5.89	3.66	10.41	**0.03**	0.63	0.52	0.72
Anthidium manicatum	1	2	10.81	5.21	3.19	9.68	0.08	0.44	0.50	1.00
Halictus scabiosae	1	3	14.07	8.75	4.90	17.52	0.23	0.63	0.77	0.26
Hoplitis leucomelana	1	1	11.49	9.36	4.31	22.43	0.38	0.71	0.73	0.99
Lasioglossum fulvicorne	1	1	7.80	4.94	2.78	9.12	0.18	0.29	0.30	0.66

Lasioglossum leucozonium	1	3	8.40	8.01	4.05	22.70	0.51	0.29	0.60	0.38
Megachile pilidens	1	2	10.82	5.19	3.18	9.63	0.08	0.44	0.50	1.00
Megachile willughbiella	**1**	**1**	10.88	4.70	2.64	8.99	**0.05**	0.44	0.39	0.11
Osmia leaiana	1	3	14.18	6.44	3.18	17.43	0.13	0.63	0.46	0.74
Pseudoanthidium	1	2	14.33	6.58	3.91	17.19	0.12	0.63	0.60	1.00
Seladonia submediterranea	1	4	12.48	8.48	4.44	17.56	0.21	0.71	0.74	1.00
Trachusa byssina	**1**	**2**	14.76	5.18	3.19	9.48	**0.03**	0.63	0.50	0.55
Apis mellifera	2	2	7.80	4.94	2.78	9.12	0.18	0.33	0.34	0.66
Andrena flavipes	3	11	14.36	14.53	6.75	40.38	0.50	0.39	0.56	0.59
Ceratina cucurbitina	**3**	**6**	29.14	10.07	5.49	18.39	**0.03**	0.67	0.59	0.76
Bombus terrestris	4	5	12.54	8.73	4.53	18.70	0.32	0.49	0.49	1.00
Osmia rufohirta	**4**	**7**	27.26	8.36	5.00	14.68	**0.02**	0.41	0.56	**0.04**
Lasioglossum morio	7	13	10.80	12.42	6.99	26.03	0.68	0.49	0.51	0.95
Seladonia tumulorum	8	19	16.37	14.89	8.35	29.64	0.50	0.45	0.49	0.78
Lasioglossum calceatum	17	22	14.92	12.12	7.83	20.25	0.35	0.55	0.52	0.74
Bombus lapidarius	**20**	**33**	45.40	13.81	8.21	25.26	**0.01**	0.54	0.63	0.30
Lasioglossum interruptum	**50**	**84**	38.95	16.92	9.83	30.34	**0.04**	0.54	0.56	0.78

Plant species	nb links Visit-based network	nb links Pollen-based network	Distance Visit-based network	Distance Simulated network (mean)	2.5% quantile	97.5% quantile	p-value adjusted positions	d' Visit-based network	d' Simulated network (mean)	p-value adjusted d'
Asperula cynanchica	1	7	9.04	11.52	6.30	23.64	0.78	0.65	0.52	0.55
Echium vulgare	**1**	**3**	19.80	8.47	4.62	15.21	**0.04**	0.30	0.41	0.67
Helianthemum nummularium	1	4	8.60	11.22	7.20	19.95	0.90	0.65	0.63	0.84
Euphrasia stricta	2	4	16.59	10.33	6.10	18.09	0.13	0.38	0.57	0.76
Origanum vulgare	**3**	**1**	19.87	6.70	3.57	11.86	**0.01**	0.37	0.31	0.08
Leontodon hispidus	5	10	22.07	15.16	7.36	39.29	0.30	0.70	0.77	0.59
Scabiosa columbaria	**5**	**5**	34.98	11.36	7.03	19.99	**0.02**	0.39	0.58	**0.00**
Allium sphaerocephalon	**8**	**12**	27.96	9.66	5.94	16.19	**0.02**	0.40	0.45	0.67
Centaurea scabiosa	**8**	**5**	30.03	8.10	4.29	24.27	**0.00**	0.53	0.55	0.91

Teucrium sp.	**8**	**15**	31.50	9.80	5.68	18.27	**0.03**	0.58	0.63	0.78
Phyteuma orbiculare	13	15	18.74	15.82	8.95	29.95	0.44	0.65	0.51	0.27
Ononis natrix	22	51	27.70	13.42	8.16	25.79	0.09	0.54	0.61	0.35
Anthericum ramosum	54	81	27.16	13.80	8.60	24.39	0.08	0.66	0.65	0.98
Hypericum perforatum	0	4	NA	NA	NA	NA	NA	NA	0.51	NA
Ononis pusilla	0	5	NA	NA	NA	NA	NA	NA	0.51	NA
Reseda lutea	0	3	NA	NA	NA	NA	NA	NA	0.34	NA
Senecio jacobaea	0	2	NA	NA	NA	NA	NA	NA	0.46	NA
Seseli libanotis	0	1	NA	NA	NA	NA	NA	NA	0.81	NA

Table S3. 2. Insect and plant species in the site of Château Gaillard. Species are ranged following the number of links in the visit-based network (first column). Species underlined and in bold showed a significant change both in their position (column p-value adjusted position) AND in the specialization degree (column p-value adjusted d'). Species in bold showed a significant change in their position (column p-value adjusted position) OR in the specialization degree (column p-value adjusted d'). Plant species which show NA values were not observed in interaction in the visit-based network (nb of links Visit-based network, first column) or in the pollen network (nb of links Pollen-based network, second column). For these species were not possible to calculate the species positions.

Insect species	nb links Visit-based network	nb links Pollen-based network	Distance Visit-based network	Distance Simulated network (mean)	2.5% quantile	97.5% quantile	p-value adjusted positions	d' Visit-based network	d' Simulated network (mean)	p-value adjusted d'
Andrena ovatula	1	1	7.51	5.03	2.66	9.12	0.23	0.28	0.30	0.63
Bombus hortorum	1	3	9.58	6.38	3.73	11.67	0.23	0.52	0.53	0.85
Ceratina cyanea	**1**	**3**	4.05	5.84	4.05	11.13	0.78	1.00	0.76	**0.00**
Lasioglossum laticeps	1	2	10.49	6.67	3.78	12.78	0.23	0.79	0.54	0.51
Lasioglossum lativentre	1	2	10.26	6.37	3.59	11.91	0.22	0.52	0.67	0.21
Lasioglossum leucozonium	1	1	5.76	9.07	4.21	36.79	0.78	0.63	0.69	0.75
Lasioglossum morio	1	1	7.48	7.01	4.00	13.63	0.57	0.51	0.60	0.28
Lasioglossum xanthopus	1	3	6.96	7.30	3.69	19.37	0.59	0.63	0.65	0.99
Bombus terrestris	2	3	12.33	10.58	5.53	24.11	0.48	0.50	0.47	0.85
Lasioglossum fulvicorne	**2**	**1**	16.77	4.94	2.67	14.49	0.11	0.59	0.97	**0.00**
Andrena wilkella	3	6	8.24	8.37	5.13	14.87	0.68	0.36	0.47	0.59
Lasioglossum villosulum	4	5	8.35	14.04	7.05	38.58	0.93	0.83	0.80	0.77

Osmia aurulenta	4	4	7.51	5.03	2.66	9.12	0.23	0.39	0.41	0.63
Osmia bicolor	**4**	**4**	19.17	5.03	2.66	9.12	**0.00**	0.32	0.41	**0.00**
Lasioglossum pauxillum	7	18	17.07	15.19	8.38	32.07	0.49	0.63	0.60	0.83
Bombus pascuorum	13	27	19.30	11.64	7.44	19.46	0.16	0.41	0.50	0.23
Osmia rufohirta	**14**	**32**	27.38	14.08	8.71	25.70	0.13	0.33	0.48	**0.00**
Seladonia tumulorum	15	34	19.43	15.30	8.90	29.91	0.33	0.41	0.56	0.11
Bombus lapidarius	17	23	19.03	11.11	6.24	20.06	0.20	0.46	0.55	0.07
Plant species	nb links Visit-based network	nb links Pollen-based network	Distance Visit-based network	Distance Simulated network (mean)	2.5% quantile	97.5% quantile	p-value adjusted positions	d' Visit-based network	d' Simulated network (mean)	p-value adjusted d'
Achillea millefolium	**1**	**2**	26.99	5.39	2.59	16.64	**0.03**	0.87	0.95	0.78
Campanula rapunculus	1	2	12.74	8.65	4.63	16.69	0.25	0.48	0.52	0.83
Hieracium pilosella	1	2	11.02	14.54	8.16	37.83	0.77	0.73	0.67	0.61
Prunella vulgaris	**1**	**1**	0.00	0.00	0.00	0.00	**0.00**	1.00	1.00	**0.00**
Asperula cynanchica	3	7	14.37	10.17	5.94	19.77	0.26	0.57	0.64	0.71
Thymus praecox	4	11	18.04	11.53	6.57	22.49	0.23	0.44	0.57	0.26
Centaurium erythraea	7	12	16.15	12.00	7.02	22.49	0.26	0.63	0.59	0.78
Leontodon hispidus	7	9	10.34	13.78	6.71	38.59	0.75	0.70	0.77	0.75
Trifolium pratense	12	9	21.73	9.57	6.01	16.42	0.08	0.49	0.63	0.36
Ononis repens	13	14	21.38	13.85	8.06	25.58	0.23	0.51	0.53	0.88
Lotus corniculatus	42	63	20.25	15.05	9.20	26.98	0.26	0.66	0.66	0.95
Hippocrepis comosa	0	5	NA	NA	NA	NA	NA	NA	0.46	NA
Hypericum perforatum	0	3	NA	NA	NA	NA	NA	NA	0.52	NA
Rubus plicatus	0	17	NA	NA	NA	NA	NA	NA	0.57	NA
Trifolium repens	0	16	NA	NA	NA	NA	NA	NA	0.65	NA
Leucanthemum vulgare	1	0	NA	NA	NA	NA	NA	NA	NA	NA

Table S3. 3. Insect and plant species in the site of Riez. Species are ranged following the number of links in the visit-based network (first column). Species underlined and in bold showed a significant change both in their position (column p-value adjusted position) AND in the specialization degree (column p-value adjusted d'). Species in bold showed a significant change in their position (column p-value adjusted position) OR in the specialization degree (column p-value adjusted d'). Plant species which show NA values were not observed in interaction in the visit-based network (nb of links Visit-based network, first column) or in the pollen network (nb of links Pollen-based network, second column). For these species were not possible to calculate the species positions and the specialisation degree.

Chapter IV

Geographical variation of floral scent in four calcareous grassland species with generalist pollination: genetic adaptation vs. phenotypic plasticity

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Abstract

Floral scents are considered major non-visual attractants for many pollinator species. Thus, as other floral traits, the composition of floral scents is likely to be under pollinator-mediated selection. However, despite recent advances and an increasing interest in odours emitted by flowers, little is known about the mechanism (genetic adaptation vs. phenotypic plasticity) behind geographical variation of floral scents. Among-populations differences are also often interpreted as the result of local adaptation to pollinators. One way to investigate this question is to compare floral scents both in natural population and in controlled conditions. In the present study, we compared floral scent composition in four generalist plant species among three distinct regions, both *in natura* and on plants reared in common garden. We also collected data on geographical variation in pollinator communities associated with each plant species. Our results show that floral scent composition can differ within and among species and between environments (natural vs. common garden), arguably due to genetic differences among species and populations, and phenotypic plasticity between environments. On the four studied species, only *Ranunculus bulbosus* showed significant differences in the floral scent emissions between the tested populations in both conditions, suggesting local genetic adaptation. We also found geographical variation in VOCs composition and emission among natural population of *Anthyllis vulneraria* and *Pilosella officinorum*. However, these variation *in natura* were not consistently found in controlled condition, due to the emission of different floral scents in the greenhouse, suggesting the occurrence of phenotypic plasticity. Moreover, we did not detect any obvious variation in the pollinator communities associated with each plant species, since the only differences were observed at the species level. Thus, we cannot interpret the floral scent variation as pollinator-mediated selection. Overall, this suggests that floral bouquet variation is species-specific, *i.e.* each species has its own scent profile that is context-dependent, since the volatile compounds emitted in controlled conditions were different from those detected in natural conditions. Hence, it is important to consider different environmental conditions to have a better representation of ecological situations and to clarify the mechanisms behind the floral scent variation.

Introduction

Because plants are sessile organisms, they have to cope with the problem of distant reproduction by dispersing pollen. A majority of angiosperms depend on animal pollinators for the dispersal of their pollen (Ollerton *et al.*, 2011) and often invest in a suite of floral signals to attract pollinators. Volatile organic compounds (VOCs) are considered as major non-visual attractants for many pollinator species (Schiestl 2010). Floral scents are often a complex mix of several compounds which are produced by biochemical pathways of secondary metabolism (Knudsen *et al.*, 2006; Junker & Parachnowitsch, 2015). Several studies have shown that both quality (*i.e.* VOC identity) and quantity (often analysed as VOC proportions) of floral scents can mediate the interaction with floral visitors (Galen *et al.*, 2011; Burger *et al.*, 2013; Beyaert & Hilker, 2014). Indeed, the inhibition of a particular group of compounds can mediate a shift between two groups of pollinators or modify their behaviour (Huber *et al.*, 2005; Shuttleworth & Johnson, 2010; Larue-Kontic & Junker, 2016). Floral scent composition is thus expected to be under pollinator-mediated selection (Schiestl & Johnson, 2013; Delle-Vedove *et al.*, 2017). Although most studies rely on indirect evidence for such selective role of pollinators, this has been more directly documented by recent studies based on selective gradients (Majetic *et al.*, 2009a; Ehrlén *et al.*, 2012; Parachnowitsch *et al.*, 2012; Gross *et al.*, 2016) or experimental evolution (Gervasi & Schiestl, 2017). Thereby, one expects divergent floral scents among populations of the same plant species if pollinator assemblages vary geographically. This has been found in several pollination systems, although (i) specialized pollination systems seem to have been more often investigated compared to generalist plant species and (ii) scent variation is not always consistent with pollinator variation (reviewed in Delle-Vedove *et al.* 2017a).

Although variation in floral scent composition detected in the field is often interpreted as the result of pollinator-mediated selection, which should retain genotypes that emit the most efficient floral signals, other mechanisms could be involved in geographic variation of floral scents. Indeed, several studies have shown evidence for phenotypic plasticity of floral odour, related to environmental conditions. Both total emission rates and composition have been shown to vary with nitrogen supply (Majetic *et al.*, 2017), temperature (Farré-Armengol *et al.*, 2014), water availability (Campbell *et al.*, 2019), and between night and day (Dötterl *et al.*, 2012). The level of plasticity appears to vary from one compound to another, with even some VOCs specifically found in some environmental conditions but not in others (Farré-Armengol *et al.*, 2014; Campbell *et al.*, 2019).

Despite recent advances and increasing interest in floral scent variation, little is known about the mechanism behind this variation, especially the relative importance of genetic adaptation vs. phenotypic plasticity in determining floral scent bouquets is still not clear. Studies using common garden approaches to provide the genetic bases of floral scent variation (Dobson *et al.*, 1997; Parachnowitsch *et al.*, 2012) usually describe scent composition obtained in experimental (garden or lab) populations, which may be not representative of scents produced in natural populations. On the other hand, studies which focus on natural conditions explain variation as the result of local adaptation, often excluding phenotypic plasticity because these approaches cannot tease apart genetic adaptation from plasticity (Huber *et al.*, 2005; Breitkopf *et al.*, 2013). Only the comparison between natural populations and controlled conditions can help disentangle the origin of the variation (Delle-Vedove *et al.*, 2017), and this has been done by only a few studies that focused on one plant species or one plant genus so far (Ellis & Johnson, 2009; Majetic *et al.*, 2009b; Friberg *et al.*, 2013; Chapurlat *et al.*, 2018).

In this study, we compared the floral bouquets (compound identity and proportions) in four annual plant species, largely distributed along an environmental and diversity gradient in France and found in calcareous grasslands. Calcareous grasslands, when compared to other types of habitats, produce the greatest amount of nectar per unit area and are characterized by highly diverse plant communities with a high proportion of entomophilous species (Baude *et al.*, 2016) and richer in VOCs (Cornu *et al.*, 2015). Hence, they are convenient models to study plant-pollinator interactions and floral scent variation. We compared VOCs emitted in natural and controlled conditions. We addressed three main questions: (i) How do plant floral scents vary along the environmental gradient? (ii) How do floral scents co-vary with pollinator communities? (iii) What is the mechanism inducing floral scent variation?

To answer these questions, we realized *in situ* floral scent extractions to perform comparisons among wild populations and among regions (*i.e.* along a latitudinal gradient). We also recorded pollinator visits on the focal species, to document any possible variation of the pollinating fauna among regions. At the end of the flowering season, we collected plant seeds from the focal species in all sites to rear them under controlled conditions. We then performed a second study of floral bouquet to investigate whether observed variation in the field was due to genetic variation among populations or to phenotypic plasticity.

Materials and methods

Study sites and study systems

We selected six calcareous grassland areas of 1 hectare each in three different regions along a latitudinal gradient in France (Fig. S4.1): two populations in the French region of Hauts-de-France (Les Larris de Grouches-Luchuel, noted LAR 50°11'22.5"N 2°22'02.9"E and Regional natural reserve Riez de Noeux les Auxi, called Riez, noted R 50°14’51.85”N 2°12’05.56”E), two populations in the region of Normandie (Château Gaillard – le Bois Dumont, CG 49°14'7.782"N 1°24'16.445"E and les Falaises d’Orival, FAL 49°04'40.08"N 1°33'07.254"E) and two populations in the region of Occitanie (Fourches, F 43°56'07.00"N 3°30'46.1"E and Bois de Fontaret, BF 43°55'17.71"N 3°30'06.06"E). Although populations in Normandie are geographically closer to the northern populations of Hauts-de-France than to populations in Occitanie, this collection of sites showed important climatic differences among them, which can influence community composition. We can then consider that the sites in Normandie are a sort of “crossroads” of diversity for both plant and insect species, with some plant and insect species that reach their northern limit of repartition in these sites (*e.g. Eucera* species for insects and *Astragalus* for plants).

In these 6 sites, we focused on four annual plant species which were widely spread along the environmental gradient and present in at least some of the study sites: *Anthyllis vulneraria* (AV, Family: Fabaceae), *Globularia vulgaris* (GV, Family: Plantaginaceae), *Pilosella officinarum* (PO, family: Asteraceae) and *Ranunculus bulbosus* (RB, Family: Ranunculaceae). Even if the four species display different morphologies and phenologies (Table S4.1), all of them are entomophilous and considered potentially attractive for local pollinators. GV populations were present in both sites in Occitanie (BF and F) and both sites in Normandie (CG and FAL), RB and AV populations were found in both sites in Occitanie and Hauts-de-France (LAR and R), PO populations were found only in the southern site of Fourches (Occitanie) and in both sites in the other two regions (CG and FAL in Normandie and LAR and R in Hauts-de-France. The populations of AV display different colours along the gradient (yellow in Hauts-de-France and pink in Occitanie). In the context of a larger study investigating plant-pollinator networks in all six study sites, we characterised the local pollinator populations from April to October 2016 and again in 2017 (focusing on native bees, Anthophila and hoverflies, Syrphidae). We performed pollinator sampling on all flowering plants and we recorded plant-pollinator

interactions for 4 hours each day. All sampled pollinators were put in a killing vial, pinned in the laboratory and identified by expert taxonomists. For the present study, we extracted the data on pollinators associated with the four focal plant species.

Floral scents

We studied volatile compounds emitted *in natura* during spring 2017. At the end of the flowering seasons, we collected plant seeds from all different species, in order to grow focal species in greenhouse and repeat the study of volatile compounds in controlled conditions during spring 2018. This was done for all species except *Globularia vulgaris*, whose plants did not flower in the greenhouse.

Sampling - Floral scent extractions were performed in the field between April and June 2017, during the species flowering peak, and at the greenhouse between April and May 2018 (Tab S2). Floral scents were collected when the selected plant species had reached receptivity, *i.e.* when they attracted pollinators. In natural conditions (field), we selected 5-15 individuals per site and species, according to flowering plant availability. Under controlled conditions (greenhouse), we selected from 5-8 plant individuals per site and species, all belonging to different maternal families. All surveyed plants in greenhouse were excluded from pollination events since they were placed in a separated chamber cell (Tab S2). Odour collection was always performed under natural light in the field and at maximum ambient temperature (which ranged from 10°C in April to 29°C in June) and minimum of humidity. In the greenhouse, floral scents were collected under a mix of natural and artificial lights and we settled the cell temperature around 20°C (with a recorded maximum of 29°C). Scent collections were performed between 12:00 to 16:00 h, corresponding to the period of insect maximum activity during in the field.

Scent extraction – Scents were extracted using the dynamic Headspace technique (Grison-Pigé *et al.*, 2002; Proffit *et al.*, 2008; Soler *et al.*, 2011). We enclosed each receptive plant individual for one hour in polyethylene terephthalate bags (Nalophane®, Kalle Nalo GmbH, Wursthüllen, Germany), which have been shown not to release or adsorb VOCs, and shut tightly with cotton string to allow the accumulation of VOCs. One control (empty bag) was sampled for each combination of site, species and date, in order to collect VOCs occurring in the ambient air. These contaminant VOCs were excluded from analyses (see next section). ChromatoProbe® quartz microvials of Varian Inc. (length: 15 mm; inner diameter: 2 mm), previously cut closed-end and filled with 3 mg of a 1:1 mix of Tenax-TA and Carbotrap® (60–80 and 20–40 mesh,

respectively; Sigma Aldrich, Munich, Germany), were used as adsorbent traps. One microliter of a solution of internal standards (n-Nonane and n-Dodecane, 108 ng/µl and 114 ng/µl of each) was added to each trap before scent extraction. Traps were attached to silicone tubing within the collection bags and connected on the other end to flowmeters and a standard 12-V air pump. The 200 ml/min air flow was drawn out of the bag and over the trap for 20 minutes. All samples were kept in clean glass vials and stored in the dark, in a portable cooler, until transport to a −20°C freezer where samples remained until analysis by Gas Chromatography-Mass Spectrometry (GC-MS) in the laboratory.

Floral scent analysis – All samples collected under natural and controlled conditions were analysed at the "Platform for Chemical Analyses in Ecology" (PACE), technical facilities of the LabEx CeMEB (Centre Méditerranéen pour l'Environnement et la Biodiversité, Montpellier, France), using gas chromatograph coupled to mass spectrometer (GC, Trace™ 1310, and ISQ™ QD Single Quadrupole, Thermo Scientific™ Milan, Italy). For further details of the GC-MS analysis see Souto-Vilarós et al. (2018).

Xcalibur™ software (Thermo Scientific™, Milan, Italy) was used to identify the chromatogram peak. We converted the retention time of each compound into a retention index (IR) using the retention times of the n-alkanes (Alkanes standard solution, 04070, Sigma Aldrich®). To identify the compounds, we matched the mass spectra with a database (NIST 2007 MS library, Wiley 9th edition), and with retention indices reported in the literature (Adams, 2007), or by comparison with reference compounds. We subtracted any potential contaminant compounds from the samples that we used for statistical analysis by comparing their spectra to the controls collected on the same days of collection and under the same conditions.

Statistical analyses –Although similar studies sometimes exclude from statistical analyses VOCs with low average proportions or low occurrence, we chose to consider all detected VOCs in the present study. This is because some VOCs that were found to be rare in one extraction condition (field or greenhouse) were detected at higher proportions in the other. Excluding them from the analyses would give the false message that these VOCs were emitted in one environmental condition only. However, we also ran alternative analyses by focusing on major VOCs only to check that results were not qualitatively affected (unshown data).

For each species, we investigated the effects of population and extraction condition (field vs. greenhouse) on scent composition (proportions of all VOCs). Three datasets were analysed separately: (i) *in natura* scent data, (ii) greenhouse scent data, which were typically based on a

sub-sample of populations sampled in the field, and (iii) a merged dataset including both *in natura* and greenhouse, but restricted to the populations that were studied in both environmental conditions. We assessed the importance of explanatory factors using a PERMANOVA performed on Bray-Curtis distances between scents (Bray & Curtis, 1957) and testing the significance of individual factors (population provenance and extraction condition) using 999 permutations (*adonis* function, R-package vegan, Oksanen *et al.* 2019). Prior to permutational analysis of variance, data were square-root transformed and standardized using the Wisconsin double standardization (*wisconsin* function, R-package vegan, Oksanen *et al.* 2019). For datasets including only *in natura* or greenhouse scents, we tested for an effect of population on scent composition. For the other datasets, we simultaneously tested for population and condition. When a population effect was found significant, multiple pairwise comparisons were conducted, and p-values were adjusted using Bonferroni's correction.

We then inspected whether significant among-population differences were consistent with a regional effect, *i.e.* if populations from sites belonging to the same region clustered together. Both similarities and dissimilarities were visually assessed on a non-metric multidimensional scaling, using the function *metaMDS* in the R package *vegan*, two or three dimensions and 100 runs (Fig. S4.2 NMDS). A stress value is given to calculate how well the particular configuration produces the observed distance matrix (conventional stress cutoff of 0.2). Statistics were conducted in R using functions from *plotly* (Sievert *et al.*, 2018) and *graphics* packages. Similarity percentage analyses were also performed in R using the simper function from the *vegan* package to identify the compounds that were the most responsible for differences between the populations or growing conditions. All analyses were conducted in R version 3.3.3 (R Development Core Team 2018).

Floral reflectance

Because *Anthyllis vulneraria* presents a colour dimorphism between the two regions in which the species was surveyed (Fig. S4.3), we quantitatively analysed floral reflectance from 300 nm to 700 nm (visible spectrum) on the same 15 individuals grown in greenhouse that were surveyed for scent analysis. Two flowers were measured per individual and we used their mean for statistical analysis. We used a spectrophotometer equipped with a Xenon light source (AvaLight-XE, Avantes, Eerbeek, The Netherlands). The spectrophotometer was calibrated with a white standard (made with Barium Sulfate) prior to the measurements. We standardized the raw data using the function prospect in the package *pavo* in R (Maia *et al.*, 2019) and we

use the function *vismodel* to model the visual system of the honeybee ("*apis*") and hoverfly ("*musca*") receptor-sensitivity in order to test the different perception of the two *Anthyllis* colours. For honeybee, we used the colour hexagon for trichromatic hymenopteran view (Chittka, 1992) which is a colour-opponent model and offers a measure of perceptual distance. For hoverfly, we used the categorical fly-visual model (Troje, 1993) which assumes the involvement of all four dipteran photoreceptor classes (R7, R8, "pale" and "yellow"). All colours are perceptually grouped under one of the colour categories. For both models we extracted the XY coordinates of the calculated individual spectral loci. To test the hypothesis that bee and hoverfly perceptions of the two colours are significantly different between populations, we performed PERMANOVA analyses (package *vegan*) on the Euclidean distance matrix based on the XY coordinates, and tested the significance using 999 permutations followed by post-hoc comparisons (Bonferroni's adjustment).

Results

Floral scents

We detected a total of 38 volatile compounds, including 6 unknown compounds, belonging to four different chemical families, mainly fatty acid derivatives and monoterpenes (Table S4.3). Floral scents of surveyed species shared many volatile compounds, with 21 VOCs out of the 38 being detected in all four species. Overall, the major compounds (average proportion >10% in at least one population, occurrence in more than half of sampled individuals) found in natural populations were partly similar among species. This included the Limonene (major compound in all four species), (*E*)-beta-Ocimene (a major compound in *A. vulneraria* and *R. bulbosus*) and the Nonanal (rare in *A. vulneraria*, major in three other species). However, every species presented a specific floral bouquet, with some species-specific volatile compounds and among-species differences in VOC proportions (Fig. 4.1).

For the three species that were also studied in greenhouse, we found a significant difference between floral scents detected in natural and controlled conditions (Table 4.2), involving both qualitative and semi-quantitative variation. Regarding comparison between populations, *Globularia vulgaris* (GV) was the only species in which no significant geographical variation was detected (Table 4.1). In *Pilosella officinarum,* (PO) the variation of floral scents appeared to depend on population, but with no clear geographical trend (Table 4.1 and 4.2). Finally, in both *Anthyllis vulneraria* (AV) and *Ranunculus bulbosus* (RB), we found a geographical

variation of floral scent composition, which included significant differences between regions (Table 4.1). Whereas this geographical variation was not significant in the greenhouse study regarding *Anthyllis vulneraria*, we found fairly consistent results between nature and greenhouse studies in *Ranunculus bulbosus*. Below we present in more details these different sources of variation, for each of the four species. The volatile compounds mentioned in the text and included in Figure 1 were the ones that explained most of the statistical differences among populations and/or conditions (greenhouse vs. nature), based on the on-way Simper analyses. Figure 4.1 also includes major compounds as defined above, in order to provide a global picture of scent composition for the different samples. Complete information on average proportions of all VOCs in each sample is available in Table S4.4.

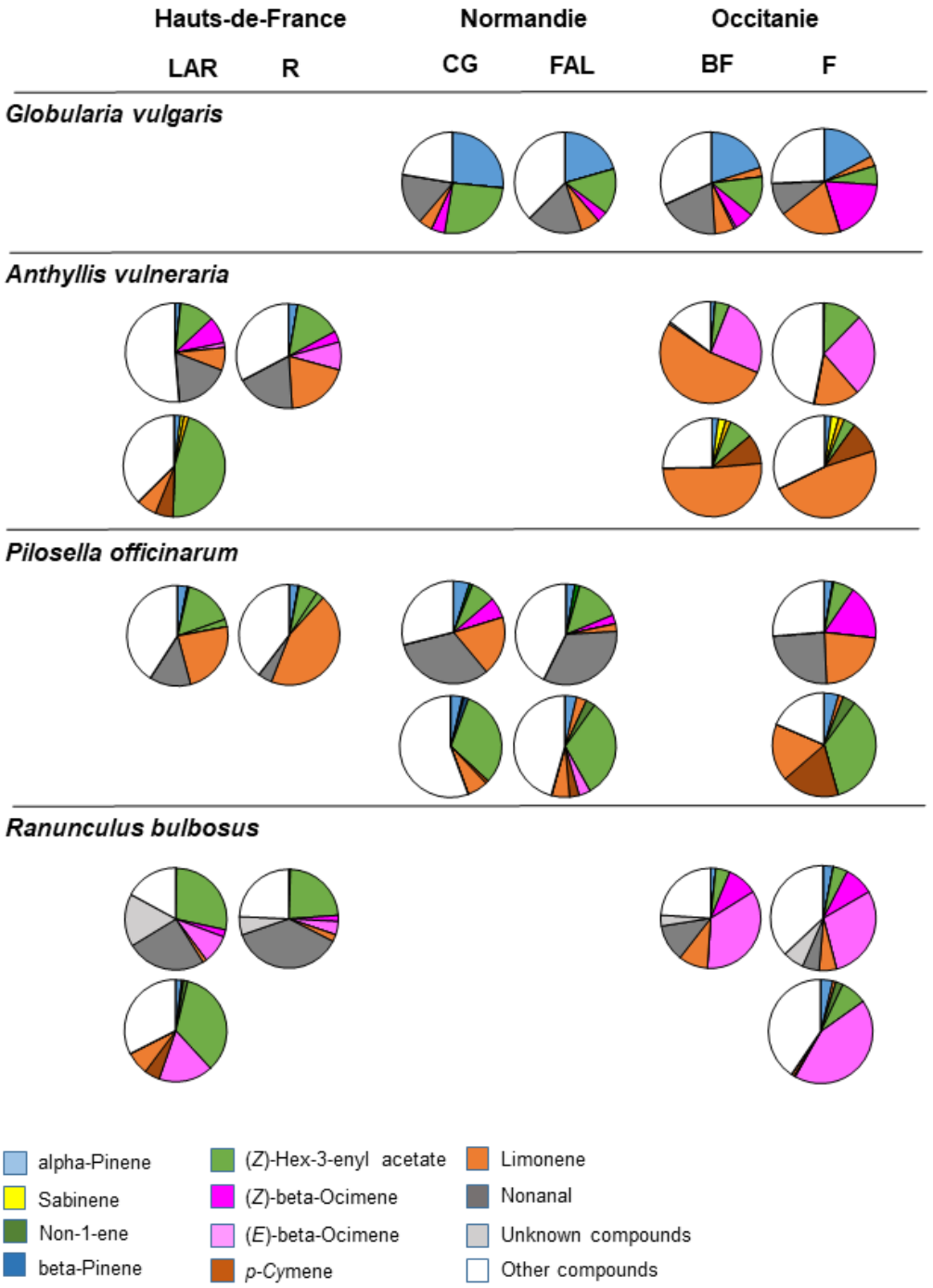

Figure 4. 1. Average proportions of major compounds and compounds involved in significant differences among populations (based on Simper analyses), in the four species. For each species, the first line depicts the floral bouquet analysed in the field, and the second line in greenhouse conditions. Only scents extracted in nature could be analysed in Globularia vulgaris.

Type	Model	Effect	Species	Df	F.Model	R2	Pr(>F)	
(i) Environment	bouquet_freq_normAV ~ Condition + Population	Condition	**AV**	1	29.16	0.39	**0.00**	***
		Population		2	3.55	0.94	**0.00**	**
	bouquet_freq_normPO ~ Condition + Population	Condition	**PO**	1	46.39	0.53	**0.00**	***
		Population		2	3.66	0.84	**0.01**	**
	bouquet_freq_normRB ~ Condition + Population	Condition	**RB**	1	29.76	0.47	**0.00**	***
		Population		1	5.94	0.09	**0.00**	**
(ii) Nature	bouquet_freq_normAV ~ Population	Population	**AV**	3	3.23	0.24	**0.00**	***
	bouquet_freq_normGV ~ Population		GV	3	1.33	0.12	0.16	n.s.
	bouquet_freq_normPO ~ Population		**PO**	4	2.15	0.23	**0.00**	***
	bouquet_freq_normRB ~ Population		**RB**	3	5.92	0.32	**0.00**	***
(iii) Greenhouse	bouquet_freq_normAV ~ Population	Population	AV	2	1.28	0.18	0.24	n.s.
	bouquet_freq_normPO ~ Population		**PO**	2	3.48	0.32	**0.01**	**
	bouquet_freq_normRB ~ Population		**RB**	1	2.66	0.18	**0.03**	*

*Table 4. 1. Results of PERMANOVA analysis on the floral bouquet (bouquet_freq_norm) in the four focal species (AV, Anthyllis vulneraria; GV, Globularia vulgaris; PO, Pilosella officinarum and RB, Ranunculus bulbosus). We performed three types of analyses: for each species we tested (i) if there were differences in the floral bouquet between nature condition vs. greenhouse condition (type environment, effect condition) and between populations that were tested in both conditions (effect population). We then tested the effect of populations (ii) in natural and (iii) in controlled conditions, separately. Significant results are showed in bold (P-value codes: '***'< 0,0001 ; '**' < 0.001 ; '*' < 0.01 and < 0,05; n.s. > 0,05).*

Type	Effect	Species	Population pairs	Df	Pseudo-F	R2	p-value	p-adjusted	
(i) Environment	Condition	**AV**	**nature vs controlled**	1	25.94	0.39	0.00	**0.00**	***
	Population		BF vs F	1	1.50	0.53	0.16	0.49	n.s.
			BF vs LAR	1	2.37	0.08	0.05	0.15	n.s.
			F vs LAR	1	2.32	0.08	0.04	0.12	n.s.
	Condition	**PO**	**nature vs controlled**	1	40.26	0.53	0.00	**0.00**	**
	Population		CG vs F	1	2.37	0.09	0.08	0.24	n.s.
			CG vs FAL	1	1.26	0.05	0.24	0.71	n.s.
			F vs FAL	1	2.84	0.12	0.06	0.19	n.s.
	Condition	**RB**	**nature vs controlled**	1	25.42	0.47	0.00	**0.00**	**
	Population		**F vs LAR**	1	3.22	0.10	0.02	**0.02**	*
(ii) Nature	Population	**AV**	**BF vs F**	1	2.70	0.14	0.00	**0.02**	*
			BF vs LAR	1	4.68	0.23	0.00	**0.01**	*
			BF vs R	1	4.05	0.22	0.00	**0.01**	*
			F vs LAR	1	3.89	0.19	0.00	**0.01**	*
			F vs R	1	2.77	0.16	0.00	**0.01**	*
			LAR vs R	1	1.58	0.10	0.15	0.91	n.s.
		GV	BF vs CG	1	0.62	0.04	0.73	1.00	n.s.
			BF vs F	1	1.36	0.06	0.22	1.00	n.s.
			BF vs FAL	1	1.34	0.08	0.19	1.00	n.s.
			CG vs F	1	1.63	0.12	0.17	1.00	n.s.
			CG vs FAL	1	0.70	0.10	0.68	1.00	n.s.
			F vs FAL	1	2.52	0.17	0.07	1.00	n.s.
		PO	CG vs F	1	1.15	0.09	0.30	1.00	n.s.
			CG vs FAL	1	2.24	0.16	0.03	0.27	n.s.
			CG vs LAR	1	1.27	0.08	0.25	1.00	n.s.
			CG vs R	1	2.14	0.12	0.04	0.35	n.s.
			FAL vs LAR	1	2.39	0.19	0.06	0.55	n.s.

			FAL vs R	1	5.42	0.33	0.00	**0.01**	*
			F vs FAL	1	5.35	0.40	0.01	0.13	n.s.
			F vs LAR	1	1.78	0.15	0.09	0.85	n.s.
			F vs R	1	2.33	0.17	0.01	0.11	n.s.
			LAR vs R	1	1.09	0.08	0.37	1.00	n.s.
		RB	BF vs F	1	2.23	0.10	0.03	0.17	n.s
			BF vs LAR	1	6.53	0.22	0.00	**0.01**	*
			BF vs R	1	6.05	0.21	0.00	**0.01**	*
			F vs LAR	1	7.09	0.32	0.01	**0.03**	*
			F vs R	1	10.77	0.42	0.00	**0.01**	*
			LAR vs R	1	5.91	0.25	0.00	**0.01**	*
(iii) Greenhouse	Population	AV	BF vs F	1	0.92	0.10	0.55	1.00	n.s.
			BF vs LAR	1	1.48	0.16	0.22	0.66	n.s.
			F vs LAR	1	1.31	0.14	0.23	0.70	n.s.
		PO	**CG vs F**	1	5.65	0.34	0.00	**0.01**	**
			CG vs FAL	1	0.99	0.11	0.43	1.00	n.s.
			F vs FAL	1	4.75	0.30	0.01	**0.03**	*
		RB	**F vs LAR**	1	2.66	0.18	0.03	**0.03**	*

*Table 4. 2. Results of pairwise comparisons between populations (BF and F in Occitanie region; CG and FAL in Normandie region; LAR and R in Hauts-de-France region) and in the three conditions following the PERMANOVA analysis (Table 4.1). In the first analysis (i), we tested if there were differences in the floral bouquet between nature condition vs. greenhouse condition (type environment, effect condition) and between populations that were tested in both conditions (type environment, effect population). We then tested the effect of populations (ii) in natural and (iii) in controlled conditions, separately, in the four focal species (AV, Anthyllis vulneraria; GV, Globularia vulgaris; PO, Pilosella officinarum and RB, Ranunculus bulbosus). Significant results are showed in bold (P-value codes: '***'< 0,0001 ; '**' < 0.001 ; '*' < 0.01 and < 0,05; n.s. > 0,05).*

Globularia vulgaris – Floral scents extracted in all natural populations were dominated by alpha-pinene, (*Z*)-hex-3-enyl acetate, Limonene and Nonanal (Fig. 4.1). No significant difference was found among surveyed populations. Because the germination experiment could not produce any flowering individual for this species, we were not able to test whether this result still holds in controlled conditions.

Pilosella officinarum – A significant population effect was detected in natural conditions, but only two populations remained significantly different after p-value adjustment (Table 4.2). Although these two populations belonged to different regions (FAL population in Normandie vs. R population in Hauts-de-France), one could not conclude to a regional effect in scent variation. Limonene was detected in all populations, with the lowest average proportion in FAL and the highest in R population. This difference in Limonene proportion was the major basis of the significant difference between the two populations.

Composition of floral scents significantly differed between nature and greenhouse conditions, involving both quantitative and qualitative variation. (*Z*)-Hex-3-enylacetate, found in all populations in both conditions, was detected at much higher frequency in the greenhouse study. Two additional minor compounds (*p*-cymene and beta-Pinene) were only found in the greenhouse study. As a result, even though we detected a significant population effect in the greenhouse study (Table 4.2), among-population differences were not consistent with the results found in natural conditions. One PO population from the South (F) was found different from the other two populations from Normandie (CG and FAL). As in the natural condition study, the geographical effect was mainly explained by the Limonene proportion, higher on average in the Southern population.

Anthyllis vulneraria - In natural populations, we found a significant population effect, reflecting a regional differentiation of floral scents. All populations belonging to different regions had different floral scents, and the two southern populations were even different from one another (Table 4.2). Most of the between-region variation was explained by the proportion of (*E*)-beta-Ocimene, detected at higher proportions in the South. In addition, the southern population BF was characterized by a high proportion of Limonene compared to the other three populations (Figure 1). The two Northern populations had a similar average chemical profile, dominated by Nonanal and (*Z*)-hex-3-enyl acetate.

The greenhouse study of floral scents in *Anthyllis vulneraria* included only three of the four populations: the two southern populations (BF and F) and only one from the Hauts-de-France

region (LAR). The composition of floral scents was significantly different from scents extracted in nature, the main difference being the complete absence of (*E*)-beta-Ocimene from the greenhouse extracts. Consequently, no significant variation in floral scents was found among populations when plants were grown in greenhouse. One can note, however, that proportions of other major volatile compounds detected in greenhouse were fairly consistent with results obtained in natural conditions, with a dominance of Limonene in southern populations and (*Z*)-Hex-3-enyl acetate in the northern one.

Ranunculus bulbosus – Quite similarly to *A. vulneraria*, we found a strong population effect, consistent with a regional variation, but with additional variation of scent within the Hauts-de-France region. Most of the regional signal was explained by higher frequencies of (*Z*) and (*E*)-beta-Ocimene in the south, whereas scent extracted in the northern populations were dominated by (*Z*)-Hex-3-enyl acetate and Nonanal. The significant difference between the two northern populations was mainly explained by an unidentified compound (Unknown compound 3), which was detected in higher proportions in one of the two populations.

Two out of these four populations (F and LAR), one in each region, were included in the greenhouse study. Although scent composition appeared to vary with the culture condition, the greenhouse and natural condition studies provided fairly consistent results regarding geographical variation. Two of the compounds involved in geographical variation in nature (Nonanal and Unknown compound 3) were not detected in the greenhouse study. In spite of this, because the other compounds globally behaved similarly, the greenhouse study also evinced a difference between the southern and the northern populations based on the same composition difference: higher frequency of (*E*)-beta-Ocimene in the southern population and higher frequencies of (*Z*)-Hex-3-enyl acetate in the northern one.

Floral reflectance

The PERMANOVA analysis of the two *Anthyllis* colours showed that the southern populations (pink-coloured) significantly differed from the northern population (yellow-coloured) in both bee (R^2 = 0.81, *P* = 1e-04) and hoverfly visual perceptions (R^2 = 0.87, *P* = 1e-04). Indeed, in the colour hexagon for bee vision (Fig. S4.4: bee and hoverfly vision), the southern and northern populations were separated, *i.e.* the two stimuli were perceived as differently coloured. However, in the categorical colour for hoverfly vision (Fig. S4.4), the populations were in the same portion, which means that the two colours are not perceived as different stimuli.

Pollinator composition

We sampled a total of 1080 pollinators on the four focal species between 2016 and 2017. The main pollinators were native wild bees (group Anthophila), mostly of the family Halictidae. When considering pollinator composition at the family level, no obvious variation could be detected among populations or regions. In case of GV, few interactions were observed, making comparisons among sites difficult. For the three other species, when the number of observed interactions largely varied among regions, the main pollinator family of a given plant species was always the same over all study sites. Moreover, the proportion of the different pollinator families was quite similar among AV populations of the Occitanie region, and even among regions in case of RB (Fig 4.2).

When investigating pollinator composition at the species level, a stronger variation was detected, with very few pollinator species shared among regions (Supp. Table S5, S6). Over the 48 observed insect species found associated with AV, only 4 were found in both Occitanie and Hauts-de-France. This proportion was as low in the other species (GV: 2 shared species over 31; PO: 15 over 53; RB: 10 over 58).

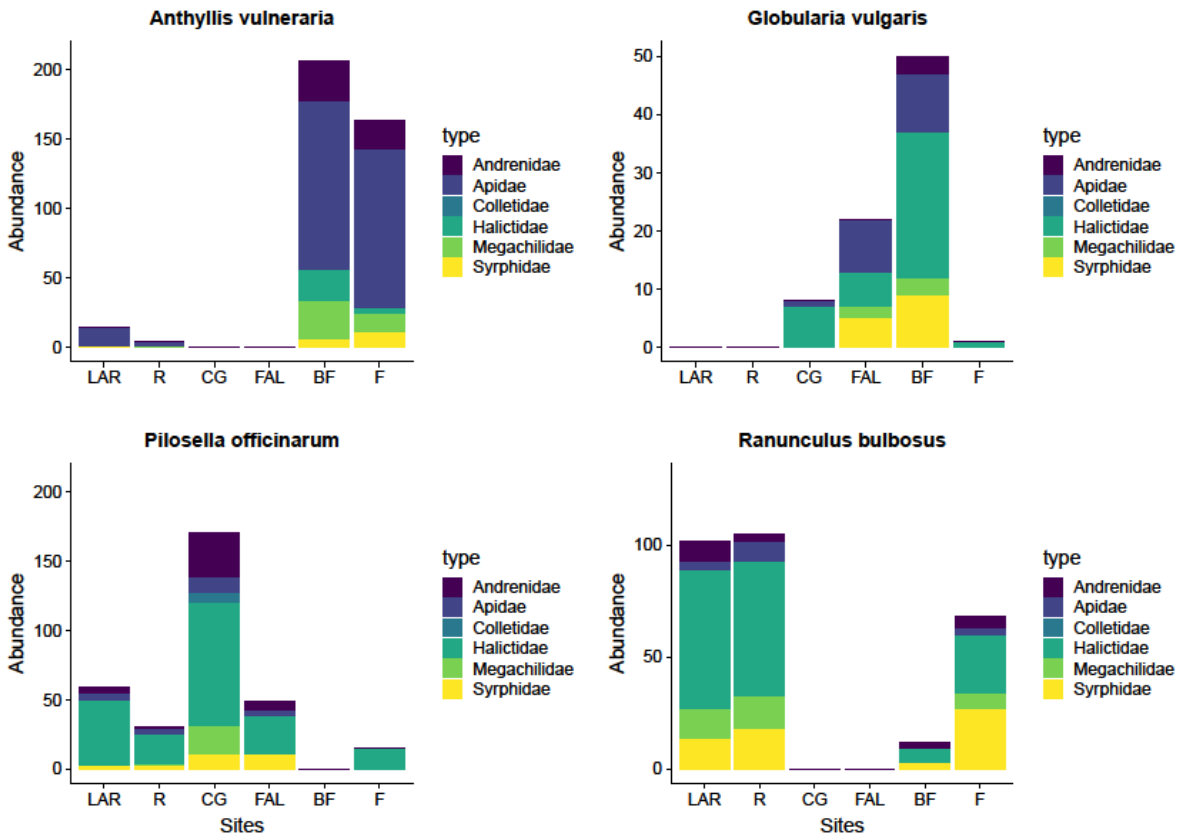

Figure 4. 2. Recorded abundances of insect visitors in the four focal plant species (different panels) and in the different populations (different bars): LAR and R, Hauts-de-France; CG and FAL, Normandie; BF and F, Occitanie. Insect visits varied among plant species and among populations, so the y-axes (abundances) are different among species. Insects are separated in wild bees (5 families: Andrenidae, Apidae, Colletidae, Halictidae and Megachilidae) and hoverflies (Syrphidae family).

Discussion

In this study, we focused on four generalist plant species, widely distributed in different regions and at different latitudes, along an environmental gradient in France. Our results evince among-species and among-population variations of floral scents, as well as differences between scents obtained for the same species and populations *in natura* vs. in common garden. Overall, these results seem to indicate that, out of the four studied species: one displayed no variation in floral scents among populations (GV); two evinced variation *in natura* that was not consistently found in greenhouse (PO, AV), thus suggesting the occurrence of phenotypic plasticity; and one species (RB) showed consistent differences in floral scents in both conditions, thus suggesting the existence of local genetic adaptation. While data on pollinator family abundances found

associated with each plant species indicate a potential for pollinator-induced selection on floral traits, no obvious variation linked to populations or regions could be evidenced.

The variation in floral scents reported here cannot be interpreted in the light of pollination biology only. Indeed, several studies have shown that both generalist and specific pollinators detect and/or are attracted by only a portion of the compounds contained in a given floral bouquet (Dötterl *et al.*, 2005a; Knudsen *et al.*, 2006; Dötterl & Vereecken, 2010; Burger *et al.*, 2013; Milet-Pinheiro *et al.*, 2013).This means that at least some of the observed variation must be either neutral or under selective pressures mediated by agents different from pollinators (such as herbivores, see Schiestl 2010).

The effect of culture condition - In all four species, we observed that scent composition varies with culture condition, involving both qualitative and semi-quantitative variation. Even if our work is not based on a true "reaction-norm" study (*i.e.* we did not use clonal individuals as in Majetic *et al.*, 2009b; Friberg *et al.*, 2013), individuals reared in common garden were likely from the same genetic pool as the plants surveyed in wild populations. With this approach, we found that the effect of culture condition differed among VOCs. Whereas some volatile compounds seem to be specifically emitted in one of the two conditions, such as Nonanal (detected in natural populations only) or Sabinene (only in the greenhouse), most compounds were found in both conditions, sometimes at different proportions. In contrast with studies that explored the impact of one particular abiotic factor on emission rates of several VOCs (Majetic *et al.*, 2009b; Chapurlat *et al.*, 2018), our study exposed plants from the same population to two different conditions. Thereby, we cannot identify the exact factors responsible for the observed variation, and this potentially includes the effect of temperature, soil composition and water availability, which have all been shown to trigger phenotypic plasticity (Majetic *et al.*, 2009b; Farré-Armengol *et al.*, 2014; Chapurlat *et al.*, 2018). Overall, this strengthens the idea that investigating floral scents in controlled conditions only can produce a biased picture of signals perceived by pollinators in the wild.

Geographical variation in floral scents - Even though this study surveyed the variation in pollinator communities among sites and regions, how this should correlate with scent divergence is not entirely clear. Indeed, we only compared pollinator communities "qualitatively" and we did not perform any statistical test which may help to explain the relation between the floral scent and pollinator variations among populations. However, for the four plant species, we observed differences in pollinator communities at the species level, but not

among genera or families. In generalist pollination systems, some variation in the response to VOCs among pollinators was found in several studies, but it always involved insects belonging to different families or orders (Shuttleworth & Johnson, 2010; Larue-Kontic & Junker, 2016). To our knowledge, the only cases of closely related pollinator species found to react differently to floral scents concern specialized pollination systems, like the mutualistic interaction between Ficus species and its wasp pollinators (Soler *et al.*, 2011; Gervasi *et al.*, 2017; Segar *et al.*, 2018; Souto-Vilarós *et al.*, 2018).

We observed that floral scent variation was species-dependent, and we observed different situations when we compared populations in natural and controlled conditions. In the case of *Globularia vulgaris* we did not detect any regional difference in the floral scent compositions. This suggests that local adaptation to different pollinator communities is not necessarily expected, possibly because different pollinator species, when they belong to the same taxonomic groups, may react to the same signal. In *Pilosella officinarum*, no clear geographical trend was found. Among the five populations considered, only two were significantly different from each other (FAL, Normandie and R, Hauts-de-France). Moreover, differences found between wild populations were not confirmed by the results in controlled conditions – hence suggesting the occurrence of phenotypic plasticity –, but this comparison was made difficult because one of the two populations did not flower in the greenhouse. Finally, we found a significant difference between other populations reared in the common environment – suggesting genetic adaptation –, which was not observed in the field. This suggests that floral scent variation in this species may be based on a mix both phenotypic plasticity and genetic differentiation, but without any clear linked with pollinator variation. This results seems similar to the results of Dötterl *et al.* (2005b) who found low consistency between pollination biology and floral scents in *Silene latifolia*, with no clear difference in scents between two groups of populations pollinated by different insect taxa, and a strong variation among population of the same region.

The other two species showed strong patterns of regional variation in floral scents. In *Ranunculus bulbosus*, the consistency between the common garden study and the study *in natura* suggests a genetic component of the floral scent variation among populations. However, no strong variation in the groups of pollinators (mostly wild bees of the family Halictidae) was found in the wild, even though we observed different insect species. This could mean that selection mediated by closely related (generalist) pollinator species can lead to genetically

determined scent divergence. Alternatively, other mechanisms, such as genetic drift or selection mediated by natural enemies, may also be involved in this variation.

Finally, in *A. vulneraria*, floral scents were found to vary among regions, but this difference was statistically significant only when scents were extracted in natural populations. The inconsistency between *in situ* and greenhouse studies can be explained by two facts: (i) the lack of production in the greenhouse of one of the major compound found in the southern natural population ((*E*)-beta-Ocimene) and (ii) the higher emission of a stress compound, the (*E*)-Hex-3-enyl-acetate, in the northern population which was highly variable among individuals (ranging between 0 and 92% of the total floral bouquet). However, other major volatile compounds detected in greenhouse were consistent with the study *in natura*, thus suggesting a blend of genetic adaptation and phenotypic plasticity. In *A. vulneraria*, genetically based scent differentiation could have been expected, mostly because of the clear colour dimorphism between northern and southern populations. First, differences in the pollinator communities between the two regions were probably more marked in this species: southern populations were mostly visited by several species of genus *Eucera* (family Apidae) which are not present in the northern sites, while in the northern populations the most observed visitors where bumblebees (genus *Bombus*, Family Apoidea). Second, plants from the two study regions also showed differences in corolla pigmentation. Over its range of distribution *A. vulneraria* is known to present colour dimorphism, with yellow colour usually in moister conditions and red morphs in drier habitats (Puidet *et al.*, 2005). We were able to verify that this trait variation did not result from environmental plasticity, since it was observed in the wild and then quantified in the greenhouse study (Fig.S4.2 and S4.3). Volatile compounds and pigments involved in floral colour are key factors to attract pollinator species and often follow the same chemical pathways in the flower development (Delle-Vedove *et al.*, 2017). However, studies that investigated co-variation of floral colour and scents have found mixed results to date (Majetic *et al.*, 2008; Delle-Vedove *et al.*, 2011; Dormont *et al.*, 2014; Kantsa *et al.*, 2017). In our case, it seems that at least a part of floral scent variation is not genetically based and may not be entirely correlated with colour variation.

To conclude, our results clearly show that some variation of both qualitative and semi-quantitative scent emission, produced in natural and greenhouse-reared populations, is environmentally induced. Variation in floral scent composition and emission is species-specific, *i.e.* each species had its own context-dependent scent profile. On the four studied species, only *Ranunculus bulbosus* showed significant differences in the floral scent emissions

which were congruent in both conditions, suggesting local genetic adaptation. For the other species, the variation observed *in natura* were not consistently found in controlled condition, due to the emission of different floral scents induce by the different environment, suggesting the occurrence of phenotypic plasticity. The evidence so far does not seem to pinpoint any clear pollinator-mediated selection insofar as pollinator family abundances were very similar between sites in spite of the differences in floral scents. Overall, our results highlight the importance of comparing the floral bouquets produced in different conditions to clarify the origins of the variations and to have a better representation of the ecological situations.

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Supplementary Information

Geographical variation of floral scent in four calcareous grassland species with generalist pollination: genetic adaptation vs. phenotypic plasticity.

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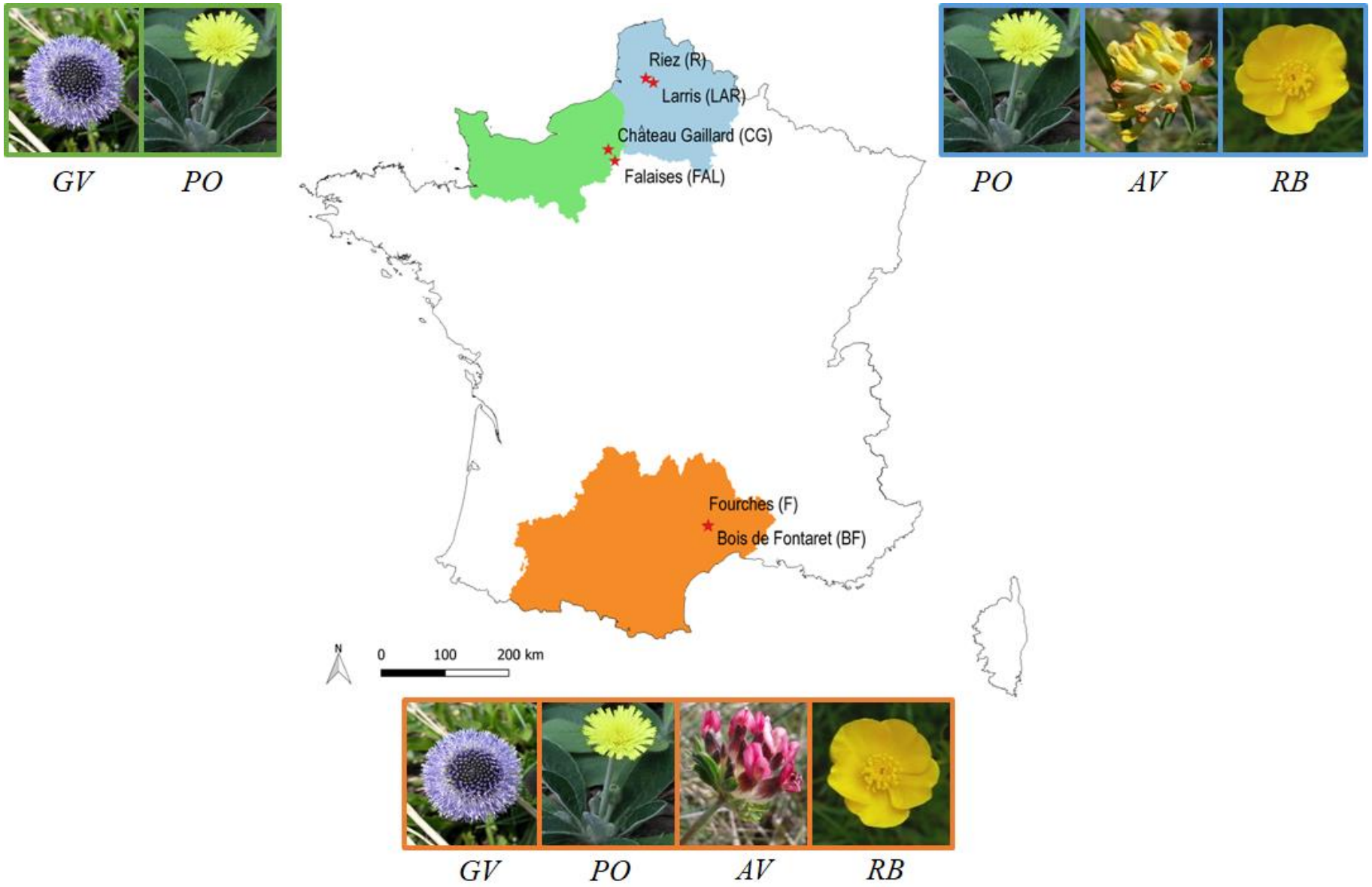
Supplementary figures

Figure S4. 1. Sites and regions location and species occurrence. In blue the French region Hauts-de-France, in green the region Normandie, in orange the region Occitanie. The six sites correspond to the red dots. Globularia vulgaris (GV) is present in the Occitanie and in the Normandie regions; Ranunculus bulbosus (RB) and Anthyllis vulneraria (AV) are present in Occitanie and Hauts-de-France regions; and Pilosella officinorum (PO) is present in the three regions. A. vulneraria shows colour dimorphism between the two regions with the pink colour in Occitanie and yellow colour in the Hauts-de-France.


GV
PO
Riez (R)
Larris (LAR)
Château Gaillard (CG)
Falaises (FAL)
PO
AV
RB
Fourches (F)
Bois de Fontaret (BF)
N
0
100
200 km
GV
PO
AV
RB

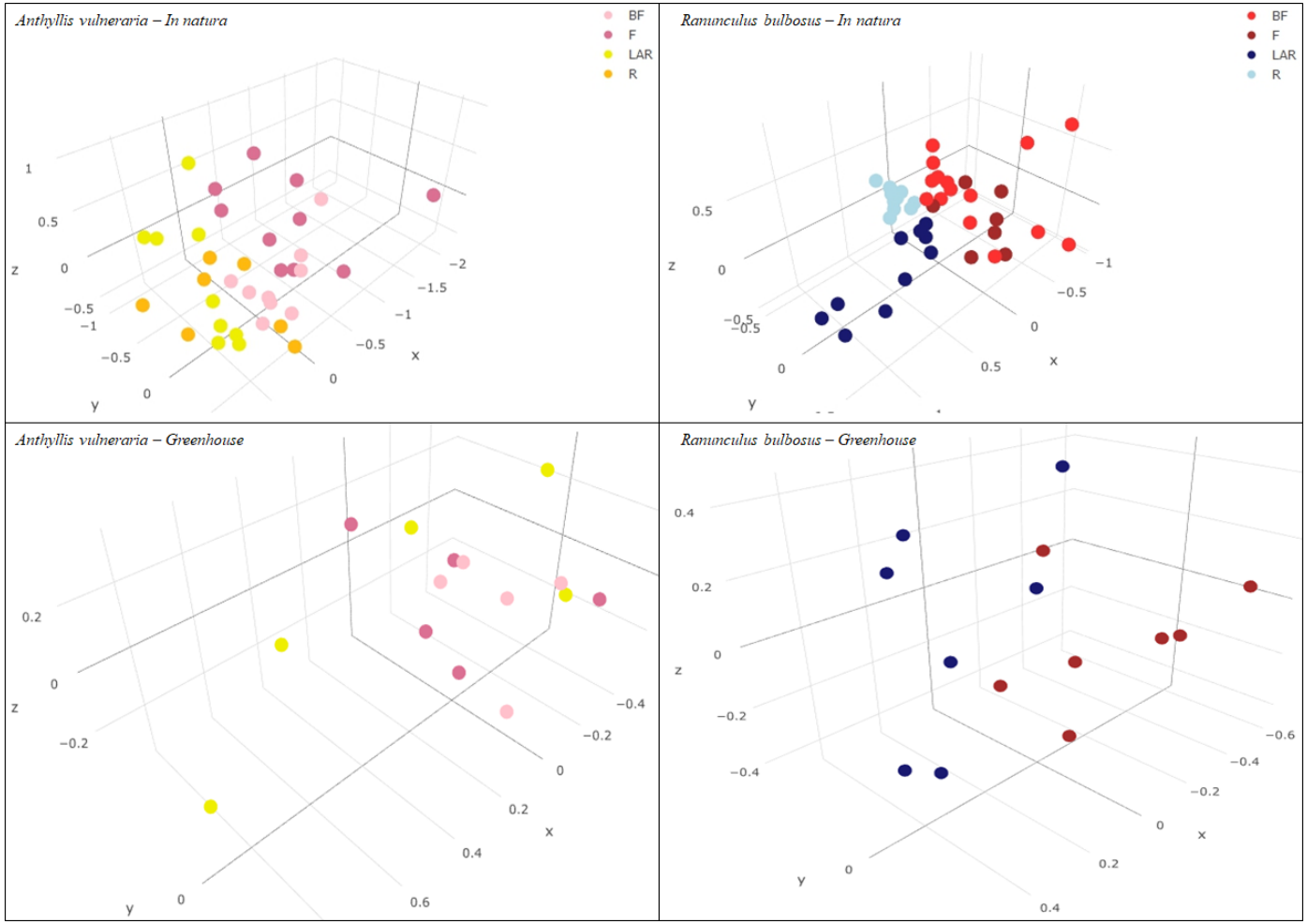


Figure S4. 2. Both similarities and dissimilarities are visually assessed on a non-metric multidimensional scaling using a 3D plot, both in natural (upper plot) and controlled conditions for Anthyllis vulneraria (on the left) and Ranunculus bulbosus species (on the right). In natural condition, both AV and RB showed differences among populations in different region, but only the two population of R. bulbosus tested in the greenhouse are significatively different.

Figure S4. 3. Anthyllis vulneraria populations showed colour dimorphism between regions both in natural and controlled conditions. Southern populations have dark pink / white flowers in natura and pale pink / white flowers when individual are reared in the greenhouse. Norther populations showed yellow colour both in natural and controlled conditions.

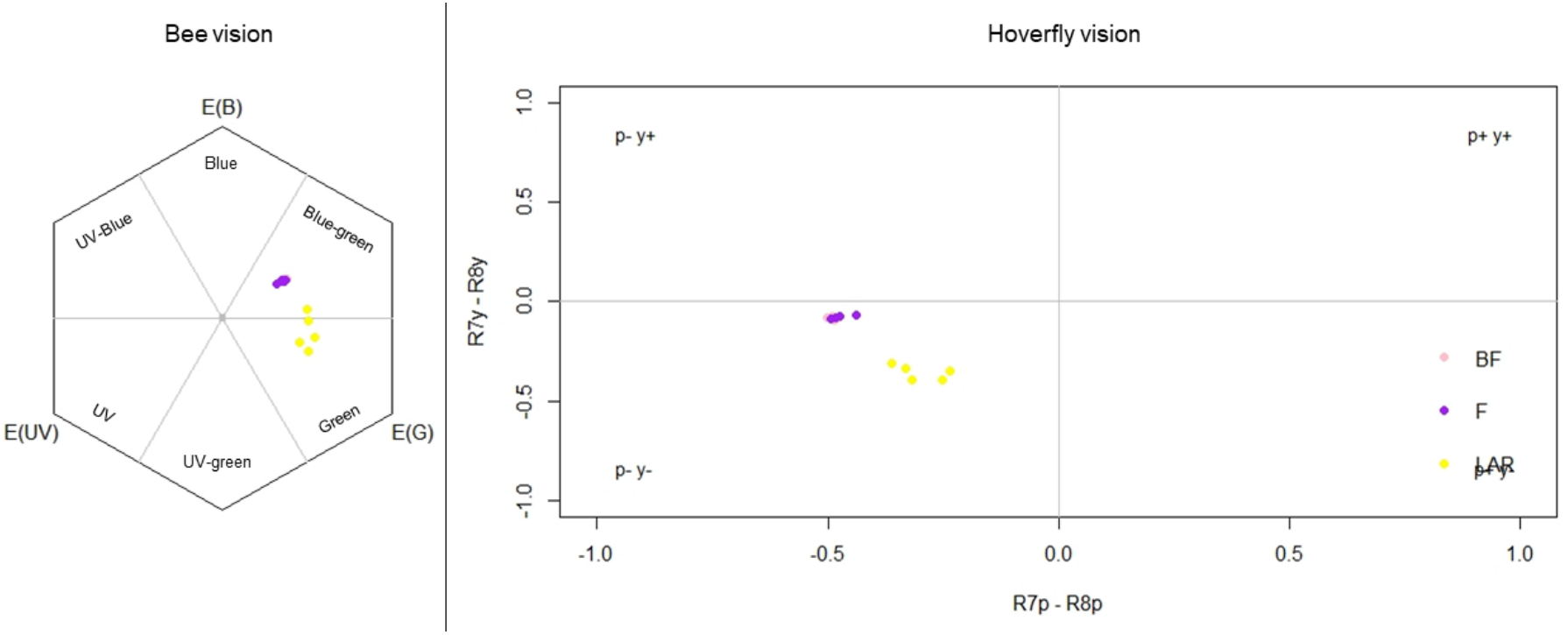


Figure S4. 4. Bee and hoverfly vision of the two-colour dimorphism in Anthyllis vulneraria populations. The southern populations, BF (pink) and F (purple), have pink/white flowers and the northern population, LAR (yellow points), have yellow flowers. Southern and northern populations are separated in the bee-vision hexagon (on the left), which means that the two stimuli are perceived as differently coloured while they reflect different wavelengths. In the categorical colour for hoverfly vision (rectangle, on the right) the populations are in the same portion, which means that the two colours are not perceived as different stimuli.

Supplementary tables

Family	Species	Symmetry	Inflorescence type	Colour	Phenology	
Plantaginaceae	*Globularia vulgaris (GV)*	zygomorphic	capitular	purple	April - June	perennial
Ranunculaceae	*Ranunculus bulbosus (RB)*	actinomorphic	single flowers	yellow	April - June	perennial
Fabaceae	*Anthyllis vulneraria (AV)*	zygomorphic	capitular	yellow / pink / white	May - July	perennial
Asteraceae	*Pilosella officinarum (PO)*	actinomorphic	capitular	yellow	April - October	perennial

Table S4. 1. Information about the floral morphology and phenology of the four focal species.

Condition	Species	Region	Sites	session	Temperature (°C)	Humidity	Nb of individuals
Nature 2017	*Anthyllis vulneraria*	Occitanie	BF	May-June	14°-28°	37-58%	9
		Hauts-de-France	F	May-June	14°-26°	47-98%	10
			LAR	May-June	22°-29°	38-60%	9
			R	May-June	22°-29°	52-57%	7
	Globularia vulgaris	Occitanie	BF	April-May	13°-20°	47-63%	14
		Normandie	F	April-May	12°-16°	35-54%	10
			CG	April-May	14°-15°	40-72%	4
			FAL	April-May	16°-18°	39-51%	4
	Pilosella officinarum	Occitanie	BF	-	-	-	-
		Normandie	F	May	19-20°	49-51%	5
			CG	May-June	14°-17°	61-69%	9
			FAL	May	23°-24°	66-69%	5

		Hauts-de-France	LAR	May-June	21°-29°	32-62%	7
			R	May-June	19°-25°	58-67%	8
	Ranunculus bulbosus	Occitanie	BF	May	19°-28°	38-49%	15
		Hauts-de-France	F	May	13°-14°	45-56%	7
			LAR	April-May	10°-22°	44-84%	10
			R	April-May	16°-20°	49-57%	10
Greenhouse 2018	*Anthyllis vulneraria*	Occitanie	BF	May	27°-29°	44-54%	5
		Hauts-de-France	F	May	27°-29°	44-54%	5
			LAR	May	27°-29°	44-54%	5
			R	-	-	-	-
	Globularia vulgaris	Occitanie	BF	-	-	-	-
		Normandie	F	-	-	-	-
			CG	-	-	-	-
			FAL	-	-	-	-
	Pilosella officinarum	Occitanie	BF	-	-	-	-
		Normandie	F	April	27°-29°	44-48%	8
			CG	April	27°-29°	44-48%	5
			FAL	April	27°-29°	44-48%	5
		Hauts-de-France	LAR	-	-	-	-
			R	-	-	-	-
	Ranunculus bulbosus	Occitanie	BF	-	-	-	-
		Hauts-de-France	F	April	25°-28°	40-64%	7
			LAR	April	25°-28°	40-64%	7
			-	-	-	-	-

Table S4. 2. This table are reported information about the environmental conditions recorded during the floral scent extraction in the two tested conditions (in natura and greenhouse). We reported for each species and each population the period of extraction (monthly session), the minimum and maximum temperatures and humidity recorded during the sessions with the Tinytag station and the number of individuals used for the statistical analyses.

Condition	Family	*VOCs*
Nature	Fatty acid derivative	*(E)-Hex-3-enol*
	Fatty acid derivative	***(Z)-Hex-3-enyl acetate***
	Fatty acid derivative	***6-methyl-5-Hepten-2-one***
	Fatty acid derivative	*Cyclohexen-1-yl ethanone*
	Fatty acid derivative	*Non-1-ene*
	Fatty acid derivative	*Nonanal*
	Monoterpenic compounds	***(E)-beta-Ocimene***
	Monoterpenic compounds	*(Z)-beta-Ocimene*
	Monoterpenic compounds	*alpha-Phellandrene*
	Monoterpenic compounds	***alpha-Pinene***
	Monoterpenic compounds	*alpha-Terpenyl acetate*
	Monoterpenic compounds	***Geranylacetone***
	Monoterpenic compounds	***Limonene***
	Monoterpenic compounds	*Nerylacetone*
	Sesquiterpenic compounds	*(E)-beta-Farnesene*
	Sesquiterpenic compounds	***(E)-Caryophyllene***
	Sesquiterpenic compounds	*alpha-Copaene*
	Sesquiterpenic compounds	*Unknown Sesquiterpene*
	Unknown_compounds	*Unknown compound 1*
	Unknown_compounds	*Unknown compound 2*
	Unknown_compounds	*Unknown compound 3*
	Unknown_compounds	*Unknown compound 4*
	Unknown_compounds	*Unknown compound 5*
Greenhouse	Fatty acid derivative	***(Z)-Hex-3-enyl acetate***
	Fatty acid derivative	***6-methyl-5-Hepten-2-one***
	Fatty acid derivative	*Dodecan-1-ol*
	Fatty acid derivative	*Hexadec-1-ene*
	Fatty acid derivative	*Methyl dodecanoate*
	Fatty acid derivative	*Pentadecane*
	Fatty acid derivative	*Tetradec-1-ene*
	Fatty acid derivative	*Tetradecan-1-ol*
	Fatty acid derivative	*Tetradecanal*
	Homoterpenic compound	*(E,E)-4,8-dimethylNona-1,3,7-triene*
	Monoterpenic compounds	***(E)-beta-Ocimene***
	Monoterpenic compounds	*(E,E)-2,6-dimethylOcta-1,3,5,7-tetraene*
	Monoterpenic compounds	***alpha-Pinene***
	Monoterpenic compounds	*beta-Pinene*
	Monoterpenic compounds	***Geranylacetone***
	Monoterpenic compounds	***Limonene***
	Monoterpenic compounds	*Myrcene*
	Monoterpenic compounds	*p-Cymene*
	Monoterpenic compounds	*Sabinene*
	Sesquiterpenic compounds	***(E)-Caryophyllene***
	Sesquiterpenic compounds	*(E,E)-alpha-Farnesene*
	Unknown_compounds	*Unknown_compound*

Table S4. 3. List of Volatile Organic Compounds (VOCs) identified the natural and controlled conditions. VOCs underlined and in bold are emitted in the two environments.

Anthyllis vulneraria (AV)		**In natura**				**Greenhouse**		
		Occitanie		Hauts-de-France		Occitanie		Hauts-de-France
Family	*VOCs*	BF	F	LAR	R	BF	F	LAR
Fatty acid derivatives	*(E)-Hex-3-enol*	0 ± 0	15.14 ± 31.04	0 ± 0	0 ± 0	-	-	-
Fatty acid derivatives	*(Z)-Hex-3-enyl acetate*	4.56 ± 6.42	12.05 ± 12.96	11.33 ± 9.42	14.41 ± 15.13	7.74 ± 7.16	3.72 ± 6.5	45.89 ± 42.5
Fatty acid derivatives	*6-methyl-5-Hepten-2-one*	1.51 ± 4.54	11.11 ± 31.06	1.87 ± 3.72	2.63 ± 6.95	0.14 ± 0.31	0.2 ± 0.31	0.07 ± 0.14
Fatty acid derivatives	*Cyclohexen-1-yl ethanone*	0.29 ± 0.78	0.1 ± 0.31	0.57 ± 0.75	0 ± 0	-	-	-
Fatty acid derivatives	*Non-1-ene*	0.13 ± 0.17	0.09 ± 0.14	0.56 ± 0.58	0.5 ± 0.55	-	-	-
Fatty acid derivatives	*Nonanal*	0.75 ± 1.28	0.1 ± 0.32	17.87 ± 24.03	18.27 ± 21.71	-	-	-
Monoterpenic compound	*(E)-beta-Ocimene*	25.48 ± 18.15	26.35 ± 18.32	1.61 ± 4.83	8.66 ± 18.49	0 ± 0	3.84 ± 7.83	0.31 ± 0.7
Monoterpenic compound	*(Z)-beta-Ocimene*	0 ± 0	1.32 ± 3.89	8.75 ± 9.05	3.46 ± 6.85	-	-	-
Monoterpenic compound	*alpha-Phellandrene*	0 ± 0	0 ± 0	0 ± 0	0.85 ± 2.26	-	-	-
Monoterpenic compound	*alpha-Pinene*	1.4 ± 2.39	0.23 ± 0.48	1.71 ± 2.68	2.75 ± 4.71	1.94 ± 0.76	2.01 ± 0.78	1.88 ± 1.7
Monoterpenic compound	*alpha-Terpenyl acetate*	0.33 ± 0.35	0.74 ± 1.34	0.57 ± 0.92	0.13 ± 0.1	-	-	-
Monoterpenic compound	*Geranylacetone*	1.31 ± 1.18	0.25 ± 0.71	3.33 ± 2.29	3.7 ± 7.12	0.26 ± 0.42	0.04 ± 0.08	0.23 ± 0.39
Monoterpenic compound	*Limonene*	53.14 ± 26.57	14.38 ± 21.17	7.45 ± 10.6	19.72 ± 33.69	51.1 ± 12.74	47.83 ± 21.97	6.41 ± 10.97
Monoterpenic compound	*Nerylacetone*	0.03 ± 0.07	1.34 ± 2.79	1.2 ± 1.93	2.41 ± 2.48	-	-	-
Sesquiterpenic compounds	*(E)-beta-Farnesene*	2.13 ± 4.12	0.53 ± 1.67	4.91 ± 4.92	7.95 ± 21.04	-	-	-
Sesquiterpenic compounds	*(E)-Caryophyllene*	1.17 ± 2.21	4.1 ± 9.27	4.93 ± 5.91	0.03 ± 0.09	1.94 ± 1.35	6.29 ± 7.07	0.79 ± 1.59
Sesquiterpenic compounds	*alpha-Copaene*	2.11 ± 4.46	6.08 ± 8.18	9.75 ± 26.68	2.34 ± 4.54	-	-	-
Sesquiterpenic compounds	*Unknown Sesquiterpene*	0.78 ± 0.9	1.2 ± 2.01	5.67 ± 4.58	4.2 ± 8.36	-	-	-
Unknown compounds	*Unknown compound 1*	3.03 ± 7.05	0 ± 0	2.69 ± 4.53	0.52 ± 0.83	-	-	-
Unknown compounds	*Unknown compound 2*	0.21 ± 0.35	0 ± 0	0.72 ± 1.18	0.66 ± 0.89	-	-	-
Unknown compounds	*Unknown compound 3*	0.38 ± 0.85	2.33 ± 7.28	12.09 ± 24.94	4.08 ± 5.81	-	-	-
Unknown compounds	*Unknown compound 4*	0.63 ± 0.8	0.27 ± 0.53	2.26 ± 2.22	2.05 ± 1.89	-	-	-
Unknown compounds	*Unknown compound 5*	0.62 ± 0.8	2.28 ± 4.4	0.15 ± 0.24	0.66 ± 0.98	-	-	-
Fatty acid derivative	*Dodecan-1-ol*	-	-	-	-	2.15 ± 1.98	1.13 ± 0.83	3.53 ± 4
Fatty acid derivative	*Hexadec-1-ene*	-	-	-	-	3.07 ± 2.15	3.88 ± 3.44	6.31 ± 7.11
Fatty acid derivative	*Methyl dodecanoate*	-	-	-	-	0.04 ± 0.09	0 ± 0	0 ± 0
Fatty acid derivative	*Pentadecane*	-	-	-	-	0.3 ± 0.3	0.36 ± 0.22	2.92 ± 5.61
Fatty acid derivative	*Tetradec-1-ene*	-	-	-	-	0.22 ± 0.2	0.1 ± 0.22	0.03 ± 0.07
Fatty acid derivative	*Tetradecan-1-ol*	-	-	-	-	2.36 ± 1.96	0.63 ± 0.73	4.89 ± 6.51
Fatty acid derivative	*Tetradecanal*	-	-	-	-	0.91 ± 0.72	0.73 ± 0.55	1.43 ± 1.59
Homoterpenic compound	*(E.E)-4.8-dimethylNona-1.3.7-triene*	-	-	-	-	0.78 ± 1.18	2.11 ± 1.5	1.2 ± 1.72
Monoterpenic compound	*(E.E)-2.6-dimethylOcta-1.3.5.7-tetraene*	-	-	-	-	-	-	-
Monoterpenic compound	*beta-Pinene*	-	-	-	-	1.61 ± 0.71	1.75 ± 1.11	1.52 ± 1.43
Monoterpenic compound	*Myrcene*	-	-	-	-	12.05 ± 7.08	12.39 ± 7.2	8.76 ± 11.92
Monoterpenic compound	*p-Cymene*	-	-	-	-	9.79 ± 6.14	10.07 ± 10.42	5.69 ± 4.68
Monoterpenic compound	*Sabinene*	-	-	-	-	2.59 ± 0.26	2.43 ± 1.14	1.17 ± 0.95
Sesquiterpenic compound	*(E.E)-alpha-Farnesene*	-	-	-	-	0 ± 0	0 ± 0	0.06 ± 0.13
Unknown_compound	*Unknown_compound*	-	-	-	-	1.05 ± 0.94	0.48 ± 0.46	6.91 ± 14.15

Pilosella officinorum (PO)		***In natura***					**Greenhouse**		
		Normandie		Hauts-de-France		Occitanie	Normandie		Occitanie
Family	*VOCs*	CG	FAL	LAR	R	F	CG	FAL	F
Fatty acid derivatives	*(E)-Hex-3-enol*	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	-	-	-
Fatty acid derivatives	*(Z)-Hex-3-enyl acetate*	7.69 ± 10.83	14.34 ± 1.88	15.93 ± 19.43	6.19 ± 7.5	6.55 ± 8.05	31.29 ± 22.66	32.02 ± 17.42	35.62 ± 7.31
Fatty acid derivatives	*6-methyl-5-Hepten-2-one*	0 ± 0	7.78 ± 7.45	0 ± 0	0 ± 0	3.01 ± 6.73	4.18 ± 6.68	5.69 ± 5.51	1.15 ± 3.24
Fatty acid derivatives	*Cyclohexen-1-yl ethanone*	0.59 ± 0.76	1.57 ± 1.35	0 ± 0	0.14 ± 0.26	0.29 ± 0.44	-	-	-
Fatty acid derivatives	*Non-1-ene*	0.99 ± 0.52	1.57 ± 0.1	0.57 ± 0.83	0.26 ± 0.35	0.33 ± 0.46	-	-	-
Fatty acid derivatives	*Nonanal*	32.11 ± 26.13	33.29 ± 12.59	13.24 ± 15.57	4.47 ± 8.16	24.42 ± 8.35	-	-	-
Monoterpenic compound	*(E)-beta-Ocimene*	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	3.67 ± 8.2	0 ± 0
Monoterpenic compound	*(Z)-beta-Ocimene*	6.49 ± 5.51	2.92 ± 1	2.44 ± 4.34	2.51 ± 5.28	17.25 ± 9.66	-	-	-
Monoterpenic compound	*alpha-Phellandrene*	1.18 ± 2.57	0 ± 0	3.51 ± 6	1.73 ± 2.39	0 ± 0	-	-	-
Monoterpenic compound	*alpha-Pinene*	5.06 ± 6.27	2.75 ± 1.89	3.12 ± 3.92	2.82 ± 2.78	2.6 ± 5.81	3.67 ± 1.72	3.62 ± 4.66	4.52 ± 1.77
Monoterpenic compound	*alpha-Terpenyl acetate*	0.47 ± 0.35	0.12 ± 0.12	0.04 ± 0.06	7.6 ± 20.62	0.76 ± 1.58	-	-	-
Monoterpenic compound	*Geranylacetone*	6.32 ± 7.71	3.14 ± 4.02	2.81 ± 3.81	4.03 ± 6.77	11.86 ± 16.61	4.14 ± 6.52	4.78 ± 3.42	0.03 ± 0.07
Monoterpenic compound	*Limonene*	18.71 ± 21.12	2.47 ± 1.66	23.79 ± 31.13	44.13 ± 34.18	22.59 ± 11	6.5 ± 8.91	5.69 ± 12.71	17.84 ± 8.3
Monoterpenic compound	*Nerylacetone*	0.54 ± 0.56	0.63 ± 0.46	0.34 ± 0.44	0.35 ± 0.56	1.03 ± 1.07	-	-	-
Sesquiterpenic compounds	*(E)-beta-Farnesene*	3.07 ± 6.21	13.05 ± 8.36	8.97 ± 12.86	3.34 ± 7.8	0 ± 0	-	-	-
Sesquiterpenic compounds	*(E)-Caryophyllene*	1.67 ± 1.95	0.79 ± 1.11	7.14 ± 13.55	2.83 ± 5.58	1.31 ± 1.15	-	-	-
Sesquiterpenic compounds	*alpha-Copaene*	4.99 ± 10.28	4.58 ± 3.89	5.96 ± 9.81	0.76 ± 2.16	0.47 ± 1.06	-	-	-
Sesquiterpenic compounds	*Unknown Sesquiterpene*	3.3 ± 6.12	5.12 ± 5.25	3.86 ± 4.83	7.08 ± 9.23	0 ± 0	-	-	-
Unknown compounds	*Unknown compound 1*	1.71 ± 1.69	0.64 ± 1.15	4.91 ± 7.36	3.67 ± 1.32	1.78 ± 1.37	-	-	-
Unknown compounds	*Unknown compound 2*	0.99 ± 0.58	0.43 ± 0.26	1.31 ± 1.58	1.18 ± 0.75	0.99 ± 1.67	-	-	-
Unknown compounds	*Unknown compound 3*	2.68 ± 3.85	4.03 ± 4.59	0.8 ± 1.91	2.35 ± 3.38	3.25 ± 6.91	-	-	-
Unknown compounds	*Unknown compound 4*	1.17 ± 1.7	0.67 ± 0.64	0.83 ± 1.36	1.24 ± 0.79	1.19 ± 0.83	-	-	-
Unknown compounds	*Unknown compound 5*	0.26 ± 0.49	0.12 ± 0.21	0.42 ± 0.58	3.33 ± 7.22	0.31 ± 0.25	-	-	-
Fatty acid derivative	*Dodecan-1-ol*	-	-	-	-	-	2.31 ± 3.25	9.2 ± 8.68	1.51 ± 2.96
Fatty acid derivative	*Hexadec-1-ene*	-	-	-	-	-	14.6 ± 15.85	0 ± 0	5.59 ± 7.78
Fatty acid derivative	*Methyl dodecanoate*	-	-	-	-	-	0 ± 0	0.82 ± 1.83	0.06 ± 0.1
Fatty acid derivative	*Pentadecane*	-	-	-	-	-	5.86 ± 5.17	2.23 ± 2.12	1.35 ± 1.72
Fatty acid derivative	*Tetradec-1-ene*	-	-	-	-	-	7.44 ± 5.93	2.39 ± 1.85	2.07 ± 3.23
Fatty acid derivative	*Tetradecan-1-ol*	-	-	-	-	-	6.09 ± 7.02	10.57 ± 11.82	5.31 ± 4.84
Fatty acid derivative	*Tetradecanal*	-	-	-	-	-	2.61 ± 2.28	0.89 ± 1.29	1.33 ± 1.93
Homoterpenic compound	*(E.E)-4.8-dimethylNona-1.3.7-triene*	-	-	-	-	-	0.47 ± 1.06	1.74 ± 2.45	0 ± 0
Monoterpenic compound	*(E.E)-2.6-dimethylOcta-1.3.5.7-tetraene*	-	-	-	-	-	-	-	-
Monoterpenic compound	*beta-Pinene*	-	-	-	-	-	1.48 ± 0.66	2.95 ± 3.82	3.97 ± 1.35
Monoterpenic compound	*Myrcene*	-	-	-	-	-	-	-	-
Monoterpenic compound	*p-Cymene*	-	-	-	-	-	0.99 ± 2.21	3.08 ± 5.96	17.95 ± 5.44
Monoterpenic compound	*Sabinene*	-	-	-	-	-	0.48 ± 1.07	3.3 ± 4.59	1.55 ± 0.84
Sesquiterpenic compound	*(E.E)-alpha-Farnesene*	-	-	-	-	-	-	-	-
Unknown_compound	*Unknown_compound*	-	-	-	-	-	7.91 ± 6.44	7.36 ± 9.08	0.15 ± 0.35

Ranunculus bulbosus (RB)		**In natura**				**Greenhouse**	
		Occitanie		Hauts-de-France		Occitanie	Hauts-de-France
Family	*VOCs*	BF	F	LAR	R	F	LAR
Fatty acid derivatives	*(E)-Hex-3-enol*	0 ± 0	0 ± 0	0.42 ± 0.87	0 ± 0	-	-
Fatty acid derivatives	*(Z)-Hex-3-enyl acetate*	4.64 ± 7.17	4.46 ± 6.16	28.21 ± 18	23.36 ± 10.32	8.2 ± 12.17	34.39 ± 23.1
Fatty acid derivatives	*6-methyl-5-Hepten-2-one*	5.76 ± 7.18	0 ± 0	1.49 ± 3.1	3.13 ± 5.38	1.24 ± 1.86	1.22 ± 1.28
Fatty acid derivatives	*Cyclohexen-1-yl ethanone*	0.52 ± 0.58	0.32 ± 0.57	0.5 ± 0.64	1.05 ± 0.57	-	-
Fatty acid derivatives	*Non-1-ene*	0.63 ± 0.58	1.34 ± 3	0.4 ± 0.53	1.53 ± 0.76	-	-
Fatty acid derivatives	*Nonanal*	11.93 ± 8.66	5.2 ± 8.95	25.21 ± 7.45	37.11 ± 10.06	-	-
Monoterpenic compound	*(E)-beta-Ocimene*	35.01 ± 18.78	29.34 ± 16.54	9.12 ± 10.01	4.34 ± 4.02	43.06 ± 21.04	17.17 ± 13.88
Monoterpenic compound	*(Z)-beta-Ocimene*	9.84 ± 6.66	9.11 ± 4.63	2.26 ± 2.05	2.01 ± 1.7	-	-
Monoterpenic compound	*alpha-Phellandrene*	1.56 ± 4.88	0 ± 0	0 ± 0	0 ± 0	-	-
Monoterpenic compound	*alpha-Pinene*	1.61 ± 2.31	3.05 ± 3.41	0.21 ± 0.67	0.53 ± 0.86	3.7 ± 4.58	1.83 ± 1.99
Monoterpenic compound	*alpha-Terpenyl acetate*	0.2 ± 0.33	0.57 ± 0.5	0.24 ± 0.45	0.17 ± 0.36	-	-
Monoterpenic compound	*Geranylacetone*	5.9 ± 5.08	10.98 ± 8.84	1.83 ± 2.18	7.38 ± 3.25	1.26 ± 1.02	0.83 ± 0.62
Monoterpenic compound	*Limonene*	9.45 ± 15.07	5.37 ± 6.37	1.29 ± 1.87	2.34 ± 1.43	0.76 ± 1.34	7.3 ± 5.82
Monoterpenic compound	*Nerylacetone*	0.75 ± 1.99	1.64 ± 1.47	0.62 ± 0.84	0.91 ± 0.39	-	-
Sesquiterpenic compounds	*(E)-beta-Farnesene*	2.23 ± 3.3	7.43 ± 9.47	0 ± 0	3.62 ± 2.65	-	-
Sesquiterpenic compounds	*(E)-Caryophyllene*	1.06 ± 4.03	0.75 ± 1.72	0.04 ± 0.11	0.05 ± 0.11	10.61 ± 6.28	16.23 ± 18.34
Sesquiterpenic compounds	*alpha-Copaene*	0.28 ± 0.61	0 ± 0	0.15 ± 0.46	0.53 ± 0.83	-	-
Sesquiterpenic compounds	*Unknown Sesquiterpene*	1.73 ± 3.95	0 ± 0	3.12 ± 4.76	3.44 ± 2.74	-	-
Unknown compounds	*Unknown compound 1*	1.45 ± 2.04	10.66 ± 9.1	5.14 ± 6.16	0.9 ± 1.37	-	-
Unknown compounds	*Unknown compound 2*	0.18 ± 0.34	1.15 ± 1.13	1.8 ± 2.09	0.46 ± 0.33	-	-
Unknown compounds	*Unknown compound 3*	3.52 ± 4.39	6.44 ± 7.03	16.63 ± 9.4	5.99 ± 6.25	-	-
Unknown compounds	*Unknown compound 4*	1.13 ± 0.96	2.07 ± 1.5	1.14 ± 1.33	1.09 ± 1.09	-	-
Unknown compounds	*Unknown compound 5*	0.63 ± 2.06	0.12 ± 0.13	0.19 ± 0.46	0.05 ± 0.1	-	-
Fatty acid derivative	*Dodecan-1-ol*	-	-	-	-	5.32 ± 4.61	1.68 ± 1.05
Fatty acid derivative	*Hexadec-1-ene*	-	-	-	-	5.67 ± 6.37	2.06 ± 3.3
Fatty acid derivative	*Methyl dodecanoate*	-	-	-	-	0 ± 0.01	0.06 ± 0.08
Fatty acid derivative	*Pentadecane*	-	-	-	-	2.54 ± 3.01	0.51 ± 0.46
Fatty acid derivative	*Tetradec-1-ene*	-	-	-	-	0.99 ± 1.54	0.46 ± 0.41
Fatty acid derivative	*Tetradecan-1-ol*	-	-	-	-	3.51 ± 4.03	1.36 ± 1.29
Fatty acid derivative	*Tetradecanal*	-	-	-	-	1.99 ± 2.18	0.92 ± 0.64
Homoterpenic compound	*(E.E)-4.8-dimethylNona-1.3.7-triene*	-	-	-	-	0.73 ± 1.02	2.19 ± 2.66
Monoterpenic compound	*(E.E)-2.6-dimethylOcta-1.3.5.7-tetraene*	-	-	-	-	2.43 ± 2.67	0.33 ± 0.87
Monoterpenic compound	*beta-Pinene*	-	-	-	-	2.26 ± 3.11	1.33 ± 1.71
Monoterpenic compound	*Myrcene*	-	-	-	-	-	-
Monoterpenic compound	*p-Cymene*	-	-	-	-	0.53 ± 1.33	4.88 ± 6.51
Monoterpenic compound	*Sabinene*	-	-	-	-	0.99 ± 0.77	0.6 ± 0.53
Sesquiterpenic compound	*(E.E)-alpha-Farnesene*	-	-	-	-	0.56 ± 0.85	3.06 ± 2.19
Unknown_compound	*Unknown_compound*	-	-	-	-	3.65 ± 3.81	1.58 ± 1.51

Globularia vulgaris (GV)		**In natura**			
		Occitanie		Normandie	
Family	*VOCs*	BF	F	CG	FAL
Fatty acid derivatives	*(E)-Hex-3-enol*	0.21 ± 0.49	2.21 ± 5.07	0 ± 0.01	0 ± 0
Fatty acid derivatives	*(Z)-Hex-3-enyl acetate*	12.83 ± 10.55	5.83 ± 5.45	25.95 ± 17.38	14.66 ± 11.44
Fatty acid derivatives	*6-methyl-5-Hepten-2-one*	2.47 ± 3.48	1.58 ± 2.59	1.56 ± 3.12	3.44 ± 6.88
Fatty acid derivatives	*Cyclohexen-1-yl ethanone*	0.66 ± 0.77	0.75 ± 0.92	0.51 ± 0.59	0.95 ± 0.35
Fatty acid derivatives	*Non-1-ene*	0.55 ± 0.57	0.36 ± 0.48	0.41 ± 0.5	0.24 ± 0.27
Fatty acid derivatives	*Nonanal*	19.11 ± 18.4	9.98 ± 12.08	16.35 ± 14.53	17.65 ± 4.41
Monoterpenic compound	*(E)-beta-Ocimene*	0.8 ± 1.62	0.09 ± 0.27	0 ± 0	0 ± 0
Monoterpenic compound	*(Z)-beta-Ocimene*	6.2 ± 5.63	19.09 ± 12.89	4.36 ± 3.37	3.41 ± 3.98
Monoterpenic compound	*alpha-Phellandrene*	2.03 ± 2.91	1.5 ± 2.62	0 ± 0	0 ± 0
Monoterpenic compound	*alpha-Pinene*	20.03 ± 13.79	17.17 ± 10.25	26.61 ± 13.61	20.55 ± 16.38
Monoterpenic compound	*alpha-Terpenyl acetate*	0.22 ± 0.28	0.14 ± 0.12	0.33 ± 0.39	0.15 ± 0.18
Monoterpenic compound	*Geranylacetone*	7.18 ± 10.78	9.28 ± 12.64	3.23 ± 2.62	5.79 ± 5.89
Monoterpenic compound	*Limonene*	6.21 ± 5.63	19.09 ± 12.9	4.36 ± 3.37	6.23 ± 1.73
Monoterpenic compound	*Nerylacetone*	0.72 ± 0.59	1.11 ± 1.15	0.52 ± 0.6	1.05 ± 1.36
Sesquiterpenic compounds	*(E)-beta-Farnesene*	1.26 ± 3.24	1.41 ± 4.45	3.37 ± 4.90	1.29 ± 1.05
Sesquiterpenic compounds	*(E)-Caryophyllene*	0.01 ± 0.03	0.06 ± 0.09	0.06 ± 0.13	0.24 ± 0.44
Sesquiterpenic compounds	*alpha-Copaene*	0.29 ± 0.88	0 ± 0	3.22 ± 6.44	19.25 ± 15
Sesquiterpenic compounds	*Unknown Sesquiterpene*	1.2 ± 1.65	1.01 ± 1.14	3.37 ± 4.9	1.81 ± 1.39
Unknown compounds	*Unknown compound 1*	1.39 ± 1.46	1.92 ± 2.06	0.51 ± 0.96	0.34 ± 0.42
Unknown compounds	*Unknown compound 2*	1.13 ± 1.25	2.15 ± 1.07	1.11 ± 1.46	0.25 ± 0.17
Unknown compounds	*Unknown compound 3*	4.23 ± 6.76	1.61 ± 2.66	3.96 ± 5.4	0.71 ± 1.43
Unknown compounds	*Unknown compound 4*	7.72 ± 8.66	0.42 ± 0.72	0.15 ± 0.3	0.56 ± 0.73
Unknown compounds	*Unknown compound 5*	0.6 ± 1.22	0.17 ± 0.39	0.07 ± 0.08	1.42 ± 2.73
Fatty acid derivative	*Dodecan-1-ol*				
Fatty acid derivative	*Hexadec-1-ene*				
Fatty acid derivative	*Methyl dodecanoate*				
Fatty acid derivative	*Pentadecane*				
Fatty acid derivative	*Tetradec-1-ene*				
Fatty acid derivative	*Tetradecan-1-ol*				
Fatty acid derivative	*Tetradecanal*				
Homoterpenic compound	*(E.E)-4.8-dimethylNona-1.3.7-triene*				
Monoterpenic compound	*(E.E)-2.6-dimethylOcta-1.3.5.7-tetraene*				
Monoterpenic compound	*beta-Pinene*				
Monoterpenic compound	*Myrcene*				
Monoterpenic compound	*p-Cymene*				
Monoterpenic compound	*Sabinene*	2.95 ± 6.63	3.09 ± 4.98	0 ± 0	0 ± 0
Sesquiterpenic compound	*(E.E)-alpha-Farnesene*				
Unknown_compound	*Unknown_compound*				

Table S4. 4. Volatile compounds (mean ± sd) emitted by the four focal species, Anthyllis vulneraria, Pilosella officinorum, Ranunculus bulbosus and Globularia vulgaris, in different populations (BF, Bois de Fontaret; F, Fourches; CG, Chateau Gaillard; FAL, Falaises; LAR, Larris and R, Riez) and in the two tested conditions (in natura and greenhouse). Underlined VOCs were emitted only by AV and RB in the greenhouse condition.

Plant species	**Insects family**	**LAR**	**R**	**CG**	**FAL**	**BF**	**F**	Total
Anthyllis vulneraria (AV)	*Andrenidae*					29	21	50
	Apidae	13	3			122	114	252
	Halictidae					22	5	27
	Megachilidae		1			28	13	42
	Syrphidae	1				6	11	18
Glovularia vulgaris (GV)	*Andrenidae*					3		3
	Apidae			1	9	10		20
	Halictidae			7	6	25	1	39
	Megachilidae				2	3		5
	Syrphidae				5	9		14
Pilosella officinarum (PO)	*Andrenidae*	4	1	31	6			42
	Apidae	5	4	12	4			25
	Colletidae			7				7
	Halictidae	47	21	89	28		15	200
	Megachilidae		1	20				21
	Syrphidae	3	3	11	11			28
Ranunculus bulbosus (RB)	*Andrenidae*	9	3			3	5	20
	Apidae	4	9				3	16
	Halictidae	62	60			6	26	154
	Megachilidae	13	15				7	35
	Syrphidae	14	18			3	27	62
Total		**175**	**139**	**178**	**71**	**269**	**248**	**1080**

Table S4. 5. Number of insect visitors, at the family level, recorded for the four focal species in the two populations in the French region Hauts-de-France (LAR and R), two populations in the region Normandie (CG and FAL) and two populations in the region Occitanie (BF and F).

Plant species	Family	Species	LAR	R	CG	FAL	BF	F
Anthyllis vulneraria (AV)	Andrenidae	*Andrena combinata*					1	
		Andrena flavipes						1
		Andrena labialis					5	3
		Andrena nigroaenea					2	
		Andrena ovatula					9	10
		Andrena similis					5	
		Andrena wilkella					7	7
	Apidae	*Anthophora aestivalis*					14	5
		Apis mellifera					7	19
		Bombus gr. terrestris	1				6	17
		Bombus hortorum	3				2	2
		Bombus lucorum					6	
		Bombus pascuorum	9	3			9	
		Bombus ruderatus						6
		Bombus sylvarum					6	4
		Bombus terrestris					3	4
		Eucera caspica					17	12
		Eucera hungarica						1
		Eucera interrupta						6
		Eucera nigrescens					52	38
	Halictidae	*Halictus gr. simplex*					4	
		Lasioglossum albipes					1	
		Lasioglossum bimaculatum					1	
		Lasioglossum subhirtum					3	1
		Lasioglossum xanthopus					11	3
		Seladonia smaragdula						1
		Sphecodes albilabris					1	
		Sphecodes gibbus					1	
	Megachilidae	*Anthidium punctatum*						3
		Megachile circumcincta					3	6
		Megachile pilidens					4	
		Osmia aurulenta					16	2
		Osmia gallarum						2
		Osmia rufohirta		1				
		Osmia submicans					4	
		Osmia viridina/versicolor					1	
	Syrphidae	*Chrysotoxum bicinctum*						1
		Episyrphus balteatus						1
		Eupeodes corollae	1					1

		Merodon albifrons						3
		Merodon equestris					1	
		Merodon moenium					1	
		Paragus sp.					3	
		Pipizella sp.						2
		Pipizella zeneggenensis					1	
		Sphaerophoria scripta						1
		Sphaerophoria sp.						1
		Syrphus vitripennis						1
		Total visitors of AV	**14**	**4**			**207**	**164**
Plant species	Family	Species	LAR	R	CG	FAL	BF	F
Glovularia vulgaris (GV)	Andrenidae	*Andrena gravida*					1	
		Andrena humilis					1	
		Andrena rufula					1	
	Apidae	*Anthophora aestivalis*					2	
		Anthophora plumipes				1		
		Apis mellifera				1	7	
		Bombus gr. terrestris				2		
		Bombus pratorum					1	
		Bombus terrestris				1		
		Nomada bifasciata				2		
		Nomada fucata				1		
		Nomada succincta			1	1		
	Halictidae	*Halictus gr. simplex*				2		
		Lasioglossum bimaculatum					3	
		Lasioglossum interruptum					2	
		Lasioglossum laevigatum					1	
		Lasioglossum mediterraneum					1	
		Lasioglossum morio			4	4		
		Lasioglossum subhirtum					12	1
		Lasioglossum xanthopus					3	
		Seladonia tumulorum			3			
		Lasioglossum punctatissimum					3	
	Megachilidae	*Osmia aurulenta*					1	
		Osmia bicolor				2		
		Osmia rufohirta					2	
	Syrphidae	*Episyrphus balteatus*				3		
		Melanostoma mellinum					1	
		Merodon equestris					6	
		Pelecocera pruinosomaculata					1	
		Sphaerophoria scripta				1		

		Sphaerophoria sp.				1	1	
		Total visitors of GV			**8**	**22**	**50**	**1**
Plant species	Family	Species	LAR	R	CG	FAL	BF	F
Pilosella officinarum (PO)	Andrenidae	*Andrena cineraria*			1			
		Andrena gravida			2			
		Andrena humilis	2	1	23	6		
		Andrena minutula	2		3			
		Andrena nigroaenea			1			
		Andrena polita			1			
	Apidae	*Bombus gr. terrestris*				1		
		Bombus lapidarius	1	1		1		
		Bombus pascuorum		1	1			
		Ceratina cucurbitina			1			
		Ceratina cyanea	3	1	3			
		Epeolus variegatus			4			
		Nomada bifasciata				2		
		Nomada flavoguttata		1	3			
		Nomada goodeniana	1					
	Colletidae	*Colletes similis*			6			
		Hylaeus cf. confusus			1			
	Halictidae							
		Halictus maculatus			1			
		Halictus scabiosae			3			
		Lasioglossum aeratum			3			
		Lasioglossum albipes			4	1		
		Lasioglossum calceatum		2	1			
		Lasioglossum interruptum			12	1		
		Lasioglossum leucozonium		1	7	3		2
		Lasioglossum malachurum		1				10
		Lasioglossum medinai				1		
		Lasioglossum morio	5	6	20	1		
		Lasioglossum puncticolle				1		
		Lasioglossum pauxillum	2	3	2			
		Lasioglossum politum			1			
		Lasioglossum villosulum	40	4	14	19		3
		Lasioglossum xanthopus			1			
		Seladonia submediterranea			2			
		Seladonia tumulorum		4	18	1		
	Megachilidae	*Anthidium punctatum*			2			
		Heriades truncorum			1			
		Hoplitis leucomelana			3			
		Osmia aurulenta			1			

		Osmia bicolor		1				
		Osmia leaiana			3			
		Osmia niveata			1			
		Osmia rufohirta			1			
		Osmia spinulosa			7			
		Osmia submicans			1			
	Syrphidae	***Episyrphus balteatus***		1	3	9		
		Eupeodes corollae		1	1	1		
		Eupeodes luniger			1	1		
		Melanostoma mellinum		1				
		Paragus haemorrhous			1			
		Sphaerophoria scripta			2			
		Sphaerophoria sp.	1		3			
		Syrphidae sp.	1					
		Syrphus ribesii	1					
		Total visitors of PO	**59**	**30**	**170**	**49**		**15**
Plant species	Family	Species	LAR	R	CG	FAL	BF	F
Ranunculus bulbosus (RB)	Andrenidae	*Andrena angustior*	1					
		Andrena fabrella						1
		Andrena flavipes		2				
		Andrena gravida		1			1	
		Andrena haemorrhoa	1					
		Andrena humilis					1	
		Andrena minutula	7					1
		Andrena nigroaenea						1
		Andrena nitida						1
		Andrena simontornyella					1	1
	Apidae	*Apis mellifera*						1
		Bombus pascuorum		1				
		Bombus pascuorum		2				
		Ceratina cyanea		1				
		Nomada armata						1
		Nomada bifasciata		1				
		Nomada braunsiana						1
		Nomada fabriciana		1				
		Nomada flavoguttata	2	2				
		Nomada goodeniana	2					
		Nomada sheppardana		1				
	Halictidae	*Halictus rubicundus*		2				
		Lasioglossum albipes	3				1	4
		Lasioglossum bimaculatum						1
		Lasioglossum fulvicorne		4				

	Lasioglossum laevigatum					2	11
	Lasioglossum lativentre		1				
	Lasioglossum leucozonium		1				
	Lasioglossum malachurum	1					8
	Lasioglossum marginatum						1
	Lasioglossum morio	17	4				
	Lasioglossum pauxillum	17	7			3	
	Lasioglossum subhirtum						1
	Lasioglossum villosulum	6					
	Seladonia tumulorum	18	41				
Megachilidae	***Chelostoma florisomne***	1					6
	Hoplitis claviventris		1				
	Osmia aurulenta	1					
	Osmia bicolor	11	4				
	Osmia rufohirta		10				1
Syrphidae	***Cheilosia albitarsis/ranunculii***	12	3			1	2
	Cheilosia pagana		1				
	Cheilosia sp.	1					
	Chrysotoxum bicinctum						1
	Chrysotoxum intermedium						1
	Episyrphus balteatus		1				
	Eumerus strigatus		6				
	Eumerus strigatus/sogdanius		2				
	Eupeodes corollae						1
	Meliscaeva auricollis						2
	Merodon equestris						1
	Neoascia podagrica		1				
	Pipizella sp.						3
	Pipizella zeneggenensis						1
	Sphaerophoria scripta		2			1	8
	Sphaerophoria sp.	1	1				7
	Syrphus ribesii		1				
	Xanthogramma citrofasciatum					1	
	Total visitors of RB	**102**	**105**			**12**	**68**

Table S4. 6. Insects species and visitation rates of the four focal specie (AV, Anthyllis vulneraria; GV, Globularia vulgaris; PO, Pilosella officinorum; RB, Ranunculus bulbosus) in the two populations in the French region Hauts-de-France (LAR and R), two populations in the region Normandie (CG and FAL) and two populations in the region Occitanie (BF and F). Insect species that are shared among populations in different regions are underlined and bold

Chapter V

Discussion and Perspectives

Thesis aim and questions

The study of ecological networks, such as plant-pollinator networks, has fascinated researchers since the 18th century (Kearns & Inouye 1997; Bascompte & Jordano 2007; Mitchell *et al.* 2009; Dáttilo *et al.* 2016). Recently, concerns about global changes, such as climate change, anthropisation and biodiversity loss, have fostered reflections about the potential disruption of plant-pollinator associations (Visser & Both 2005; Biesmeijer *et al.* 2006; Memmott *et al.* 2007; Hegland *et al.* 2009; Potts *et al.* 2010). Plant-pollinator associations are complex and dynamic, with high variability in space and time. Different approaches to study interaction networks have been developed in the past decades, making use of both new empirical approaches (yielding more complete and detailed data) and statistical and theoretical models. However, relatively little is known about the mechanisms behind such interactions. New methods are thus needed to assess which factors drive the structure of networks.

Considering both spatial and temporal sources of variation at the same time, the general aim of this study was to understand and help predict the effects of environmental changes, accounting for changes on species compositions, plant-pollinator associations and species roles. We used a latitudinal gradient as a source of natural variation of biodiversity. Along this gradient we studied seasonal (from April to October) and inter-annual (for two consecutive years) plant-pollinator associations in six calcareous grasslands. We aimed to respond to four main questions (Fig. 5.1), considering different levels of complexity, from the entire network to species-specific variation among populations.

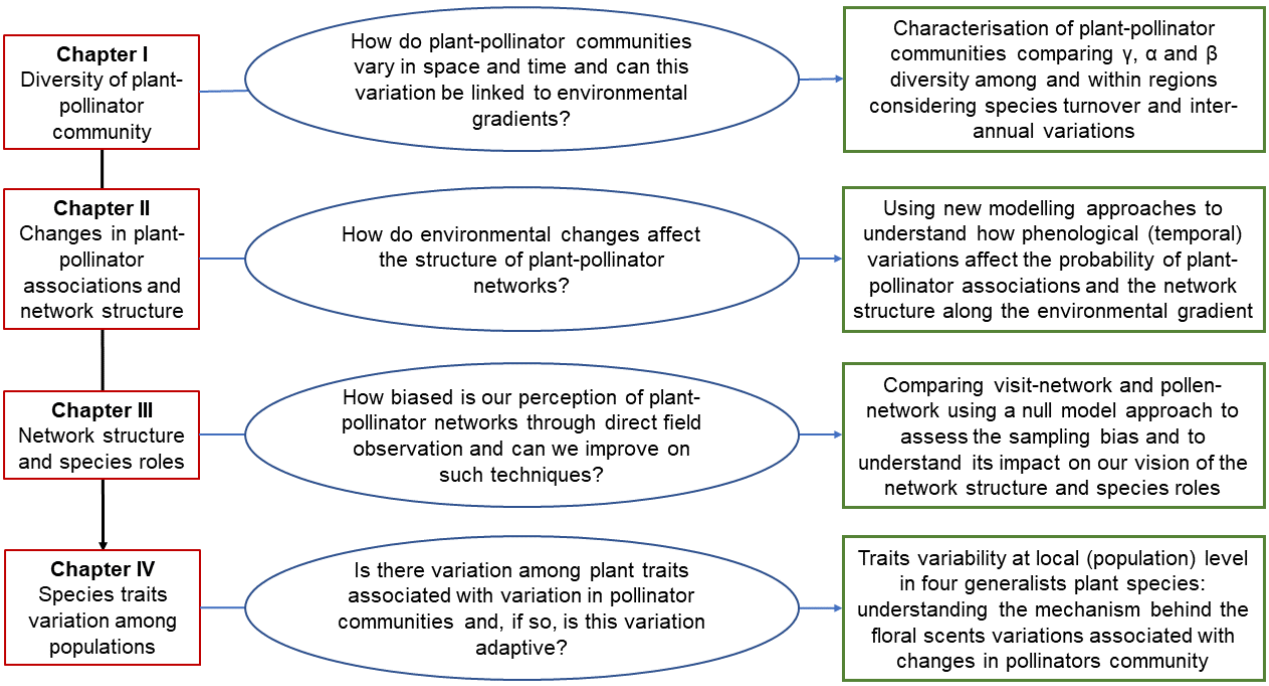


Figure 5. 1. The four main questions of the thesis (in blue), the analyses used (in green) and presented in the four chapters (in red) accounting for different level of complexity, from the entire network to species-specific variation among populations.

Characterising plant-pollinator communities and associations along an environmental gradient

To describe plant-pollinator communities, different methods can be used. The most common "active" methods are (i) observational plots and (ii) walking transects. Even if they provide information about plant-pollinator interactions, these techniques are time-consuming, resource-demanding and require surveyors with expertise in both botanical and entomological identifications (Westphal *et al.* 2008; Jordano 2016; Poisot *et al.* 2016; Bell *et al.* 2019). Passive methods such as pan traps are not biased by the "surveyor behaviour", and can theoretically provide a good assessment of the local bee diversity when used in both agricultural and natural landscapes (Westphal *et al.* 2008; Shapiro *et al.* 2014). However, pan traps are taxonomically limited and/or biased, since they are not equally attractive for all pollinator groups and their effectiveness also depend on floral abundances, which can vary among sites (Baum & Wallen 2011). Indeed, in sites with higher floral abundance and richness, pan traps may undersample and underestimate pollinator species richness and abundance (Baum & Wallen 2011). Moreover, since only insects and the pollen found on their bodies are caught into pan traps, further advances in molecular ecology are still required in order to use this method for interaction surveys – an interaction *per se* cannot be directly inferred from a species' presence in a trap. Depending on the ecological question, the season and the habitat, one methodology could be

more appropriate than the other. The use of different techniques, combined with interactions records, can give a good picture of species richness and diversity (Nielsen *et al.* 2011; Jordano 2016).

In this study we decided to record species interactions and to measure species diversity and abundances using a combination of variable walking transects and three-colour pan traps. The study was restricted to the Anthophila and Syrphidae family which are considered as the main pollinators of wild and crop species worldwide (Michener 2000; Jauker & Wolters 2008; Rader *et al.* 2011; Albrecht *et al.* 2012). While a wide variety of plant-pollinator studies are plant-based, often considering the pollinators of one focal species or a group of plants (Bosch *et al.* 2009; Gibson *et al.* 2011), we instead followed whole plant and pollinator communities throughout the entire flowering season and pollinator life cycles. We also accounted for spatial variability considering three regions in France, and we chose two sites for each region as "replicates" of regional diversity. Changes in climate and floral resource availability among years can affect pollinator assemblages (Petanidou *et al.* 2008, 2014; CaraDonna *et al.* 2014). Thus, to identify these variations efficiently, only the use of repeated standardized sampling protocols allows direct comparison of plant-pollinator records across space and time (Williams *et al.* 2001). For this reason, we sampled communities using a standardized protocol, with the same number of surveyors, the same sampling hours and the same general schedule, replicated among all sites, regions, and sampling sessions.

Another important aspect which should not be underestimated in these types of study is the preparation and identification of sampled specimens. This task is also time-consuming and requires expertise in taxonomical identification, both for plant and pollinators. The identification of plants and pollinators at the lowest taxonomical level, *i.e.* species level, is fundamental to have an appropriate description of community diversity and functioning, since not all species, even within one genus, have the same ecological role. Moreover, species-level identification helps avoid biases when studies are re-used in meta-analyses and keeps from overestimating interaction generalisation in interaction networks. In the present study, plant identification was performed in the field, but pollinators were harder to tease apart. All individual insects have thus been prepared, pinned and identified to genus level in the laboratory, and then sent to expert taxonomists for species-level identification, thus delaying statistical analyses by the time it took samples to go back and forth between the lab and the experts.

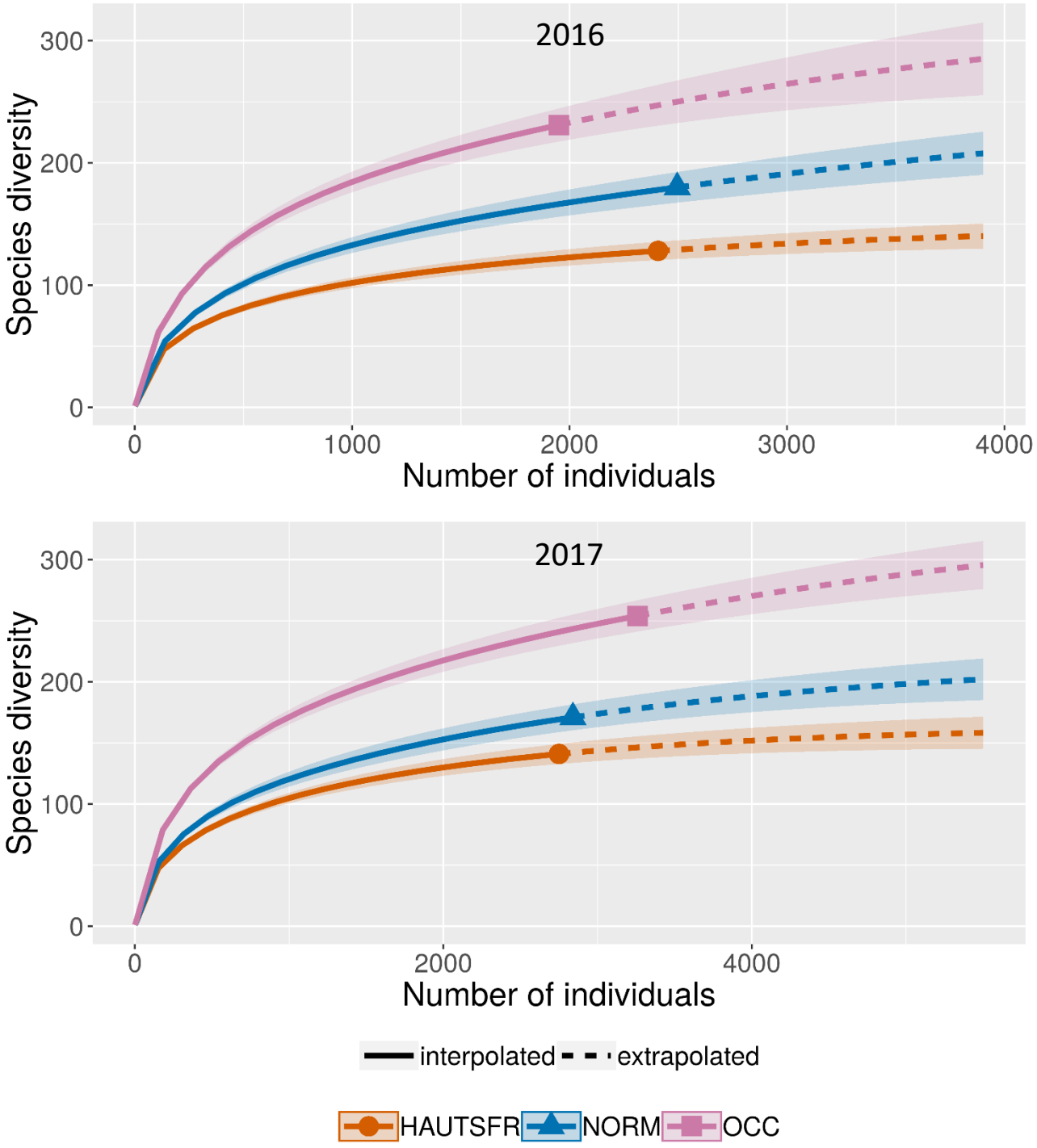


Figure 5. 2. Rarefaction curves of pollinator species richness (Anthophila + Syrphidae) sampled during the two years of fieldwork in the three regions considered in this study: OCC, Occitanie in purple; NORM, Normandie in blue and HAUTSFR, Hauts-de-France in orange.

Combining both sampling methods in this study, we obtained a good representation of pollinator species richness in the two years of fieldwork (Fig. 5.2), accounting for differences in abundances among regions and between years. After two years of fieldwork, we sampled about 33% of the French bee fauna and 20% of the French hoverfly fauna. Among the sampled species, we recorded the first

local occurrence in the Occitanie region for *Andrena florivaga* and *Eucera* (Cubitalia) sp. For other species, such as *Rophites algirus, Anthophora larvata, Tetraloniella fulvescens, Sphecodes schencki* there were very few reports in the last 20-30 years.

We recorded the highest richness using the walking transect methodology (Fig. 1.1). The lower richness and abundance recorded with pan traps was probably due to the limited number of clusters used (only two), but the differences observed in pan trap effectiveness among sites were probably linked to differences in local floral abundances along the season, with southern sites yielding quite fewer trapped insects since floral abundances and diversity could be much higher there than in the other four sites. However, some insect families were over-represented in the pan traps revealing a discriminating effect of the sampling method. In **Chapter I**, we provided a general picture of spatio-temporal diversity of plants and pollinators – from regional to local, and from seasonal to inter-annual diversity. Even if we found differences in abundance and species richness, the two year of sampling were very similar, with a low β diversity (Fig. 1.10) of both for plant (0.10) and insect species (0.09). We found a clear pattern of species diversity along the latitudinal gradient with southern sites always displaying the highest diversity of both plant and insect species. Higher β diversities were recorded between Occitanie and Normandie and between Occitanie and Hauts-de-France than between Normandie and Hauts-de-France. However, the Normandie region seems to be a "transit zone", where some species reach their limits of distribution. Among the bee species shared only between Normandie and Occitanie we found species of the genus *Eucera, Eupeolus*, *Coelioxys*, *Stelis*, *Pseudoanthidium*, *Trachusa* (group Anthophila) and *Dasysyrphus* (Family Syrphidae). Among the plant, 20 species are shared only with the Hauts-de-France, such as *Scabiosa columbaria* (Caprifoliaceae) and *Centaurea jacea* (Asteraceae), and 25 species with the Occitanie region, such as *Allium sphaerocephalon* (Liliaceae), *Astragalus monspeliensis* (Fabaceae), *Phyteuma orbiculare* (Campanulaceae), *Echium vulgare* (Boraginaceae) and *Odontites verna* (Orobancacheae). This last species is the host plant of *Melitta tricincta* (Family Melittidae, group Anthophila), which we sampled in both Normandie and Hauts-de-France region.

The temporal diversity showed regionally idiosyncratic patterns of temporal turnover for pollinator and plant species, probably linked to the high number of species with short phenologies (Fig. 1.9). We indeed observed two peaks of species originality in each site, which differed among regions, one at the beginnig and the other in the middle of the season. The most important factors influencing the β diversities between sampling sessions were the identity of the site and the sampling month (Table 1.1).

The next step of this analysis will be to look at the diversity of interactions along the environmental gradient considering seasonal and annual changes, making use of a framework recently developed to partition the diversity of interactions at different scales (Ohlmann *et al.* 2019). Switch from β diversity of species to β diversity of interactions might provide a clearer picture of the dissimilarity of plant-pollinator networks and thus help understand how these networks can vary in space and time (Poisot *et al.* 2012). Network dissimilarity can be divided into different components; for example, the dissimilarity of interactions due to the turnover of species, or the dissimilarity between networks based on the dissimilarity of interactions established between shared species. This approach can be applied to weighted networks to model the probability of interaction using the interaction strength (as described in Ohlmann *et al.* 2019). The information provided by this analysis could help assess network robustness and resilience in the face of species and interaction loss due to global changes.

In **Appendix 2** (from Adrien Berquer's Master Dissertation, whom I advised in 2018), we analysed the β diversity of interactions in the six hoverfly-plant networks recorded in 2016 along the latitudinal gradient in France. We observed that the dissimilarity of interactions increases with the geographical distance. Sites in the same region are more similar than sites in other regions, with the exception of the sites in Normandie which exhibit a relatively high dissimilarity within the region. Variation in interactions due to species turnover (β_{ST}) is higher when a Normandie site is involved (Table 3, in Appendix 2), probably due to the high number of interactions recorded in both sites from this region (Table 2.1, Chapter II). These findings highlight a promising future for the use of the β diversity of interactions, particularly when integrated in the comparison of networks along environmental gradients.

Network variation in space and time

Despite important methodological advances, our perception of how and why plant-pollinator networks vary in time and space is still limited. Part of this limitation is caused by the lack of extensive interaction data sets, especially in species-rich regions. Most European regions show plant and pollinator data deficit (Bilz *et al.* 2011; Nieto *et al.* 2014). which can only be assessed with new ecological studies providing exhaustive information of plant-pollinator interactions, richness and abundances considering seasonal dynamics and different spatial units. The comparison of networks along environmental gradients is a promising new tool to develop a more accurate perception of changes in network structure related to changes in natural conditions (Pellissier *et al.* 2017), and space-for-time substitution approaches would help predict future changes, as is usually done in studies on species adaptation to global change (Merilä & Hendry 2014; Van Dijk & Hautekèete 2014).

In our study we used the environmental/latitudinal gradient to predict the effects of global changes on plant-pollinator associations.

Phenological mismatches between plant and pollinator species have the potential to threaten the existence of interactions and thus pollination (Hutchings *et al.* 2018). In **Chapter II**, we studied the consequences of environmental gradients on plant-hoverfly interactions, focusing on how phenology overlap and species abundances affect the probability of interaction between plants and insects. We modelled the probability of species interactions using an innovative approach (Bayesian Structural Equation Models) that allows us to make comparisons between networks with different sizes, avoiding the use of classic metrics which are affected by network dimensions (Bascompte 2010; Staniczenko *et al.* 2013; Astegiano *et al.* 2015). Another innovative approach, now widely used in functional ecology, is the use of traits – real or latent – to explain the general mechanism driving pairwise interactions (Dalla Riva & Stouffer 2015; Bartomeus *et al.* 2016, 2017; Laigle *et al.* 2017; Joffard *et al.* 2019). We used a latent trait-based approach (random-dot product graph model followed by redundancy analysis, RDPG x RDA), considering species phenology, morphology, behaviour, phylogeny, habitat type and geographical differences, to assess whether the variation observed in the plant-hoverfly network can be explained by several factors (phylogeny, phenology, etc.). We also tried another modelling approach (exponential random graph models) to tease apart the effects of "extrinsic" factors, such as the characteristics of the studied organisms, from those of "intrinsic" factors, explaining the interactions based on other interactions in the network (**Appendix 2**: Adrien Berquer's Master Dissertation).

The SEM and RDPG x RDA analyses eventually led to similar results:

With the SEM analysis, we highlighted that plant phenology drives the duration of the phenology overlap (PO) between plant and insects, which in turn influences either the probability of interaction or the expected number of visits. However, the phenology overlap alone was not sufficient to explain interactions and its explanatory power decreased with latitude. Phenology was indeed a stronger determinant of plant-pollinator interactions in sites where species were more generalist and network compartmentalization was weaker, *i.e.* where phenology overlap and specialisation can be distinguished. Variation in species phenologies were observed along the gradient, with plant species showing shorter phenologies in the southern sites. Plant and insect abundances played a substantial role to explain the number of visits, with more abundant species accounting for most interactions.
The results of the RDPG x RDA analysis also suggested that geographic differences explained network variation for both hoverfly and plant species. Moreover, each individual network was

explained by one or more different extrinsic factors (*i.e.* different traits). For example, in the site of Fourches, plant phenology explained part of the network structure, but this was essentially due to the covariance between phenology and species degree (*i.e.* R^2 were not significant when compared with results obtained from simulated networks with degrees equal to those of the observed network). This result is coherent with the results of the SEM, in which the best model found in this site was the one that considered a positive effect of the PO on the probability of interaction.

The RDPG x RDA analysis also allowed to assess which factors have an important effect to explain network topology. For example, we found a significant effect of the hoverfly phylogeny and morphology in the site of Bois de Fontaret. This suggests that the use of morphological traits (*e.g.* tongue length or inter-tegular distance) together with species richness and phylogenies, on top of variables already used in the SEM, might improve the modelling of interactions and could help better understand network variations along the environmental gradient. The next step will be to apply the same models to other pollinators (*e.g.* wild bees) and more complex networks, accounting for the inter-annual variation to test if the same patterns could explain plant-pollinator interactions between the two years.

Informing plant-pollinator networks with pollen information

In **Chapter III**, we used the pollen found on insect bodies to switch from a "plant-based" network vision to a "pollinator-based" vision, and to cover the sampling bias in the field. We provided a robust estimation of the actual number of interactions within diversified ecological networks (*i.e.* with different levels of species richness and abundance). We showed that, when visit-based networks were adequately sampled, their structure were not different from pollen-based networks, which are intrinsically more exhaustive, *i.e.* showing high number of interactions. However, the undersampling inherent to visit-based networks may bias our perception of plant-pollinator networks, mostly at the species level. Rare plant or pollinator species, recorded as singletons in the visit-based network, can display significant changes from the pollen-based network regarding their specialisation level and their ecological role. Pollen-based networks provided unobserved links both with plant species observed in the sampled area but also with extra plant species in the surrounding area. Pollen records could then be used to assess species foraging ranges of floral resources, which seem to differ among pollinator families (Figs. S3.2, S3.3, S3.4). As future perspectives, we could switch from species- to individual-based networks, using the information provided by pollen, to test how individual variability can influence the establishment of species interactions and our perception of the network structure (Dupont *et al.* 2014; Tur *et al.* 2015; Lucas *et al.* 2018). We could assume that different

individuals of specialist species do not change their behaviour, since they all look for the same resource, and thus their role in the whole network structure should not change (such as for *Rophites algirus* in the network in the site of Fourches, Occitanie). However, different individuals of generalist species might display high variability and specialization in the partition of pollen resources which may not be observed at the species level. These patterns could vary if we consider (i) social species, such as bumblebees, or (ii) solitary species with different nesting behaviours (aggregation or not), (iii) species with longer phenologies, which may present seasonal specialisation, or (iv) species with shorter phenologies, (v) species hairiness (Stavert *et al.* 2016) and (vi) the sex of sampled insects. Accounting for individual-specific traits might be useful to infer the probability of interaction among species in the reconstruction of network dynamics, and it could foster reflection for species conservation purpose.

For example: (i) Social species such as bumblebees use scent mark as a guide to avoid plant species which have been already visited (Goulson *et al.* 1998; Stout *et al.* 1998). Thus, we might expect less variation among individuals which share information on the availability of flowering resources. (ii) Species with small colonies (such as species of the genus *Lasioglossum*), which nest in dense aggregations, can have cooperative behaviour among individuals of different colonies. They might help each other build nests but also provision the cells, thus potentially sharing their resources (Michener 1966); while solitary polylectic species might display high variability in the choice of flower resources among individuals, with some individuals being more specialised in a few plant species and others showing a more opportunistic behaviour. Depending on the species, (iii) individuals of species with longer phenologies may present seasonal specialisation while (iv) individuals of species with shorter phenologies could range from more specialised to rather opportunistic (Michener 2000). Another hypothesis is that (v) hairier species might transport more different pollen types, but different individuals might display various levels of hairiness. Finally, (vi) we could expect that males can carry more diverse pollen grains than females, due to an opportunistic approach to foraging. They indeed do not provide pollen for the larval development, but they need nectar only to feed themselves and thus they might carry pollen of several different species. In our study (Chapter III), when we compared pollen on female bodies and on their pollen-carrying structures we did not find any significant difference (Fig. 5.3), suggesting that even if females can visit different plant species looking for nectar, the floral choice seems to be “restricted” to those species that they visited to actively collect pollen for the nest. To test this hypothesis, we could use the information of the “pollen score” which is an estimation of the insect body surface cover by pollen

grains (see General material and methods, "Palynological analysis") to compare female and male individuals, and then compare pollen diversity on the associated pollen slides.

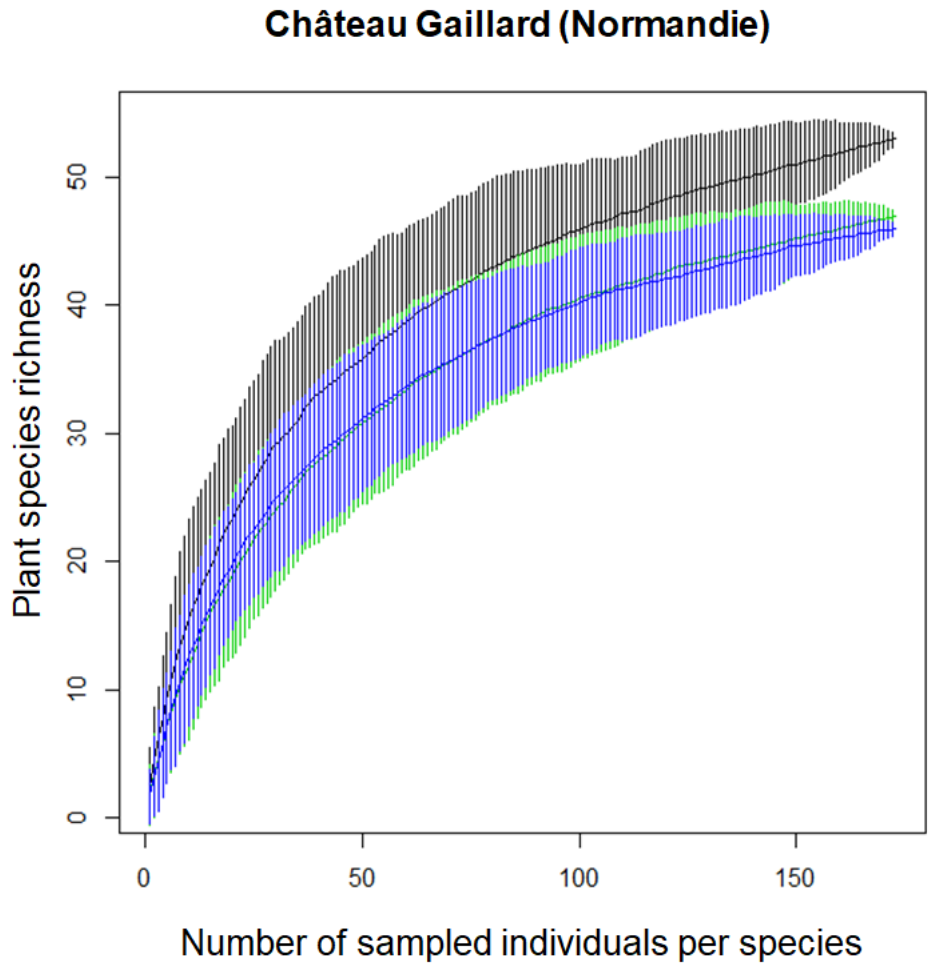


Figure 5. 3. Species accumulation curve in the site of Château Gaillard (Paul Moreau's Master Dissertation, whom I supervised). The accumulation curve of the plant richness obtained as a function of the number of the sampled insect individuals (Anthophila). The black curve represents the total information obtained pooling the visits-based and the pollen-based interactions; the green curve represents the interactions recorded using the pollen found on the insect bodies (PS); and the blue curve represents the interactions obtained using pollen from the specialized structures (PC).

Pollen microscopic identification is also time-consuming and dependent on a few experts with palynological skills (Bell *et al.* 2017). Alternative and now available methodologies, such as DNA metabarcoding, might really improve pollen identification (Clare *et al.* 2013; Keller *et al.* 2015), but also pollinator identifications. To reduce the time committed to sampling and species identification, we could switch to alternative methodologies, such as pan trap and malaise trap for sampling and DNA metabarcoding for species identification. Even if passive sampling will not give information about plant-pollinator associations, new inferential statistical models can be used to analyse the unobserved interactions in ecological interaction networks (Jordano 2016). Thus, the combination of less-demanding sampling and identification techniques and the use of inference models might

enhance the study of plant-pollinator networks and predictions of interactions from species co-occurrence and trait matching, without relying on observed visit-based networks in the field (Bartomeus *et al.* 2016; Poisot *et al.* 2016). However, DNA-barcoding is still a new methodology not widely used in the study of plant-pollinator networks and it can have some limits (Bell *et al.* 2016, 2017; Macgregor *et al.* 2019). For example, in species-rich ecosystems that contain many closely related plant species, the use of DNA barcodes may be insufficient to identify all species present (Bell *et al.* 2017). However, this technique might simplify and accelerate pollen identification in the future if improved with new specific (regional or local) botanic databases.

Changes in plant and pollinator species traits along an environmental gradient

We have already discussed how latent or functional traits can be used to explain linkage rules in ecological interaction networks (Dalla Riva & Stouffer 2015; Bartomeus *et al.* 2016). However, most studies use general functional traits from available datasets on species (at large spatial scales), which may not be an accurate estimate of the trait values if these traits vary from the same species among sites. To reach a good ecological understanding, the collection of individual functional traits combined with interaction records may help highlight intraspecific co-variation between phenotypic traits and network structure along environmental gradients (Pellissier *et al.* 2017).

In **Chapter IV**, we measured volatile compound emission on four selected plant species distributed along the latitudinal gradient (*Globularia vulgaris*, *Anthyllis vulneraria*, *Pilosella officinarum*, *Ranunculus bulbosus*), to compare floral bouquets among populations in relation to changes in pollinator communities and other environmental conditions. To understand the nature of volatile organic compound (VOC) variation, we measured VOC emission under controlled conditions on plants reared from seeds sampled in the study populations. We also measured floral morphological traits in natural populations for two of the selected species, *G. vulgaris* and *R. bulbosus* (**Appendix 3** "Morphological methods", Hineiti Lou Chao's Master Dissertation). In particular, we measured (i) the number of inflorescences per plant, (ii) the number of flowers per inflorescence or plant, (iii) flowers and inflorescence size and (iv) the surface covered by the basal rosette.

The original idea was to obtain a picture of both visual and olfactory trait variation for the study species, but the data obtained to date only provide preliminary information for a subsample of study populations. The analysis of morphological traits needs to be deepened, in particular regarding their

potential co-variation with volatile compounds. So far, this work revealed that both types of traits varied within and/or between regions, with no obvious consistency between them. Any observed variation in floral traits is usually interpreted as the result of local adaptation to pollinators. At least for the volatile compounds released by the study species, we were able to investigate the possible factors responsible for their geographical variation. In *G. vulgaris*, we did not find any variation in the volatile compound emission. For the other three species, the variation of both qualitative and semi-quantitative scent emission, produced in natural and reared populations, was found to be – at least partly – environmentally induced. *R. bulbosus* was the only species for which we observed significant variations in the greenhouse experiment, which were consistent with variations recorded in nature. This would suggest a genetic basis of the variation of its floral bouquet. Pollinator communities differed among sites only at the species level, while family abundances were very similar between sites. In this context, one does not particularly expect strong differences between sites in terms of pollinator-mediated selection. This suggests (i) that at least some of the observed variations are neutral or under selective pressures mediated by agents different from pollinators (such as herbivores, see Schiestl 2010). and/or (ii) that closely related insect species may differ in their sensitivity or attraction to VOCs, leading to divergent selective pressures. The relative weight of these two mechanisms is poorly known to date. Some studies have shown or suggested a role of genetic drift and/or herbivores in selective pressures acting on VOC composition but their integration in pollination-focused studies is extremely limited (Delle-Vedove *et al.* 2017). On the other hand, the potential for closely related species of insects for driving local adaptation in COVs proportions in plants with generalist pollination has never been investigated to my knowledge.

A stimulating perspective of the current study would be to analyse the traits of the pollinators sampled visiting these species (*e.g.* tongue length, body size), and combine this with the intensity of the interactions (and potentially with measured plant traits). Such an approach could help disentangle the causes of floral trait variation.

Pollinator functional traits (mean value at the community scale) are also expected to vary along the latitudinal gradient in response to changes in environmental conditions. Variation in body size in many organisms has been linked to changes in temperature. Larger sizes are usually found in colder conditions, following Bergmann's rule (Bergmann 1847). The inter-tegular distance (ITD) is a measure of distance between the tegulae, *i.e.* the insertion points of wings on the thorax, and has been used as measure of pollinator body size (dry weight, Cane 1987). ITD is also used as an estimator of pollinator foraging ranges and dispersal ability (Greenleaf *et al.* 2007; Jauker *et al.* 2016; Kendall *et al.* 2019). In general, West Palearctic solitary and social bees follow Bergman's rule (Gérard *et al.*

2018). Even if we did not measure any pollinator traits yet, we could expect that differences in pollinator sizes will be found along the latitudinal gradient that we considered in our study. However, in some species, *e.g.* bumblebees, body sizes show a trend opposite to Bergmann's rule, probably because resource availability and other environmental pressures might have a larger impact on body size than temperature (Gérard *et al.* 2018). Higher temperatures, limited resource availability and habitat fragmentation could act in synergy and shape species traits (Shelomi 2012). For example, in summer months when temperature increases and resource availability is scarce, pollinators may be forced to expand their foraging range, especially in southern sites. As a future perspective, ITD measurements and landscape analysis could be used to clarify the interactions recorded in the field and the interactions revealed by the pollen found on insect bodies, which also include plant species living outside the study site.

Conclusions

This study has highlighted the importance of simultaneously considering the entire flowering season and insects flying period, using species-level identifications, to disentangle the species' ecological roles and network variations. Even if fieldwork sampling and taxonomical identifications could be time-consuming, they provide framework picture of inestimable value regarding complex plant-pollinator associations and can be used in most ecological situations. The use of different methodological approaches has allowed us to compare networks along the environmental and diversity gradient, avoiding the use of classic metrics that suffer from their sensitivity to network size. We have provided new quantitative data, both in terms of number of species and in terms of number and type of interactions, to test different ecological hypotheses and not only to compare networks from a qualitative point of view. The large amount of data provided in this thesis will permit to cover the lack of data for both plants and pollinator insects in different regions in France. This is particularly true in Mediterranean sites, where the lack of data is high, due to – at least partly – the very high species richness. These findings could foster reflections on species conservation and management of natural areas, and could lay the foundation of specific programs to preserve rare plant and pollinator species and their interactions. All the perspectives proposed will further improve our knowledge of plant-pollinator networks considering different levels of associations, from the entire network to species-specific and individual variation among populations.

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Appendix

Appendix 1

Plant and pollinator species lists.

Appendix 2

Extract from Adrien Berquer's Master Dissertation. « Inférence des facteurs affectant la structure des réseaux plantes-pollinisateurs : le cas des réseaux plantes-syrphes le long d'un gradient latitudinal de pelouses calcaires en France »

Appendix 3

Extract from Hineiti Lou Chao's Master Dissertation - Morphological analyses of *Globularia vulgaris* and *Ranunculus bulbosus*

Annex I

Species lists

Annex 3. 1. Plant species list recorded during the two year of fieldwork (2016 and 2017). *Species are ranged by Family. We reported the occurrence (presence / absence) in each region: Hauts-de-France (HFR), Normandie (NOR) and Occitanie (OCC).*

Family	*Species*	HFR	NOR	OCC	Number of occurrences
Amaryllidaceae	*Allium sphaerocephalon*		1	1	**2**
Amaryllidaceae	*Narcissus assoanus*			1	**1**
Apiaceae	*Anthriscus sylvestris*	1			**1**
Apiaceae	*Bupleurum baldense*			1	**1**
Apiaceae	*Bupleurum falcatum*		1		**1**
Apiaceae	*Daucus carota*	1	1	1	**3**
Apiaceae	*Eryngium campestre*	1		1	**2**
Apiaceae	*Heracleum sphondylium*	1			**1**
Apiaceae	*Pimpinella saxifraga*	1	1		**2**
Apiaceae	*Seseli libanotis*		1		**1**
Apiaceae	*Seseli montanum*			1	**1**
Apiaceae	*Torilis japonica*			1	**1**
Apiaceae	*Trinia glauca*			1	**1**
Apocynaceae	*Vincetoxicum hirundinaria*		1	1	**2**
Asparagaceae	*Anthericum liliago*			1	**1**
Asparagaceae	*Anthericum ramosum*		1		**1**
Asparagaceae	*Aphyllanthes monspeliensis*			1	**1**
Asparagaceae	*Leopoldia comosa*			1	**1**
Asparagaceae	*Muscari botryoides*			1	**1**
Asparagaceae	*Muscari neglectum*		1	1	**2**
Asparagaceae	*Ornithogalum umbellatum*			1	**1**
Asparagaceae	*Prospero autumnale*			1	**1**
Asteraceae	*Achillea millefolium*	1	1	1	**3**
Asteraceae	*Bellis perennis*	1		1	**2**
Asteraceae	*Carduus nigrescens*			1	**1**
Asteraceae	*Carlina acanthifolia*			1	**1**
Asteraceae	*Carlina vulgaris*	1	1	1	**3**
Asteraceae	*Carthamus lanatus*			1	**1**
Asteraceae	*Carthamus mitissimus*			1	**1**

Asteraceae	*Catananche caerulea*			1	**1**
Asteraceae	*Centaurea jacea*	1	1		**2**
Asteraceae	*Centaurea nigra*	1	1		**2**
Asteraceae	*Centaurea pectinata*			1	**1**
Asteraceae	*Centaurea scabiosa*	1	1	1	**3**
Asteraceae	*Centaurea stoebe*			1	**1**
Asteraceae	*Centaurea thuillieri*		1		**1**
Asteraceae	*Chondrilla juncea*			1	**1**
Asteraceae	*Cirsium acaulon*	1	1	1	**3**
Asteraceae	*Cirsium arvense*			1	**1**
Asteraceae	*Cirsium tuberosum*			1	**1**
Asteraceae	*Cirsium vulgare*	1		1	**2**
Asteraceae	*Crepis capillaris*	1		1	**2**
Asteraceae	*Crepis foetida*	1		1	**2**
Asteraceae	*Crepis pulchra*	1		1	**2**
Asteraceae	*Crepis vesicaria*			1	**1**
Asteraceae	*Crupina vulgaris*			1	**1**
Asteraceae	*Echinops ritro*			1	**1**
Asteraceae	*Galatella linosyris*		1		**1**
Asteraceae	*Helichrysum stoechas*			1	**1**
Asteraceae	*Hieracium glaucinum*			1	**1**
Asteraceae	*Hieracium lachenalii*	1			**1**
Asteraceae	*Hieracium murorum*			1	**1**
Asteraceae	*Hypochaeris radiata*	1			**1**
Asteraceae	*Inula conyza*	1	1	1	**3**
Asteraceae	*Inula montana*			1	**1**
Asteraceae	*Jurinea humilis*			1	**1**
Asteraceae	*Lactuca perennis*			1	**1**
Asteraceae	*Lactuca serriola*			1	**1**
Asteraceae	*Lasiospora hirsuta*			1	**1**
Asteraceae	*Leontodon crispus*			1	**1**
Asteraceae	*Leontodon hispidus*	1	1	1	**3**
Asteraceae	*Leontodon hyoseroides*		1		**1**

Asteraceae	*Leontodon saxatilis*			1	**1**
Asteraceae	*Leucanthemum graminifolium*			1	**1**
Asteraceae	*Leucanthemum vulgare*	1	1		**2**
Asteraceae	*Picris hieracioides*	1	1		**2**
Asteraceae	*Pilosella officinarum*	1	1	1	**3**
Asteraceae	*Podospermum purpureum*			1	**1**
Asteraceae	*Rhaponticum coniferum*			1	**1**
Asteraceae	*Senecio jacobaea*	1	1		**2**
Asteraceae	*Solidago virgaurea*	1	1		**2**
Asteraceae	*Sonchus arvensis*			1	**1**
Asteraceae	*Sonchus asper*	1			**1**
Asteraceae	*Taraxacum sp.*	1	1	1	**3**
Asteraceae	*Tragopogon crocifolius*			1	**1**
Asteraceae	*Tragopogon pratensis*	1	1		**2**
Boraginaceae	*Echium vulgare*		1	1	**2**
Boraginaceae	*Lithospermum officinale*			1	**1**
Boraginaceae	*Myosotis arvensis*	1		1	**2**
Boraginaceae	*Onosma fastigiata*			1	**1**
Brassicaceae	*Alyssum alyssoides*			1	**1**
Brassicaceae	*Arabis hirsuta*		1	1	**2**
Brassicaceae	*Biscutella laevigata*			1	**1**
Brassicaceae	*Brassica repanda*			1	**1**
Brassicaceae	*Capsella bursa-pastoris*			1	**1**
Brassicaceae	*Cardamine hirsuta*			1	**1**
Brassicaceae	*Erophila verna*			1	**1**
Brassicaceae	*Erysimum x cheiri*		1		**1**
Brassicaceae	*Hornungia petraea*			1	**1**
Brassicaceae	*Iberis pinnata*			1	**1**
Brassicaceae	*Isatis tinctoria*		1		**1**
Brassicaceae	*Thlaspi montanum*		1		**1**
Brassicaceae	*Thlaspi perfoliatum*		1	1	**2**
Campanulaceae	*Campanula glomerata*		1	1	**2**
Campanulaceae	*Campanula patula*			1	**1**

Campanulaceae	*Campanula rapunculus*	1		1	**2**
Campanulaceae	*Campanula rotundifolia*	1	1		**2**
Campanulaceae	*Legousia speculum-veneris*			1	**1**
Campanulaceae	*Phyteuma orbiculare*		1	1	**2**
Caprifoliaceae	*Knautia arvensis*	1		1	**2**
Caprifoliaceae	*Knautia purpurea*			1	**1**
Caprifoliaceae	*Lonicera etrusca*			1	**1**
Caprifoliaceae	*Scabiosa columbaria*	1	1		**2**
Caprifoliaceae	*Scabiosa triandra*			1	**1**
Caprifoliaceae	*Valeriana tuberosa*			1	**1**
Caryophyllaceae	*Arenaria aggregata*			1	**1**
Caryophyllaceae	*Arenaria controversa*			1	**1**
Caryophyllaceae	*Arenaria serpyllifolia*	1	1		**2**
Caryophyllaceae	*Cerastium fontanum*	1		1	**2**
Caryophyllaceae	*Dianthus longicaulis*			1	**1**
Caryophyllaceae	*Dianthus sylvestris*			1	**1**
Caryophyllaceae	*Minuartia capillacea*			1	**1**
Caryophyllaceae	*Minuartia mediterranea*			1	**1**
Caryophyllaceae	*Minuartia mutabilis*			1	**1**
Caryophyllaceae	*Petrorhagia prolifera*			1	**1**
Caryophyllaceae	*Silene conica*			1	**1**
Caryophyllaceae	*Silene italica*			1	**1**
Caryophyllaceae	*Silene latifolia*	1		1	**2**
Caryophyllaceae	*Stellaria media*			1	**1**
Cistaceae	*Fumana ericoides*			1	**1**
Cistaceae	*Fumana procumbens*			1	**1**
Cistaceae	*Helianthemum apenninum*		1	1	**2**
Cistaceae	*Helianthemum nummularium*		1	1	**2**
Cistaceae	*Helianthemum oelandicum*		1	1	**2**
Cistaceae	*Helianthemum salicifolium*			1	**1**
Convolvulaceae	*Convolvulus arvensis*	1			**1**
Convolvulaceae	*Convolvulus cantabrica*			1	**1**
Convolvulaceae	*Cuscuta epithymum*			1	**1**

Convolvulaceae	*Cuscuta planiflora var. godronii*			1	**1**
Crassulaceae	*Sedum acre*		1		**1**
Crassulaceae	*Sedum album subsp. micranthum*			1	**1**
Crassulaceae	*Sedum dasyphyllum*			1	**1**
Crassulaceae	*Sedum ochroleucum*			1	**1**
Crassulaceae	*Sedum sediforme*			1	**1**
Euphorbiaceae	*Euphorbia cyparissias*			1	**1**
Euphorbiaceae	*Euphorbia esula*		1		**1**
Euphorbiaceae	*Euphorbia exigua*			1	**1**
Euphorbiaceae	*Euphorbia helioscopia*			1	**1**
Euphorbiaceae	*Euphorbia nicaeensis*			1	**1**
Euphorbiaceae	*Euphorbia seguieriana*			1	**1**
Fabaceae	*Anthyllis montana*			1	**1**
Fabaceae	*Anthyllis vulneraria*	1		1	**2**
Fabaceae	*Argyrolobium zanonii*			1	**1**
Fabaceae	*Astragalus monspessulanus*		1	1	**2**
Fabaceae	*Coronilla minima*	1	1	1	**3**
Fabaceae	*Cytisophyllum sessilifolium*			1	**1**
Fabaceae	*Cytisus decumbens*			1	**1**
Fabaceae	*Dorycnium pentaphyllum*			1	**1**
Fabaceae	*Genista pilosa*			1	**1**
Fabaceae	*Genista pulchella*			1	**1**
Fabaceae	*Genista tinctoria*		1		**1**
Fabaceae	*Hippocrepis comosa*	1	1	1	**3**
Fabaceae	*Lathyrus cicera*			1	**1**
Fabaceae	*Lathyrus latifolius*		1		**1**
Fabaceae	*Lathyrus pratensis*	1			**1**
Fabaceae	*Lathyrus sphaericus*			1	**1**
Fabaceae	*Lotus corniculatus*	1	1		**2**
Fabaceae	*Lotus delortii*			1	**1**
Fabaceae	*Medicago falcata*			1	**1**
Fabaceae	*Medicago lupulina*	1		1	**2**
Fabaceae	*Medicago minima*			1	**1**

Fabaceae	*Medicago sativa*			1	**1**
Fabaceae	*Melilotus altissimus*	1			**1**
Fabaceae	*Onobrychis supina*			1	**1**
Fabaceae	*Onobrychis viciifolia*	1		1	**2**
Fabaceae	*Ononis minutissima*		1	1	**2**
Fabaceae	*Ononis natrix*		1	1	**2**
Fabaceae	*Ononis pusilla*		1	1	**2**
Fabaceae	*Ononis spinosa*	1		1	**2**
Fabaceae	*Ononis striata*			1	**1**
Fabaceae	*Trifolium campestre*			1	**1**
Fabaceae	*Trifolium incarnatum*			1	**1**
Fabaceae	*Trifolium pratense*	1			**1**
Fabaceae	*Trifolium repens*	1			**1**
Fabaceae	*Vicia cracca*	1		1	**2**
Fabaceae	*Vicia parviflora*			1	**1**
Fabaceae	*Vicia sativa*	1		1	**2**
Fabaceae	*Vicia tetrasperma*	1			**1**
Gentianaceae	*Blackstonia perfoliata*	1	1	1	**3**
Gentianaceae	*Centaurium erythraea*	1		1	**2**
Gentianaceae	*Gentianella amarella*	1			**1**
Geraniaceae	*Erodium cicutarium*			1	**1**
Geraniaceae	*Geranium columbinum*			1	**1**
Geraniaceae	*Geranium robertianum*	1			**1**
Hypericaceae	*Hypericum perforatum*	1	1	1	**3**
Lamiaceae	*Ajuga chamaepitys*			1	**1**
Lamiaceae	*Ajuga genevensis*			1	**1**
Lamiaceae	*Clinopodium acinos*		1	1	**2**
Lamiaceae	*Clinopodium vulgare*	1			**1**
Lamiaceae	*Lavandula angustifolia*			1	**1**
Lamiaceae	*Melittis melissophyllum*			1	**1**
Lamiaceae	*Origanum vulgare*	1	1		**2**
Lamiaceae	*Prunella grandiflora*		1		**1**
Lamiaceae	*Prunella vulgaris*	1	1		**2**

Lamiaceae	*Salvia pratensis*			1	**1**
Lamiaceae	*Stachys recta*		1	1	**2**
Lamiaceae	*Teucrium botrys*			1	**1**
Lamiaceae	*Teucrium chamaedrys*		1	1	**2**
Lamiaceae	*Teucrium montanum*		1	1	**2**
Lamiaceae	*Teucrium rouyanum*			1	**1**
Lamiaceae	*Thymus dolomiticus*			1	**1**
Lamiaceae	*Thymus praecox*	1	1		**2**
Lamiaceae	*Thymus pulegioides*	1			**1**
Lamiaceae	*Thymus serpyllum*			1	**1**
Lamiaceae	*Thymus vulgaris*			1	**1**
Liliaceae	*Tulipa sylvestris*			1	**1**
Linaceae	*Linum catharticum*	1	1	1	**3**
Linaceae	*Linum narbonense*			1	**1**
Linaceae	*Linum suffruticosum*			1	**1**
Linaceae	*Linum tenuifolium*		1	1	**2**
Orchidaceae	*Anacamptis coriophora*			1	**1**
Orchidaceae	*Anacamptis morio*			1	**1**
Orchidaceae	*Anacamptis pyramidalis*	1	1	1	**3**
Orchidaceae	*Epipactis atrorubens*		1		**1**
Orchidaceae	*Gymnadenia conopsea*	1	1		**2**
Orchidaceae	*Himantoglossum hircinum*	1	1	1	**3**
Orchidaceae	*Neotinea ustulata*			1	**1**
Orchidaceae	*Neottia ovata*	1			**1**
Orchidaceae	*Ophrys apifera*	1	1	1	**3**
Orchidaceae	*Ophrys fuciflora*		1		**1**
Orchidaceae	*Ophrys insectifera*	1		1	**2**
Orchidaceae	*Ophrys scolopax*			1	**1**
Orchidaceae	*Ophrys sphegodes*	1	1		**2**
Orchidaceae	*Orchis anthropophora*			1	**1**
Orchidaceae	*Orchis cf militaris*	1			**1**
Orchidaceae	*Orchis mascula*			1	**1**
Orchidaceae	*Orchis purpurea*	1	1		**2**

Orchidaceae	*Orchis simia*			1	**1**
Orchidaceae	*Orchis x bergonii*			1	**1**
Orchidaceae	*Platanthera bifolia*			1	**1**
Orchidaceae	*Platanthera chlorantha*	1	1	1	**3**
Orchidaceae	*Spiranthes spiralis*			1	**1**
Orobanchaceae	*Euphrasia nemorosa*		1		**1**
Orobanchaceae	*Euphrasia salisburgensis*			1	**1**
Orobanchaceae	*Euphrasia stricta*	1	1	1	**3**
Orobanchaceae	*Odontites verna*	1	1		**2**
Orobanchaceae	*Orobanche gracilis*		1		**1**
Orobanchaceae	*Orobanche minor*		1		**1**
Orobanchaceae	*Rhinanthus alectorolophus*			1	**1**
Orobanchaceae	*Rhinanthus mediterraneus*			1	**1**
Orobanchaceae	*Rhinanthus minor*			1	**1**
Papaveraceae	*Corydalis solida*			1	**1**
Papaveraceae	*Papaver rhoeas*			1	**1**
Plantaginaceae	*Chaenorhinum origanifolium*			1	**1**
Plantaginaceae	*Chaenorhinum rubrifolium*			1	**1**
Plantaginaceae	*Globularia vulgaris*		1	1	**2**
Plantaginaceae	*Kickxia spuria*			1	**1**
Plantaginaceae	*Linaria repens*	1			**1**
Plantaginaceae	*Linaria supina*			1	**1**
Plantaginaceae	*Plantago lanceolata*	1		1	**2**
Plantaginaceae	*Plantago media*	1		1	**2**
Plantaginaceae	*Veronica austriaca subsp. teucrium*		1	1	**2**
Plantaginaceae	*Veronica chamaedrys*	1			**1**
Plantaginaceae	*Veronica persica*	1		1	**2**
Plantaginaceae	*Veronica serpillyfolia*			1	**1**
Plumbaginaceae	*Armeria arenaria*			1	**1**
Polygalaceae	*Polygala calcarea / vulgaris*	1	1	1	**3**
Primulaceae	*Anagallis arvensis*			1	**1**
Primulaceae	*Primula veris*	1	1	1	**3**
Ranunculaceae	*Anemone hepatica*			1	**1**

Ranunculaceae	*Anemone pulsatilla*		1		**1**
Ranunculaceae	*Helleborus foetidus*		1	1	**2**
Ranunculaceae	*Helleborus viridis*			1	**1**
Ranunculaceae	*Ranunculus bulbosus*	1	1	1	**3**
Ranunculaceae	*Ranunculus gramineus*			1	**1**
Resedaceae	*Reseda lutea*	1	1	1	**3**
Resedaceae	*Reseda phyteuma*			1	**1**
Rosaceae	*Agrimonia eupatoria*	1			**1**
Rosaceae	*Geum urbanum*	1			**1**
Rosaceae	*Potentilla neumanniana*		1	1	**2**
Rosaceae	*Potentilla reptans*	1			**1**
Rosaceae	*Sanguisorba minor*	1	1	1	**3**
Rubiaceae	*Asperula cynanchica*	1	1	1	**3**
Rubiaceae	*Cruciata laevipes*	1			**1**
Rubiaceae	*Galium conf. timeroyi subsp. fleuroti*	1			**1**
Rubiaceae	*Galium lucidum subsp. corrudifolium*			1	**1**
Rubiaceae	*Galium mollugo*	1	1		**2**
Rubiaceae	*Galium pumilum*	1	1	1	**3**
Rubiaceae	*Galium verum*	1			**1**
Rubiaceae	*Sherardia arvensis*			1	**1**
Santalaceae	*Thesium humifusum*		1		**1**
Saxifragaceae	*Saxifraga tridactylites*		1	1	**2**
Scrophulariaceae	*Verbascum sp.*	1			**1**
Verbenaceae	*Verbena officinalis*			1	**1**
Violaceae	*Viola cf reichenbachiana*			1	**1**
Violaceae	*Viola pseudomirabilis*			1	**1**
Violaceae	*Viola suavis*			1	**1**
Xanthorrhoeaceae	*Asphodelus albus*			1	**1**
Total Family (n = 38)	**Total species (n = 288)**	**94**	**92**	**221**	

Annex 3. 2. List of species for the group Anthophila (Superfamily Apoidea). *This list shows the totality of bee species (separated in Family and Genus) sampled using both hand net and pan traps in the two years of fieldwork. For each Family, Genus and species we reported the abundances in the three regions Hauts-de-France (HFR), Normandie (NOR) and Occitanie (OCC).*

Family/Genus/Species	HFR	NOR	OCC	Total (abundance)
Andrenidae	**334**	**357**	**498**	**1189**
Andrena	**328**	**300**	**498**	**1126**
Andrena alutacea	1	1		2
Andrena angustior	1			1
Andrena bicolor		4	2	6
Andrena carantonica	8	1	9	18
Andrena chrysopyga			1	1
Andrena chrysosceles	2			2
Andrena cineraria	30	9		39
Andrena cinerea			2	2
Andrena combinata			12	12
Andrena curvungula			2	2
Andrena distinguenda			1	1
Andrena dorsata	2	8	14	24
Andrena fabrella			16	16
Andrena flavipes	55	52	4	111
Andrena florivaga			4	4
Andrena fulva	4		7	11
Andrena fulvago		1		1
Andrena fulvata	2	1		3
Andrena granulosa			5	5
Andrena gravida	28	26	25	79
Andrena haemorrhoa	13	3	50	66
Andrena hattorfiana	7		1	8
Andrena hesperia			1	1
Andrena humilis	12	30	11	53
Andrena labialis			10	10
Andrena labiata	1		9	10
Andrena minutula	42	30	21	93

Andrena minutuloides	21	4		25
Andrena nigroaenea	70	107	61	238
Andrena nitida	7		18	25
Andrena nitidula			2	2
Andrena ovatula	3	1	41	45
Andrena pandellei			1	1
Andrena paucisquama			16	16
Andrena polita		10		10
Andrena potentillae			4	4
Andrena propinqua	1	2	3	6
Andrena rhenana			52	52
Andrena rufula			5	5
Andrena saxonica			16	16
Andrena semilaevis	2			2
Andrena similis			6	6
Andrena simontornyella			23	23
Andrena sp.		3	1	4
Andrena strohmella	2	1		3
Andrena subopaca	2	3	1	6
Andrena vaga		2		2
Andrena variabilis			1	1
Andrena ventricosa			1	1
Andrena vulpecula			14	14
Andrena wilkella	12	1	25	38
Panurgus	**6**	**57**		**63**
Panurgus calcaratus		1		1
Panurgus dentipes	6	56		62
Apidae	**1100**	**1361**	**1003**	**3464**
Amegilla			**5**	**5**
Amegilla albigena			4	4
Amegilla magnilabris			1	1
Anthophora	**5**	**35**	**53**	**93**
Anthophora aestivalis			39	39

Anthophora affinis			1	1
Anthophora crassipes			1	1
Anthophora dispar			1	1
Anthophora larvata			8	8
Anthophora plumipes		13	2	15
Anthophora quadrimaculata		8		8
Anthophora retusa	5	14		19
Anthophora salviae			1	1
Apis	**30**	**154**	**208**	**392**
Apis mellifera	30	154	208	392
Bombus	**873**	**1069**	**312**	**2254**
Bombus barbutellus		1	1	2
Bombus gr. terrestris	55	134	67	256
Bombus hortorum	22	3	12	37
Bombus humilis			2	2
Bombus hypnorum	3	23		26
Bombus lapidarius	420	416	16	852
Bombus lucorum	7	23	9	39
Bombus magnus	1			1
Bombus pascuorum	305	341	34	680
Bombus pratorum	4	27	18	49
Bombus ruderarius		1		1
Bombus ruderatus			59	59
Bombus rupestris	1			1
Bombus sp.		1		1
Bombus sylvarum		1	42	43
Bombus sylvestris	2			2
Bombus terrestris	30	75	51	156
Bombus vestalis	23	23	1	47
Ceratina	**52**	**36**	**110**	**198**
Ceratina chalcites			3	3
Ceratina chalybea			7	7
Ceratina cucurbitina		27	18	45

Ceratina cyanea	52	8	59	119
Ceratina dallatorreana			3	3
Ceratina dentiventris			10	10
Ceratina gravidula			4	4
Ceratina mocsaryi			3	3
Ceratina nigrolabiata			3	3
Ceratina sp.		1		1
Epeolus		**19**	**2**	**21**
Epeolus cruciger		7		7
Epeolus variegatus		12	2	14
Eucera		**2**	**224**	**226**
Eucera (Cubitalia) sp.			3	3
Eucera caspica			66	66
Eucera cineraria			5	5
Eucera clypeata			2	2
Eucera hungarica			4	4
Eucera interrupta			7	7
Eucera nigrescens		2	136	138
Eucera taurica			1	1
Melecta		**2**		**2**
Melecta albifrons		2		2
Nomada	**140**	**43**	**21**	**204**
Nomada agrestis			1	1
Nomada armata			2	2
Nomada bifasciata	18	9		27
Nomada braunsiana			1	1
Nomada cf. distinguenda		1		1
Nomada discedens			1	1
Nomada distinguenda	1			1
Nomada fabriciana	6	1	2	9
Nomada flava	14			14
Nomada flavoguttata	45	11	1	57
Nomada fucata	8	2		10

Nomada goodeniana	17			17
Nomada lathburiana	10	3		13
Nomada maculicornis			1	1
Nomada marshamella	3			3
Nomada mutica		1		1
Nomada panurgina		1		1
Nomada panzeri	2			2
Nomada panzeri/signata	1			1
Nomada ruficornis	11		4	15
Nomada sexfasciata			1	1
Nomada sheppardana	3			3
Nomada striata	1			1
Nomada succincta		8	7	15
Nomada zonata		6		6
Tetraloniella			**35**	**35**
Tetraloniella fulvescens			35	35
Xylocopa		**1**	**33**	**34**
Xylocopa iris			1	1
Xylocopa vaga			1	1
Xylocopa violacea		1	31	32
Colletidae	**42**	**141**	**221**	**404**
Colletes	**10**	**48**	**96**	**154**
Colletes abeillei			1	1
Colletes cunicularius	4	3		7
Colletes foveolaris			2	2
Colletes gallicus			15	15
Colletes hederae	6	10		16
Colletes hylaeiformis			1	1
Colletes similis		35	77	112
Hylaeus	**32**	**93**	**125**	**250**
Hylaeus angustatus		3	1	4
Hylaeus brevicornis agg.	9	4	3	16
Hylaeus cf. imparilis			16	16

Hylaeus clypearis		5	3	8
Hylaeus communis	2	4	7	13
Hylaeus dilatatus	14	5		19
Hylaeus gibbus agg.	1	10	6	17
Hylaeus gr. conformis		1		1
Hylaeus hyalinatus		8	17	25
Hylaeus lineolatus			51	51
Hylaeus nigritus		22		22
Hylaeus punctatus/hyalinatus			1	1
Hylaeus punctulatissimus		24	13	37
Hylaeus signatus	6	6	4	16
Hylaeus sp.			1	1
Hylaeus variegatus		1	2	3
Halictidae	**2149**	**1679**	**1913**	**5741**
Halictus	**616**	**276**	**432**	**1324**
Halictus cochleariatarsis			1	1
Halictus fulvipes			3	3
Halictus maculatus	1	14	3	18
Halictus patella			3	3
Halictus patellatus			20	20
Halictus quadricinctus		1	5	6
Halictus rubicundus	133	4	3	140
Halictus scabiosae	4	50	102	156
Halictus sexcinctus		2		2
Halictus simplex		67	48	115
Halictus sp.			1	1
Seladonia kessleri			4	4
Seladonia smaragdula			117	117
Seladonia subaurata		1	60	61
Seladonia submediterranea		4	62	66
Seladonia tumulorum	478	133		611
Lasioglossum	**1390**	**1386**	**1448**	**4224**
Lasioglossum aeratum		3	6	9

Lasioglossum albipes	14	18	50	82
Lasioglossum albocinctum			9	9
Lasioglossum bimaculatum			63	63
Lasioglossum bluethgeni		1		1
Lasioglossum brevicorne			1	1
Lasioglossum calceatum	80	89		169
Lasioglossum clypeare			3	3
Lasioglossum corvinum			3	3
Lasioglossum costulatum		17	17	34
Lasioglossum discum			4	4
Lasioglossum fulvicorne	122	11	1	134
Lasioglossum glabriusculum		1	8	9
Lasioglossum griseolum	1		118	119
Lasioglossum interruptum		319	86	405
Lasioglossum laevigatum	2		76	78
Lasioglossum laticeps	90	22	7	119
Lasioglossum lativentre	3	1		4
Lasioglossum leucopus	1			1
Lasioglossum leucozonium	15	21	12	48
Lasioglossum lineare	4	36	19	59
Lasioglossum malachurum	83	37	327	447
Lasioglossum marginatum			3	3
Lasioglossum maurusium			1	1
Lasioglossum medinai		1	1	2
Lasioglossum mediterraneum			19	19
Lasioglossum minutissimum		12		12
Lasioglossum morio	628	556	24	1208
Lasioglossum nigripes		1	42	43
Lasioglossum nitidulum	1	58		59
Lasioglossum pallens	1	1	2	4
Lasioglossum parvulum	1		1	2
Lasioglossum pauxillum	146	15	47	208
Lasioglossum politum		87		87

Lasioglossum punctatissimum	4		29	33
Lasioglossum puncticolle		1	1	2
Lasioglossum pygmaeum		6	4	10
Lasioglossum quadrinotatum			2	2
Lasioglossum quadrisignatum			1	1
Lasioglossum semilucens	1			1
Lasioglossum setulellum			4	4
Lasioglossum smeathmanellum			1	1
Lasioglossum sp.		2	4	6
Lasioglossum subhirtum	21	5	280	306
Lasioglossum submediterraneum		2		2
Lasioglossum transitorium planulum			7	7
Lasioglossum tricinctum			1	1
Lasioglossum villosulum	157	46	13	216
Lasioglossum xanthopus	15	16	150	181
Lasioglossum zonulum		1	1	2
Nomiapis			**5**	**5**
Nomiapis diversipes			5	5
Rophites			**12**	**12**
Rophites algirus			12	12
Sphecodes	**143**	**17**	**16**	**176**
Sphecodes albilabris	3	1	2	6
Sphecodes crassanus			1	1
Sphecodes crassus	15	1		16
Sphecodes ephippius	7	5	8	20
Sphecodes ferruginatus	30	1		31
Sphecodes geofrellus	1			1
Sphecodes gibbus	16	1	1	18
Sphecodes hyalinatus	36			36
Sphecodes monilicornis	8	5	1	14
Sphecodes niger	16	3		19
Sphecodes pseudofasciatus			1	1
Sphecodes ruficrus	2			2

Sphecodes schencki			1	1
Sphecodes sp.	9		1	10
Megachilidae	**380**	**367**	**551**	**1298**
Aglaoapis		**1**		**1**
Aglaoapis tridentata		1		1
Anthidiellum	**20**	**4**	**30**	**54**
Anthidiellum strigatum	20	4	30	54
Anthidium	**4**	**23**	**60**	**87**
Anthidium cingulatum			1	1
Anthidium florentinum			2	2
Anthidium manicatum		11	13	24
Anthidium oblongatum			1	1
Anthidium punctatum	4	12	43	59
Chelostoma	**5**	**3**	**18**	**26**
Chelostoma campanularum	1	1		2
Chelostoma distinctum		1	4	5
Chelostoma florisomne	1		7	8
Chelostoma nasutum			1	1
Chelostoma rapunculi	3	1	6	10
Coelioxys		**23**	**12**	**35**
Coelioxys afrus		10	7	17
Coelioxys conica		2		2
Coelioxys conoidea		11	4	15
Coelioxys haemorrhoa			1	1
Heriades	**1**	**8**	**18**	**27**
Heriades crenulatus			16	16
Heriades truncorum	1	8	2	11
Hoplitis	**23**	**32**	**18**	**73**
Hoplitis adunca		16	1	17
Hoplitis brachypogon			3	3
Hoplitis claviventris	11			11
Hoplitis leucomelana	12	16	2	30
Hoplitis papaveris			9	9

Hoplitis praestans			3	3
Lithurgus			**1**	**1**
Lithurgus chrysurus			1	1
Megachile	**9**	**85**	**168**	**262**
Megachile albisecta			8	8
Megachile centuncularis	1	3	6	10
Megachile circumcincta			16	16
Megachile ericetorum		9		9
Megachile flabellipes			3	3
Megachile giraudi			1	1
Megachile lagopoda		19	1	20
Megachile maritima		2	20	22
Megachile melanopyga			4	4
Megachile octosignata			15	15
Megachile papaveris			1	1
Megachile pilicrus			3	3
Megachile pilidens	1	41	73	115
Megachile pyrenaica			2	2
Megachile rotundata		2	1	3
Megachile rufescens			12	12
Megachile versicolor	3	2		5
Megachile willughbiella	4	7	2	13
Osmia	**318**	**170**	**171**	**659**
Osmia andrenoides		10	2	12
Osmia aurulenta	38	33	77	148
Osmia bicolor	177	42		219
Osmia bicornis		8		8
Osmia c.f. labiatis			1	1
Osmia caerulescens	1	2	2	5
Osmia claviventris	2			2
Osmia crenulatus			1	1
Osmia emarginata			9	9
Osmia gallarum			6	6

Osmia leaiana	4	8		12
Osmia leucomelana	2	1		3
Osmia melanogaster			1	1
Osmia niveata		1	1	2
Osmia rufohirta	68	25	34	127
Osmia scutellaris			2	2
Osmia spinulosa	26	27		53
Osmia submicans		13	13	26
Osmia tergestensis			5	5
Osmia versicolor			1	1
Osmia viridina			7	7
Osmia viridina/versicolor			9	9
Pseudoanthidium		**12**	**15**	**27**
Pseudoanthidium melanurum			1	1
Pseudoanthidium reticulatum			7	7
Pseudoanthidium sp.		12	7	19
Rhodanthidium			**16**	**16**
Rhodanthidium infuscatum			3	3
Rhodanthidium septemdentatum			9	9
Rhodanthidium sticticum			4	4
Stelis		**1**	**4**	**5**
Stelis phaeoptera		1		1
Stelis punctulatissima			1	1
Stelis signata			3	3
Trachusa		**5**	**20**	**25**
Trachusa byssina		5	20	25
Melittidae	**32**	**22**	**21**	**75**
Dasypoda		**2**		**2**
Dasypoda hirtipes		2		2
Melitta	**32**	**20**	**21**	**73**
Melitta dimidiata			21	21
Melitta haemorrhoidalis	19	10		29
Melitta leporina		1		1

Melitta tricincta	13	9		22
Total abundance	**4037**	**3927**	**4207**	**12171**

Annex 3. 3. List of species of the Family Syrphidae. *This list shows the totality of hoverfly species (separated in Family and Genus) sampled using both hand net and pan traps in the two years of fieldwork. For each Family, Genus and species we reported the abundances in the three regions Hauts-de-France (HFR), Normandie (NOR) and Occitanie (OCC).*

Family/Genus/Species	HFR	NOR	OCC	Total (abundance)
Syrphidae	**1120**	**1408**	**1003**	**3531**
Callicera			**2**	**2**
Callicera aurata			1	1
Callicera macquarti			1	1
Cheilosia	**28**	**10**	**42**	**80**
Cheilosia albitarsis	4			4
Cheilosia albitarsis/ranunculii	12		3	15
Cheilosia mutabilis			3	3
Cheilosia pagana	3			3
Cheilosia ranunculii	1			1
Cheilosia scutellata		2	2	4
Cheilosia soror	2	3	4	9
Cheilosia sp.	1		1	2
Cheilosia urbana		4	29	33
Cheilosia vernalis	5	1		6
Chrysotoxum	**3**	**10**	**32**	**45**
Chrysotoxum bicinctum	3	4	4	11
Chrysotoxum cautum			8	8
Chrysotoxum cisalpinum			9	9
Chrysotoxum elegans		5	4	9
Chrysotoxum octomaculatus		1	2	3
Chrysotoxum vernale			1	1
Chrysotoxum intermedium			4	4
Dasysyrphus		**1**	**5**	**6**
Dasysyrphus albostriatus		1	5	6
Epistrophe		**1**		**1**

Epistrophe nitidicollis		1		1
Episyrphus	**85**	**219**	**15**	**319**
Episyrphus balteatus	85	219	15	319
Eristalinus	**1**			**1**
Eristalinus sepulchralis	1			1
Eristalis	**149**	**236**	**74**	**459**
Eristalis arbustorum	31	25	7	63
Eristalis horticola	2			2
Eristalis nemorum	21	3		24
Eristalis pertinax	3	5		8
Eristalis similis			3	3
Eristalis tenax	92	203	64	359
Eumerus	**15**	**2**	**8**	**25**
Eumerus amoenus		1		1
Eumerus basalis			2	2
Eumerus clavatus			3	3
Eumerus clavatus/uncipes			1	1
Eumerus hungaricus			1	1
Eumerus ornatus			1	1
Eumerus sp.	2			2
Eumerus strigatus	10			10
Eumerus strigatus/sogdanius	3			3
Eumerus tricolor		1		1
Eupeodes	**55**	**85**	**72**	**212**
Eupeodes corollae	42	56	56	154
Eupeodes latifasciatus	8	10	2	20
Eupeodes luniger	5	19	14	38
Ferdinandea		**10**	**14**	**24**
Ferdinandea aurea			14	14
Ferdinandea cuprea		10		10
Helophilus	**39**	**160**	**1**	**200**
Helophilus hybridus		2		2
Helophilus pendulus	29	109	1	139

Helophilus trivittatus	10	49		59
Melanostoma	**77**	**32**	**39**	**148**
Melanostoma mellinum	74	28	32	134
Melanostoma scalare	2	3	7	12
Melanostoma sp.	1	1		2
Meliscaeva		**7**	**32**	**39**
Meliscaeva auricollis		7	32	39
Merodon	**4**	**38**	**233**	**275**
Merodon albifrons			84	84
Merodon avidus			10	10
Merodon cf. obscuritarsis			1	1
Merodon elegans			1	1
Merodon equestris		2	25	27
Merodon geniculatus			57	57
Merodon moenium			20	20
Merodon nigritarsis			8	8
Merodon rufus		30	4	34
Merodon serrulatus			5	5
Merodon sp.	4	6	18	28
Microdon			**4**	**4**
Microdon analis			4	4
Milesia			**5**	**5**
Milesia craboniformis			4	4
Milesia semiluctifera			1	1
Myathropa	**1**	**4**	**1**	**6**
Myathropa florea	1	4	1	6
Neoascia	**17**	**1**		**18**
Neoascia podagrica	17	1		18
Orthonevra	**1**			**1**
Orthonevra nobilis	1			1
Paragus	**4**	**36**	**42**	**82**
Paragus albifrons			1	1
Paragus bicolor		1	1	2

Paragus haemorrhous	2	7	7	16
Paragus pecchioli		2		2
Paragus quadrifasciatus		1		1
Paragus sp.	2	12	19	33
Paragus tibialis		13	14	27
Pelecocera		**1**	**12**	**13**
Pelecocera pruinosomaculata			12	12
Pelecocera tricincta		1		1
Pipiza	**1**	**1**		**2**
Pipiza austriaca		1		1
Pipiza sp.	1			1
Pipizella	**5**	**29**	**83**	**117**
Pipizella annulata	2	2		4
Pipizella divicoi		1	2	3
Pipizella sp.		22	41	63
Pipizella viduata	3	4		7
Pipizella virens			1	1
Pipizella zeneggenensis			39	39
Platycheirus	**29**	**7**	**18**	**54**
Platycheirus albimanus	22	5	8	35
Platycheirus albimanus/muelleri			5	5
Platycheirus clypeatus	7			7
Platycheirus scutatus		2		2
Platycheirus sp.			5	5
Rhingia	**75**	**3**		**78**
Rhingia campestris	75	3		78
Scaeva	**9**	**14**	**4**	**27**
Scaeva dignota			3	3
Scaeva pyrastri	9	13	1	23
Scaeva selenitica		1		1
Sericomyia	**1**	**1**		**2**
Sericomyia silentis	1	1		2
Sphaerophoria	**384**	**420**	**206**	**1010**

Sphaerophoria scripta	238	224	100	562
Sphaerophoria sp.	143	196	106	445
Sphaerophoria taeniata	3			3
Syritta	**73**	**45**	**5**	**123**
Syritta pipiens	73	45	5	123
Syrphus	**39**	**26**	**45**	**110**
Syrphus ribesii	38	17	17	72
Syrphus torvus			7	7
Syrphus vitripennis	1	9	21	31
Volucella	**3**	**8**	**1**	**12**
Volucella bombylans	2	1		3
Volucella inanis	1	4		5
Volucella pellucens		1		1
Volucella zonaria		2	1	3
Xanthogramma	**19**	**1**	**8**	**28**
Xanthogramma citrofasciatum	19		8	27
Xanthogramma dives		1		1
Xylota	**3**			**3**
Xylota segnis	3			3
Total abundance	**1120**	**1408**	**1003**	**3531**

Annex II

Inférence des facteurs affectant la structure des réseaux plantes-pollinisateurs : le cas des réseaux plantes-syrphes le long d'un gradient latitudinal de pelouses calcaires en France

Extract from Adrien Berquer's Master Dissertation

Glossaire

ACP : Analyse en Composantes Principales

AIC : Akaike Information Criterion – Critère d'Information d'Akaike

BIC : Bayesian Information Criterion – Critère d'Information Bayésien

COI : Cytochrome Oxydase-I – Séquence d'ADN mitochondrial conservée permettant de créer les arbres phylogénétiques des métazoaires

ERGM : Exponential Random Graph Models – Modèles exponentiels de graphes aléatoires

MEM : Moran Eigenvectors Maps – Ensemble de vecteurs contenant les valeurs propres issus d'une décomposition d'une matrice de distance géographique

PCNM : Principal Coordinate analysis of Neighbour Matrice – Analyse de coordonnées principales de matrices de distance

RDA : Redundancy Analysis – Analyse de redondance

SVD : Singular Value Decomposition – Décomposition d'une matrice en une triplette de matrices (orthogonale, diagonale, orthogonale). Les valeurs de la matrice diagonale ainsi obtenue sont les valeurs singulières.

Matériel et Méthodes

Échantillonnage

L'étude effectuée durant le stage porte sur les interactions observées sur le terrain entre des syrphidés et des angiospermes herbacés sur six sites répartis dans trois régions : Normandie, Hauts-de-France et Occitanie (Fig.1). Chaque site a été visité une fois par mois d'avril à octobre 2016, à l'exception du site des Riez (mai à octobre 2016). Seules les espèces pour lesquelles une interaction a été constatée sont incluses dans l'analyse, qui porte donc sur 77 taxons de syrphes et 116 de plantes (Tabl.1).

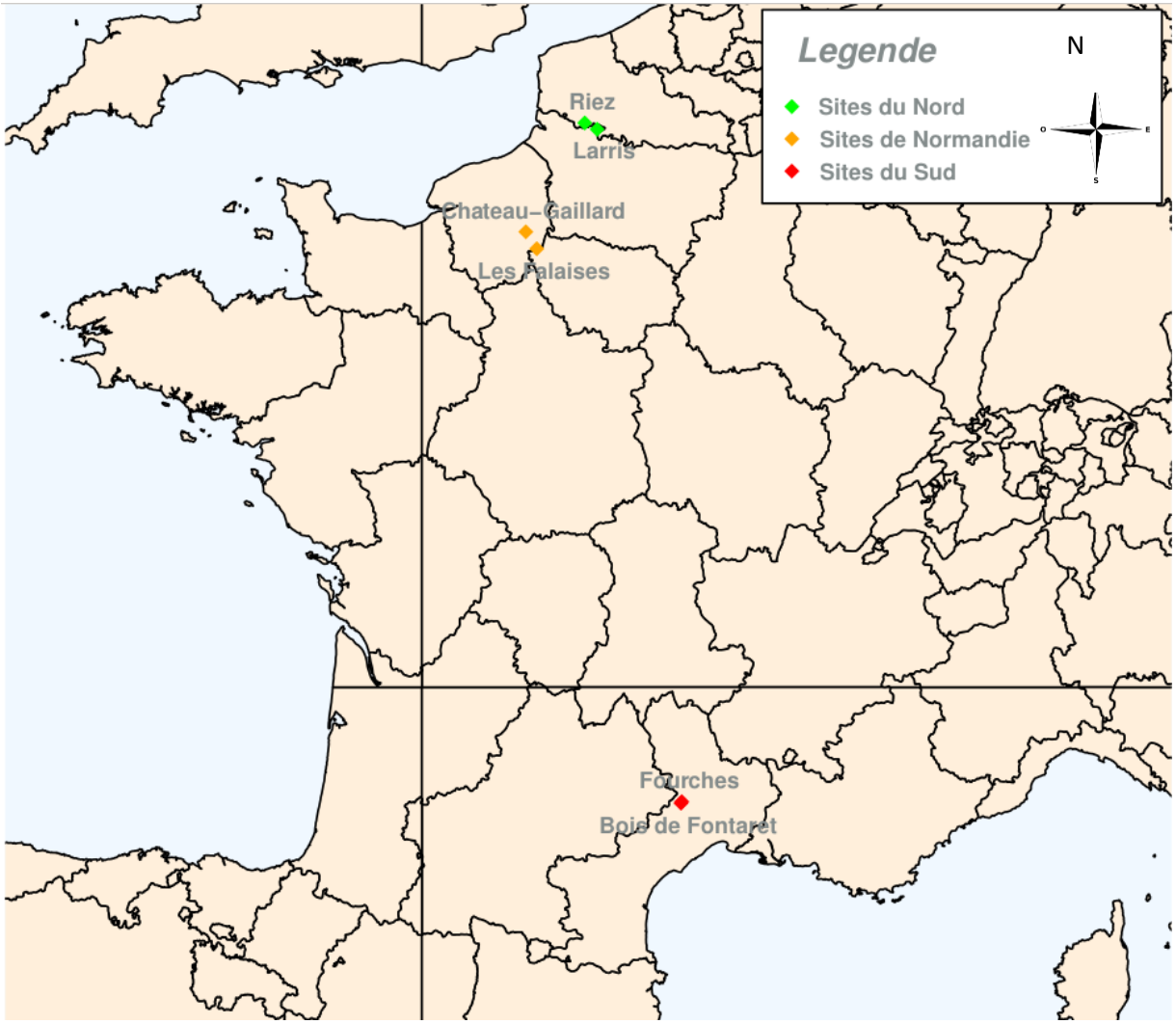

Figure 1 : Carte représentant la localisation des sites échantillonnés. En vert, dans les Hauts-de-France, en jaune, en Normandie et en rouge, en Occitanie. Deux sites par région sont sélectionnés, les régions sont sélectionnées selon un gradient latitudinal.

Localisation	Région	Occitanie		Normandie		Hauts-de-France		TOTAL
	Site (Abbréviation)	Bois de Fontaret (BF)	Fourches (F)	Château-Gaillard (CG)	Les Falaises (FAL)	Le Larris (LAR)	Le Riez (R)	
Nombre d'espèces de syrphes interagissant		40	36	32	34	24	22	77
Nombre d'espèces de syrphes observées		42	37	32	34	24	23	
Nombre d'espèces de plantes interagissant		43	49	25	30	33	29	116
Nombre d'espèces de plantes observées		137	202	94	83	74	74	
Nombre de paires d'espèces interagissant		120	143	103	128	105	104	561
Nombre d'interactions		198	286	297	374	223	293	1671

Tableau 1 : Caractéristiques par site des richesses spécifiques en syrphes et en plantes, et de leurs interactions respectives. Le total correspond au nombre d'espèces ou de paires d'espèces différentes déterminées.

Données

Phénologie

Nous avons déterminé la phénologie des syrphes à partir d'une base de données (Speight *et al.*, 2016) et d'observations de terrain. La phénologie des plantes, quant à elle, représente la période durant laquelle une floraison a été observée sur le site. La phénologie ne s'étend donc que sur les mois pour lesquels il y a eu un échantillonnage, soit d'avril (ou mai) à octobre.

Traits

Les données portant sur les traits de chaque taxon de syrphe étudié ont été obtenues de la bibliographie (Speight *et al.*, 2016), dans laquelle nous avons relevé le régime alimentaire larvaire et adulte, le lieu d'activité larvaire, la longueur des adultes, les stade et lieu d'hibernation de l'espèce, sa durée de développement, le commensalisme et le voltinisme (Tabl.2).

Regroupés pour l'analyse spatiale, ces traits sont divisés en deux tableaux distincts pour les analyses par site : l'un ne prenant en compte que la taille, l'autre regroupant le reste des traits, liés au comportement de l'espèce.

Les traits des plantes ont été répartis dans deux groupes différents : la morphologie, principalement florale, et l'habitat, obtenus après collecte de données de la bibliographie (Julve, 1998 ; Lambinon *et al.*, 2012 ; Tison *et al.*, 2014; Tison et de Foucault, 2014 ; Le Driant, 2018 ; Piquot Y. et Hautekeète N., *comm. pers.*, 2018 ; Tela Botanica, 2018). Parmi les données disponibles, nous avons sélectionné le mode de pollinisation, la forme, la couleur et le ton de l'inflorescence, ainsi que la forme de la corolle (Tabl.2). Bien qu'on ne travaille que sur des pelouses calcaires, les plantes qu'on peut y retrouver sont tolérantes à différents facteurs climatiques et géologiques. Nous avons donc décidé d'inclure dans l'analyse des données d'habitat théorique (*Ibid.*) telles que le milieu, l'humidité et le pH du sol dans leur milieu typique. (Tabl.2).

Syrphes - Comportement	
Régime alimentaire larvaire	Plantes, Animaux, Micro-organismes
Régime alimentaire adulte	Nectar, Pollen
Lieu d'activité larvaire	Plantes, Arbres, Lianes, Ourlets, Débris, Nids, Racines, Plantes aquatiques, Sédiments submergés, Sol saturé en eau
Stade d'hibernation	Larve, Pupe, Adulte
Lieu d'hibernation	Au dessus du sol, Surface du sol, Racines, Sol saturé en eau, Nids, Eaux
Durée de développement	< 2 mois, 2 à 6 mois, 7 à 12 mois, > 1 an
Commensalisme	Absent, Partiel, Total
Voltinisme	<1, 1, 2, >2 générations par an

Syrphes - Taille	
Taille du corps de l'adulte	Taille possible entre 3 et 25 mm, précision à 0.5mm

Plantes - Morphologie	
Pollinisation	Anémogame, Apogame, Autogame, Entomogame
Forme (inflorescence)	Dense (capitule), Plate (ombelle), Longue (épi, grappe), Autre

Couleur	Blanc, Bleu, Jaune, Mauve, Marron, Rose, Vert, Violet
Ton	Clair, Neutre, Foncé
Forme (corolle)	Actinomorphe, Zygomorphe

Plantes - Habitat

Milieu	Anthropique, Broussaille, Humide, Pelouse, Prairie, Rocaille
Humidité du sol	Hygrophile, Mésophile, Xérophile
pH du sol	Acide, Neutre, Basique

Tableau 2 : Présentation des modalités de traits prises en compte dans chaque facteur concernant un trait d'espèce : « Comportement des syrphes », « Taille des syrphes », « Morphologie des plantes » et « Habitat optimal des plantes »

Lors de l'analyse prenant en compte le facteur « spatial », les traits « habitat » et « morphologie » sont regroupés en un seul facteur. Ces traits sont codés sous forme de quatre matrices binaires : deux pour les syrphes (« Comportement » et « Taille »), et deux pour les plantes (« Morphologie et Habitat optimal »). Chaque modalité de trait est une colonne binaire de la matrice répondant à la question : « L'espèce considérée a-t-elle cette modalité ? ». Les traits rapportés dans cette partie s'appliquent pour l'espèce donnée, quelle que soit la station.

Phylogénie

Syrphes

Nous avons récupéré les séquences du gène de la Cytochrome oxydase I (COI) pour permettre la création d'un arbre phylogénétique. Des séquences de 651 nucléotides (Ståhls *et al.*, 2003, Young *et al.* 2016) publiées dans Genbank ont été utilisées. Si l'espèce n'est pas dans Genbank, d'autres espèces du genre sont utilisées pour pouvoir insérer une espèce proche phylogénétiquement. Aucune séquence n'étant disponible pour *Myathropa florea*, nous l'avons regroupé avec les taxons les plus proches, en l'occurence les *Helophilus* avec qui *Myathropa* partage la sous-tribu des Helophinae (Thompson, 2000). De même, le genre *Pelecocera* a été placé au même rang que les autres *Rhingiini*. Les genres étant bien placés mais les espèces souvent non différenciés, notre arbre est précis jusqu'aux genres. Il a été créé avec le logiciel Seaview v.6.4.6 (Gouy *et al.*, 2010) selon la méthode s'approchant

de Young *et al.* (2016). Les nucléotides utilisés dans l'analyse sont ceux des premières et deuxièmes bases du codon. Le modèle GTR a été choisi avec un bootstrap de 80 réplicats. Les fréquences nucléotidiques et le taux de variation entre sites pris en compte dans l'analyse sont optimisés automatiquement, et les sites invariants sont fixés à 0.2.

Plantes

Nous utilisons pour la phylogénie des plantes l'arbre « Daphne », créé par Durka et Michalski (2012) qui regroupe 4685 espèces végétales d'Allemagne, des Îles Britanniques, des Pays-Bas et de Suisse. Neuf espèces de notre étude, la plupart méditerranéennes, n'en font cependant pas partie, mais nous pouvons en inclure certaines, grâce à des publications concernant d'autres espèces phylogénétiquement proches déjà référencées dans Daphne :

- *Euphorbia nicaeensis* au même rang que *E. falcata*, *E. seguieriana* et *E. myrsinites* (Frajman et Schönswetter, 2013)

- *Inula montana* au même rang que *I. conyzae* et *I. britannica* (Englund *et al.* 2009)

- *Leucanthemum graminifolium* au même rang que *L. halleri* (Konowalik *et al.* 2015)

- *Linum suffruticosum* au même rang que *L. flavum*, proche de *L. catharticum* (McDill, 2009)

- *Helichrysum stoechas* au même rang que *H. arenarium* et *Anaphalis margaritacea* (Galbany-Casals *et al.* 2009)

- *Arenaria aggregata* au même rang que *A. biflora* (Sadeghian *et al.* 2015)

- *Brassica repanda* au même rang que *Eruscastrum nasturtiifolium* et *Raphanus raphanistrum* (Arias et Pires, 2009)

- *Rhinanthus pumilus* au même rang que le seul autre *Rhinanthus* de Daphne (Bennett et Mathews, 2006)

Seule l'espèce *Thymus dolomiticus* ne comporte ni phylogénie publiée, ni séquence disponible et l'espèce est insérée au même rang que *T. vulgaris* et *T. praecox,* déjà inclus dans l'arbre.

Mise en forme des données

Analyser un réseau d'interaction nécessite de pouvoir le mettre dans une forme analysable par les logiciels de statistiques et de modélisations (Fig.2,3). Bien qu'ils soient couramment et intuitivement représentés par un ensemble de liens reliant des nœuds, la transformation en une matrice d'incidence est un moyen de retranscrire de façon numérique un réseau. Par analogie, les facteurs caractérisant les nœuds du réseau pourront être représentés sous forme de vecteurs. Les interactions rapportées entre plantes et pollinisateurs sont représentées dans une matrice d'incidence. Chaque interaction potentielle entre syrphe et fleur est codée en système binaire, 0 signifiant une absence d'interaction, 1 signifiant une interaction rapportée. Les interactions ne sont pas quantifiées.

L'analyse statistique va nécessiter, selon la méthode Random Dot Product Graph de Dalla Riva et Stouffer (2016), de procéder à la décomposition (SVD) de la matrice obtenue en un ensemble de vecteurs associés aux valeurs singulières de la matrice d'incidence. Le but de cette décomposition est de permettre une régression multivariée sur le réseau et de rendre les nœuds indépendants. Elle fournit trois matrices qui, après une opération algébrique simple, donne des vecteurs qui peuvent être interprétés comme des vecteurs de traits latents. Ces vecteurs sont rangés par ordre décroissant de valeurs singulières associées et permettent d'apprécier le niveau d'explication de la variance portée par la matrice d'incidence (Fig.3). Le nombre de vecteurs à conserver dans une analyse statistique similaire a été étudié par Bauman *et al.* (2018), recommandant la démarche à double critère de sélection de Blanchet *et al.* (2008). La matrice issue de cette décomposition est ensuite utilisée dans une analyse de redondance.

Les arbres phylogénétiques construits comportent davantage d'espèces que celles présentes dans chaque site et il faut donc dans un premier temps sélectionner les bonnes espèces à conserver. Nous obtenons alors un sous-arbre avec les espèces nécessaires à l'analyse. À partir du tableau de la phylogénie, une matrice de variance-covariance phylogénétique est créée, sur laquelle une décomposition en valeurs singulières (SVD, Fig.3) est effectuée, comme pour la matrice d'incidence (Dalla-Riva et Stouffer, 2016).

Les facteurs représentant les traits des plantes et syrphes, codés dans des tableaux binaires, sont ensuite réduits via une analyse en composantes principales (ACP). Pour cela on utilise la fonction PCA du package `FactoMineR` (Le *et al.*, 2008) sur les tableaux de phénologie, morphologie et habitat pour les plantes, et phénologie, comportement et taille pour les syrphes (Fig.3). Les colonnes pour lesquelles la variance est égale à zéro doivent être retirées des données à analyser, ce qui signifie que le trait est le même pour toutes les espèces de l'analyse et qu'il n'apporte aucune information.

Parmi les vecteurs propres de sortie de l'ACP de chaque trait, de la phénologie et des SVD des phylogénies, nous en gardons suffisamment pour que 85 % de la variance totale du tableau soit expliquée (Fig.2a). Cette valeur a été déterminée arbitrairement pour que les modèles puissent fonctionner. En effet, si l'on applique les critères documentés par Bauman *et al.*, 2018, trop de vecteurs sont conservés. Il en résulte que l'analyse ne peut fonctionner correctement car parmi ce grand nombre de vecteurs, certains d'entre eux risquent d'expliquer, à tort, le modèle alors qu'ils ne représentent qu'une petite partie de la variation du facteur analysé.

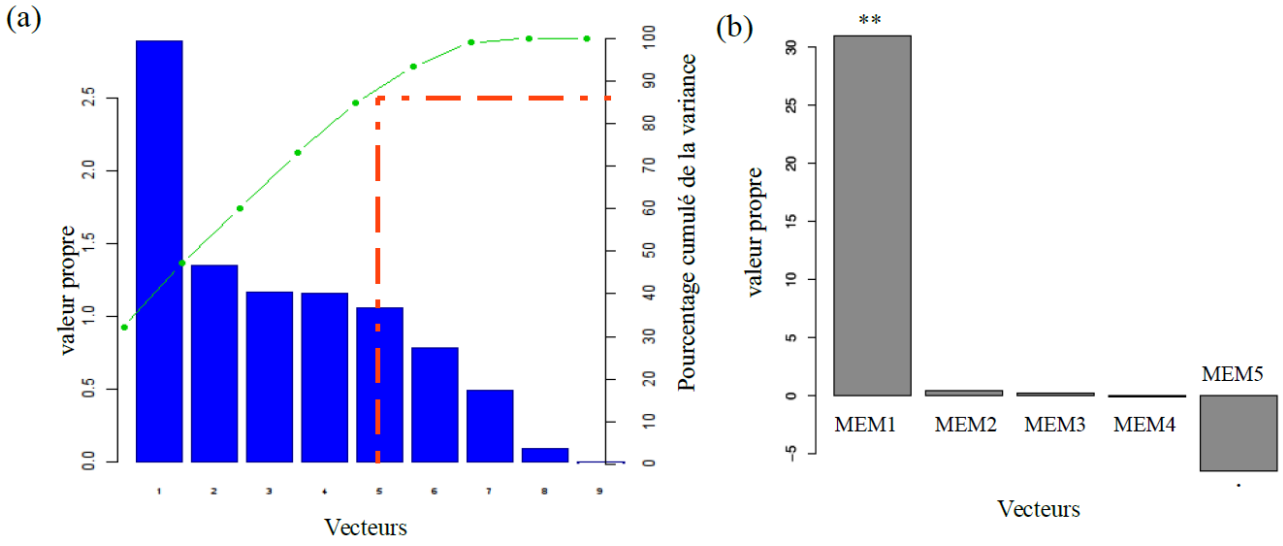

Figure 2 : Représentation graphique de la sélection du nombre de vecteurs à conserver, (a) suite à une ACP (traits, phénologie) ou une SVD (phylogénie), en prenant en compte 85 % de la variance cumulée et (b) suite à la PCNM pour le facteur « géographique », grâce au test de Mantel (**, p-value < 0.01 ; ·, p-value < 0.1).

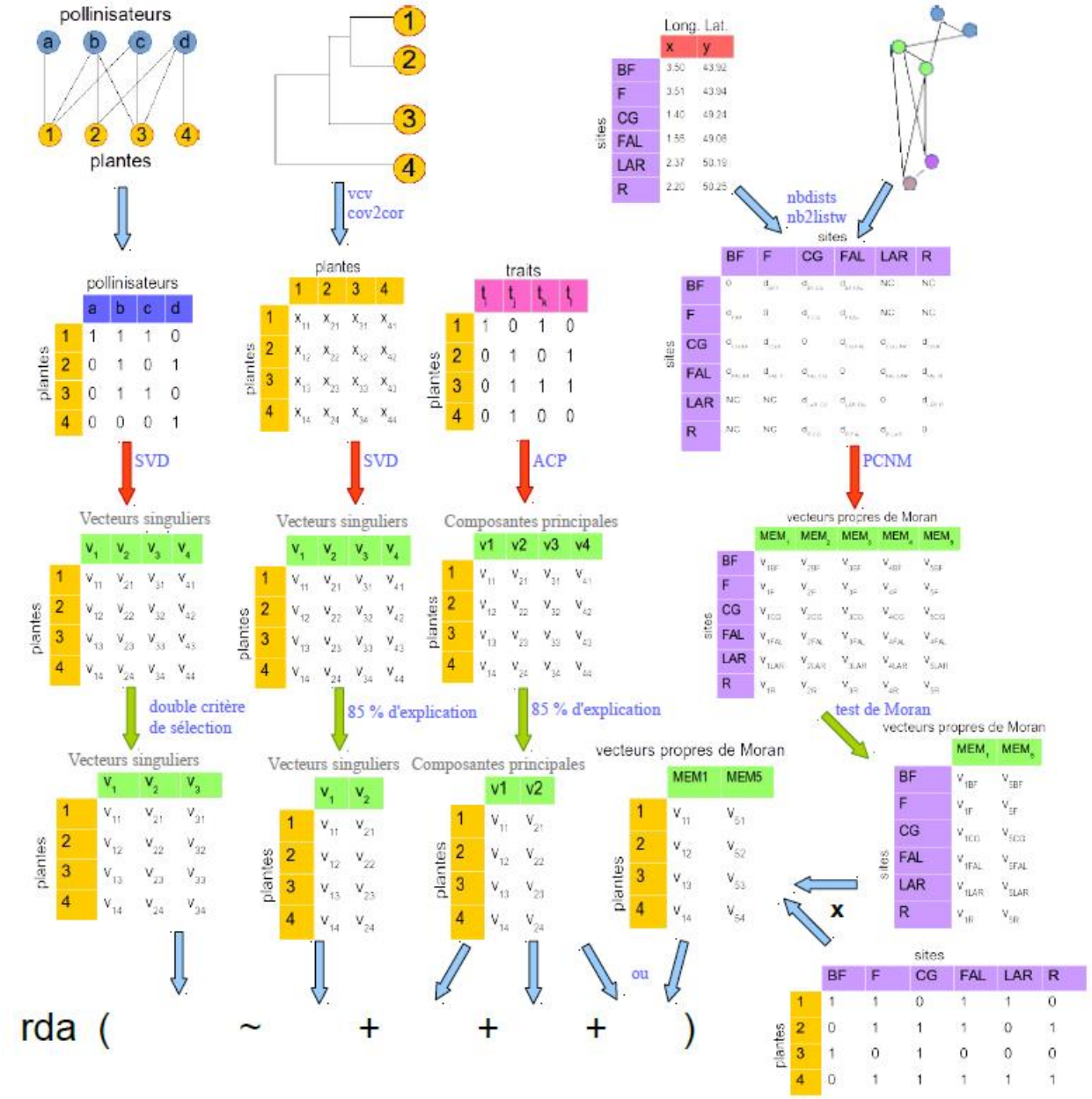

Figure 3 : Schéma de la démarche utilisée pour inclure les différents paramètres à analyser dans un modèle par analyse de redondance. Chaque facteur initial est d'abord transformé en matrice (de gauche à droite : d'incidence, de distance phylogénétique, de traits, ou de distance spatiale), elle-même décomposée en une série de vecteurs (flèches rouges) par la SVD selon Dalla-Riva et Stouffer (2016), par ACP, ou PCNM selon Dray *et al.*, (2006). Le nombre de vecteurs est ensuite sélectionné par le double-critère de sélection de Blanchet *et al.* (2008), un seuil d'explication de 85 %, ou un test d'autocorrélation de Moran (flèches vertes). Pour la matrice « Spatiale », nous devons multiplier cette dernière par la matrice de présence de l'espèce de plante sur le site.

Pour effectuer l'analyse en prenant en compte le facteur « géographie », les coordonnées par site sont transformées en une matrice de distance entre sites grâce aux fonctions `nb2listw` et `listw2mat`, suivi d'une transformation en vecteurs propres de Moran, par la fonction `mem` (package `adespatial`, Dray *et al.*, 2018), en effectuant une analyse de coordonnées principales de matrices de distance (PCNM). Le test de Moran (Fig.2b) nous permet de sélectionner les vecteurs correspondant à des autocorrélations significativement différentes de zéro (fonction `moran.randtest`, p-value < 0.1).

Analyse statistique

Toutes les analyses sont effectuées sur le logiciel R v3.5.0 (R Core Team, 2018).

Analyse de redondance (RDA)

Les jeux de données précédemment mis en forme sont utilisés pour créer des modèles analysés avec la fonction `rda` du package `vegan` (Oksanen *et al.*, 2013). La variable étudiée dans le modèle est l'ensemble des vecteurs sélectionnés issus de la SVD de la matrice d'incidence. Les facteurs sont, pour les syrphes les matrices de traits, de phénologie et les vecteurs sélectionnés issus de la SVD de la matrice de variance-covariance phylogénétique ; pour les plantes, les facteurs sont les matrices de morphologie, de phénologie, d'habitats et les vecteurs sélectionnés issus de la SVD de la matrice de variance-covariance phylogénétique. Dans une analyse de redondance, le principe général est de projeter le nuage de points du tableau à expliquer sur les bases orthonormales induites par les différents facteurs explicatifs. Un R^2 est donc associé pour chaque facteur du modèle, correspondant à la proportion de variance résiduelle une fois le tableau à expliquer projeté sur l'espace induit par ce facteur. Certains effets pouvant éventuellement être confondants (Fig.4a), une projection sur cet espace peut être effectuée en conditionnant par un facteur, c'est-à-dire en ne projetant sur l'espace du facteur d'intérêt que la partie orthogonale au facteur de conditionnement du tableau à expliquer.

Des fractions d'intérêt peuvent être alors mises en évidence (Fig.4b) parmi lesquelles la fraction totale d'un facteur (toutes les fractions dans lesquelles le facteur d'intérêt est pris en compte sans condition) et la fraction individuelle (fraction dans laquelle tous les autres facteurs sont conditionnant).

$$R^2_{adj} = 1 - \frac{n-1}{n-p-1}(1-R^2)$$

Équation 1 : Formule du R^2 ajusté (R^2_{adj}) n est la taille de l'échantillon, p le nombre de prédicteurs, R^2 le coefficient de corrélation non corrigé du modèle (Peres-Neto et al., 2006).

Tous les modèles possibles à partir de ces facteurs sont testés, y compris en les conditionnant. Pour chaque modèle testé, le R^2 ajusté (R^2_{adj} (Éq.1), Peres-Neto *et al.* 2006) est obtenu. L'ajustement du R^2 permet de prendre en compte la taille de l'échantillon *n* et le nombre de vecteurs pris en compte *p* dans le modèle. Les R^2_{adj} de chaque fraction sont étudiés par la fonction `varpart` et représentés à travers un diagramme de Venn (Fig.4b). Deux comparaisons de l'analyse à des modèles nuls sont utilisées pour l'analyse statistique, comparaisons déjà documentées par Joffard *et al.* (2018). La première, en utilisant la fonction anova sur la RDA permet de permuter les lignes de la matrice d'analyse (ici en utilisant 10000 permutations). Ces permutations permettent de tester si le R^2_{adj} est attendu lorsque les traits des espèces sont attribués au hasard (c'est-à-dire, si le R^2_{adj} est véritablement le fait de valeurs de traits particulières associées à des positions particulières du réseau). Une p-valeur significative signifie alors qu'il y a un effet du facteur sur la topologie du réseau.

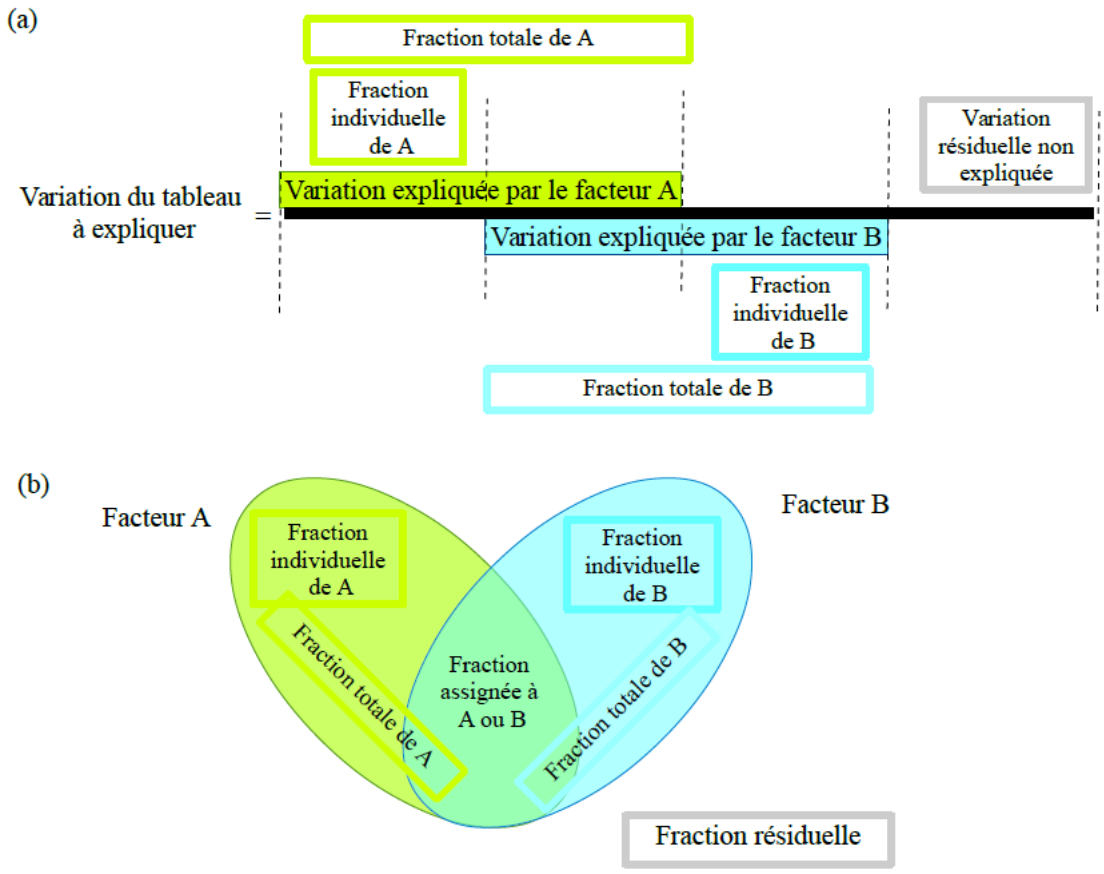

Figure 4 : (a) Schéma de l'explication de la variation d'un tableau par deux facteurs A et B. La fraction individuelle de A est la fraction de A conditionnée par le facteur B, et inversement, alors que la fraction totale est obtenue sans la condition par B. Une part de la variation non expliquée par le modèle testé est appelée variation résiduelle. Modifié d'après Peres-Neto *et al*. (2006). (b) Diagramme de Venn correspondant.

Le deuxième test procède à 10 000 simulations d'un réseau d'interactions aléatoires en conservant cependant le nombre d'interactions total pour chaque ligne et colonne (le degré de chaque espèce est conservé par la randomisation du réseau). Le R^2_{adj} observé pour chaque facteur dans notre modèle est comparé aux 10 000 R^2_{adj} des réseaux simulés. S'il est supérieur à 95 % de ces derniers, on considérera qu'il y a un effet significatif du facteur sur la topologie du réseau autre que le seul effet dû aux degrés des espèces. Pour réaliser les randomisations de réseau bipartite, nous avons utilisé l'algorithme de Strona *et al.*, (2014), implémenté dans le package `vegan`.

Analyse de beta-diversité

La β-diversité est une mesure de comparaison de la biodiversité entre plusieurs sites (Éq.2), que Poisot *et al.* (2012) ont adapté aux liens. La comparaison de ces indices de dissimilarité permettent de classer les sites en fonction de leur dissemblance. Quatre indices de dissimilarité ont été retenus dans l'analyse : la dissimilarité dans la composition en espèces (β_S), la dissimilarité totale des interactions (β_{WN}), la dissimilarité dans les interactions due au changement de composition (turnover) en espèces (β_{ST}) et la dissimilarité dans les interactions entre les espèces communes aux deux communautés (β_{OS}). Alors que les dissimilarités β_S ou β_{WN} permettent d'étudier quels sites sont les plus similaires vis-à-vis des espèces ou des interactions, nous pouvons assigner la part de variation des interactions du réseau due au turnover en quantifiant β_{ST} et due à autre chose que le seul turnover en espèces par β_{OS}.

(a) $$\beta_W = \frac{a+b+c}{\frac{2a+b+c}{2}} - 1$$ (b) $$\beta_{WN} = \beta_{OS} + \beta_{ST}$$

Équations 2 : Formules de la dissimilarité selon Whittaker (1960), (a). *a* est le nombre d'espèces ou d'interactions communes aux deux sites entre *b* et *c*. Pour β_S ou β_{WN}, *b* et *c* sont les nombres d'espèces respectif pour chaque site ; pour β_{OS}, *b* et *c* sont les nombres d'espèces desquels ont été retirés les espèces non communes. β_{ST} a été déduit de la formule (b) (Poisot *et al.*, 2012).

Étude de la modularité

Les facteurs dont l'effet n'est pas seulement sur le degré sont représentés en réorganisant les réseaux par groupes (Fig. annexes A1, A2, A3, A4). Ces groupes sont basés sur les résultats des ACP pour la morphologie et l'habitat optimal des plantes (site du Riez, Fig. annexes A3, A4), et sur les grands groupes phylogénétiques (familles ou sous-familles) des syrphes sur les sites du Bois de Fontaret (Fig.A1) et du Larris (Fig.A2). Pour chaque groupe nous étudions la moyenne du degré, de l'intermédiarité (*betweenness*) et de la centralité ainsi que ces mêmes moyennes sur les groupes de partenaires. Un groupe partenaire regroupe toutes les espèces partageant des interactions avec au moins une espèce du groupe.

Modèles exponentiels de graphes aléatoires

Les ERGM permettent d'inclure dans les modèles à la fois des facteurs intrinsèques et extrinsèques au réseau. La démarche de cette analyse repose sur la comparaison de différents modèles afin, tout d'abord, d'isoler le meilleur modèle fondé sur les propriétés intrinsèques au réseau. À partir de ce premier modèle, il est possible, dans un second temps, d'ajouter les termes spécifiques aux propriétés extrinsèques au réseau. Morris *et al.* (2008) ont documenté les termes utilisés pour modéliser les réseaux d'interactions dans le package `statnet` de R (Handcock *et al.*, 2003), parmi lesquels nous avons sélectionné ceux appropriés aux réseaux bipartites, non dirigés et non valués. La matrice d'adjacence représentant les interactions est dans ce cas transformée en graphe qui peut être directement analysé avec la fonction `ERGM`. Elle est analysée en fonction de différents termes : `edges` représente les liens dans le réseau, `b1degree` et `b2degree` représentent les degrés respectifs des nœuds de plantes et de pollinisateurs, et le terme `cycle(n)` permet d'étudier si des différents chemins reliant deux mêmes points d'une certaine longueur n structurent le réseau. Du fait du temps de calcul important de ces analyses, nous n'étudions au cours de ce stage qu'un seul réseau, pour lequel des facteurs explicatifs significatifs ont été mis en évidence par RDA, le site des Riez, qui est un site par ailleurs avec relativement moins d'espèces. Trois facteurs extrinsèques sont étudiés par ERGM : la morphologie des plantes, leur habitat optimal (facteurs avec effet significatif lors des RDA), et la phénologie des plantes qui, d'après les RDA, n'a pas d'effet significatif. Ces facteurs sont, comme précédemment, transformés en vecteurs issus d'ACP, sur les mêmes critères. Les vecteurs sont analysés un à un en commençant par ceux portant le plus d'explication du facteur. Les différents modèles sont évalués en minimisant le critères d'information d'Akaike (AIC).

Résultats

Composition en espèces

L'efficacité de l'échantillonnage a été mesurée par des courbes de raréfaction (Natasha de Manincor, *comm. pers.*), concluant à un bon inventaire à la fois des espèces de syrphes et de leurs interactions.La différence entre le nombre de paires d'espèces interagissant et le nombre total d'interactions varie entre les sites. Peu de syrphes sans interaction (capturés en vol ou au repos sur de la végétation) ont été décrits alors que les plantes sans interaction sont plus nombreuses (Tabl.1).

Analyse de redondance

L'analyse spatiale, permettant de tester l'effet de la distance entre les sites, donne un effet significatif de la distance géographique (R^2_{adj} = 0.016, p-value < 0.001 *** pour les plantes (Fig.5a), R^2_{adj} = 0.011, p-value < 0.001 *** pour les syrphes (Fig.5b)).

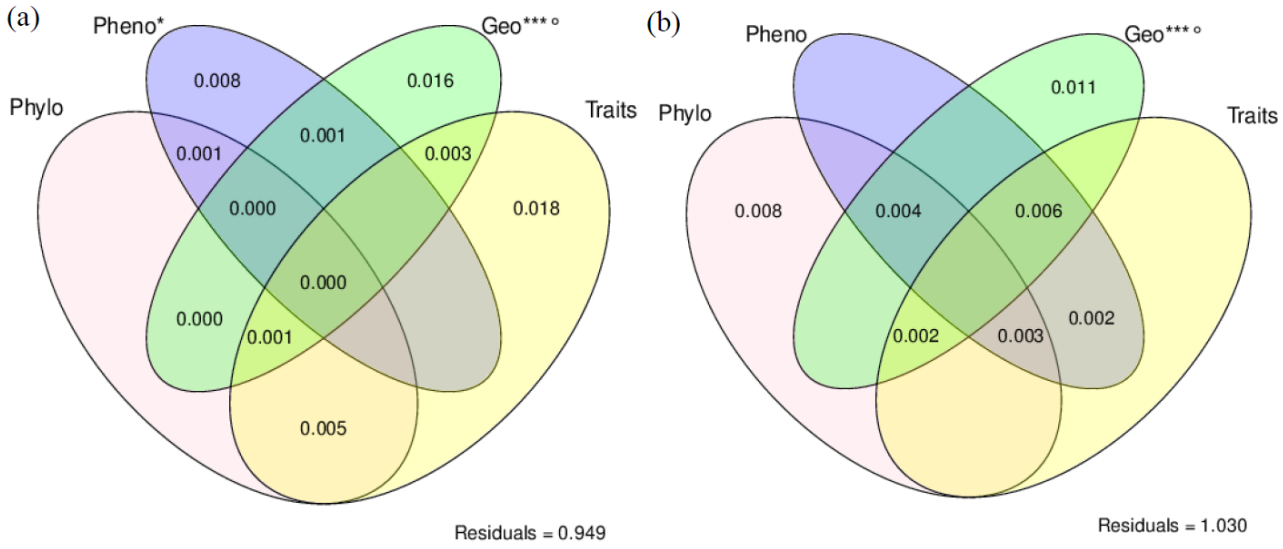

Figure 5 : Diagrammes de Venn représentant le partitionnement de la variance par R^2_{adj} en incluant un facteur spatial en plus des traits étudiés. Dans les deux cas plantes (a), comme syrphes (b), le facteur Geo (distance géographique) a un effet très significatif (*** p-value < 0.001, * p-value < 0.05, ° R^2_{adj}> 95 % des R^2_{adj} de 10 000 simulations de réseaux).

Chaque réseau est expliqué par au moins un facteur. Dans le Sud de la France, le réseau du Bois de Fontaret (Fig.6a) est expliqué par la phylogénie des syrphes (R^2_{adj} = 0.072, p-value = 0.017**) et leur taille (R^2_{adj} = 0.078, p-value = 0.0317*) et le réseau des Fourches (Fig.6b) par la phénologie des plantes (R^2_{adj} = 0.031, p-value = 0.010**) et leur phylogénie (R^2_{adj} = 0.021, p-value = 0.040*). En Normandie, le réseau de Château-Gaillard (Fig.6c,e) est expliqué par la phylogénie des syrphes (R^2_{adj}

= 0.068, p-value = 0.019*), et la phénologie et/ou la morphologie des plantes (R^2_{adj} = 0.150, p-value = 0.034*) et le réseau des Falaises (Fig.6d) par le comportement des syrphes (R^2_{adj} = 0.081, p-value = 0.040*). Enfin, sur les sites du Nord de la France, un effet de la phylogénie des syrphes (R^2_{adj} = 0.035, p-value = 0.031*) a été rapporté pour le réseau du Larris (Fig.6f), et des effets de la morphologie (R^2_{adj} = 0.155, p-value = 0.042*) et de l'habitat optimal (R^2_{adj} = 0.150, p-value = 0.018) des plantes ont été rapportés pour le réseau du Riez (Fig.6g).

La comparaison des R²adj obtenus à ceux issus des 10 000 randomisations de réseaux à distribution des degrés constante permet de mettre en évidence que les R²adj de la phylogénie des syrphes de Bois de Fontaret (R²adj = 0.072 > R²adj(Q95%) = 0.064, Fig.6a) et du Larris (R²adj = 0.070 > R²adj(Q95%) = 0.049, Fig.6f), et de la morphologie (R²adj = 0.155 > R²adj(Q95%) = 0.107) et de l'habitat optimal (R²adj = 0.150 > R²adj(Q95%) = 0.131, Fig.6g) des plantes du Riez sont supérieurs à 95 % des R²adj créés, c'est-à-dire correspondent à des effets sur la topologie du réseau qui ne se limitent pas à un effet sur les degrés des espèces.

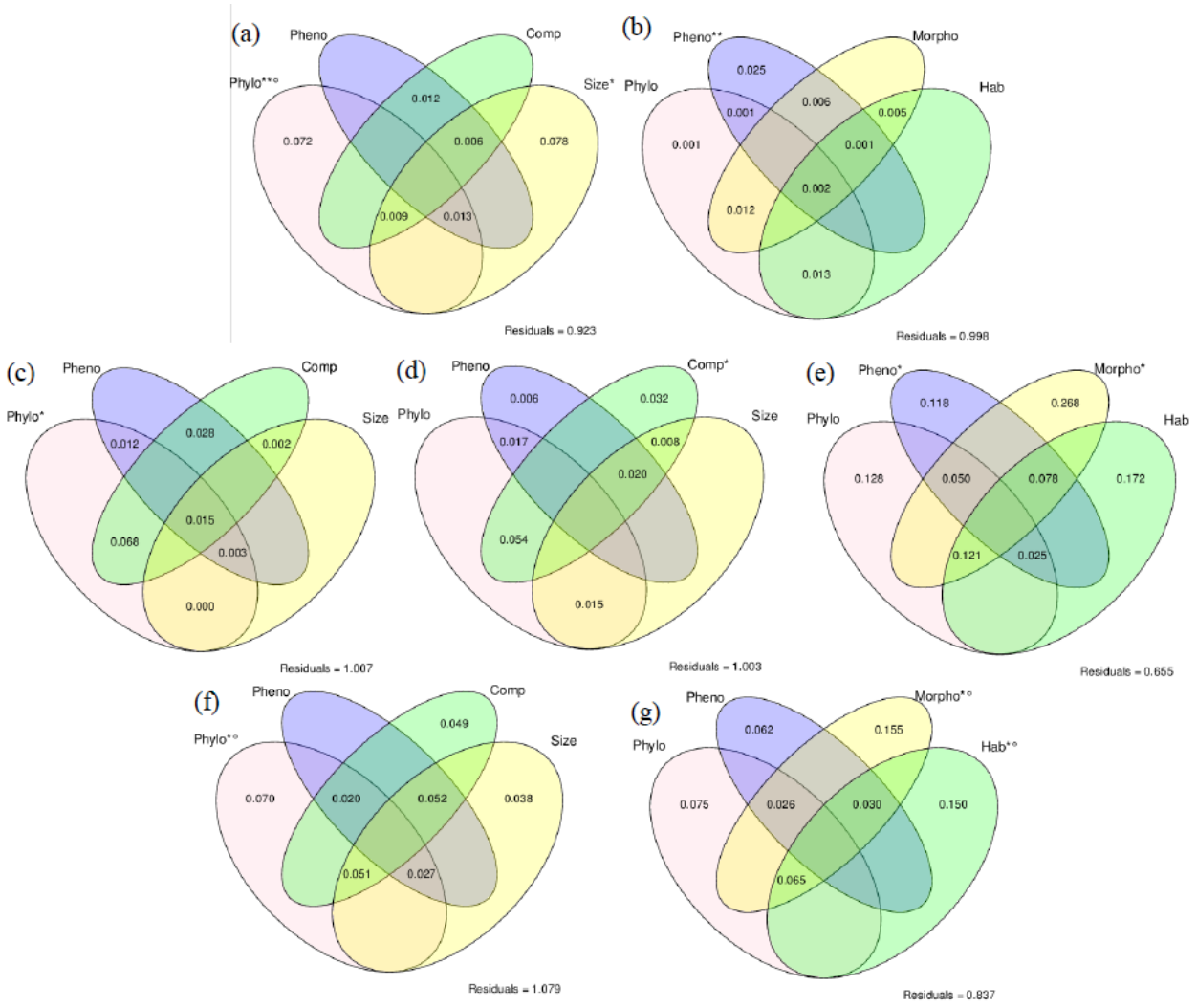

Figure 6 : Diagrammes de Venn représentant les R^2_{adj} des différentes partitions de la variable. La significativité du facteur a été représentée lorsqu'au moins une des fractions individuelle ou totale est significative : * p-value < 0.05, ** p-value < 0.01. Lorsque le R^2_{adj} est statistiquement différent des R^2_{adj} issus de 10 000 simulations de réseaux, le facteur est noté. (a) Syrphes du Bois de Fontaret, (b) Plantes des Fourches, (c) Syrphes de Château-Gaillard, (d) Syrphes des Falaises, (e) Plantes de Château-Gaillard, (f) Syrphes du Larris, (g) Plantes du Riez. Les diagrammes sur lesquels aucun R^2_{adj} significatif n'a été relevé n'ont pas été présentés.

Analyse de dissimilarité

La dissimilarité de la composition spécifique et des interactions augmente avec la distance géographique. Les sites de la même région sont davantage similaires que les sites de régions différentes. La part de variation dans les interactions due au turnover d'espèces β_{ST} est plus forte lorsqu'un site de Normandie est impliqué (Tabl.3).

type	sites	β_S	β_{WN}	β_{OS}		β_{ST}	
Sud-Sud	BF-F	0.369	0.757	0.592	78.2 %	0.164	21.7 %
Norm.-Norm.	CG-FAL	0.421	0.723	0.480	66.4 %	0.243	33.6 %
Nord-Nord	LAR-R	0.315	0.703	0.516	73.4 %	0.188	26.7 %
Sud-Norm.	BF-CG	0.714	0.982	0.555	56.5 %	0.427	43.5 %
	BF-FAL	0.741	1	1	100 %	0	0 %
	F-CG	0.704	0.975	0.739	75.8 %	0.236	24.2 %
	F-FAL	0.664	0.985	0.862	87.5 %	0.123	12.5 %
Nord-Norm.	CG-LAR	0.544	0.856	0.571	66.7 %	0.284	33.2 %
	CG-R	0.556	0.816	0.578	70.8 %	0.239	29.3 %
	FAL-LAR	0.554	0.888	0.527	59.3 %	0.361	40.7 %
	FAL-R	0.600	0.845	0.455	53.8 %	0.390	46.2 %
Nord-Sud	BF-LAR	0.728	0.982	0.750	76.4 %	0.232	23.6 %
	BF-R	0.746	0.982	0.692	70.5 %	0.290	29.5 %
	F-LAR	0.718	0.984	0.778	79.1 %	0.206	20.9 %
	F-R	0.750	0.991	0.895	90.3 %	0.097	9.8 %

Tableau 3 : Valeurs de dissimilarités β_S, β_{WN}, β_{OS} et β_{ST} entre les différents sites. Les pourcentages de dissimilarité respectifs relativement dûs au turnover ou aux interactions ont également été notés. Les paires de sites ont été classées pour différencier les sites intra-region des inter-régions. BF : Bois de Fontaret (Sud), F : Fourches (Sud), CG : Château-Gaillard (Normandie), FAL : les Falaises (Normandie), LAR : le Larris (Nord), R : le Riez (Nord), Norm. : Normandie

Dans les réseaux dont un des facteurs montre un R^2_{adj} significatif à la fois pour le test de permutation des lignes et pour le test de randomisation du réseau, nous avons trié les espèces selon le facteur d'intérêt, selon la phylogénie nous avons trois groupes principaux : les *Syrphinae*, les *Pipizini* et le reste des *Eristalinae* (*Eristalini*, *Milesiini*, *Merondontini*, *Rhingiini*) au Bois de Fontaret et deux groupes principaux sur le Larris (*Syrphinae* et *Eristalinae*). Selon l'habitat optimal des plantes du Riez, nous avons quatre groupes et deux groupes selon la morphologie. Les degré, intermédiarité et centralité moyens par groupe ou par partenaires préférentiels des espèces d'un groupe ont été rapportés dans le Tabl.4.

Site	Facteur	Module	Moyenne du module			Moyenne du module partenaire		
			degré	intermé-diarité	centralité	degré	intermé-diarité	centralité
Bois de Fontaret	Phylogénie des syrphes	*Eristalinii, Milesiini, Merodontini, Rhingiini*	1.95	42.8	0.136	3.86	154	0.261
		Syrphinae	3.53	135	0.271	3.45	127	0.300
		Pipizini	7.33	316	0.551	2.09	36.7	0.242
Larris		*Syrphinae*	5.08	93.3	0.346	3.07	90.5	0.230
		Eristalinae	3.55	35.9	0.263	3.40	112	0.264
Riez	Habitat des plantes	Pelouse xérique	3.50	33.9	0.277	6.00	80.4	0.450
		Prairie ou pelouse mésique à xérique	6.75	132	0.450	5.44	69.3	0.415
		Milieux divers xériques et plutôt basiques	2.50	8.18	0.273	9	125	0.656
		Pelouse ou prairie mésique à humide	2.91	20.1	0.282	7.72	106	0.559
	Morphologie	Plutôt zygomorphe	2.88	8.12	0.275	8.00	113	0.580
		Plutôt autogame, actinomorphe à ombelles ou capitules	4.18	56.3	0.33	4.73	59.0	0.362

Tableau 4 : Étude de la moyenne des degrès, intermédiarités et centralités des espèces des principaux modules et de leurs partenaires, pour les facteurs sur lesquels un effet statistiquement significatif a été trouvé.

Modèles exponentiels de graphes aléatoires (ERGM)

En ajoutant les facteurs intrinsèques au réseau, dans les modèles, nous avons isolé le meilleur modèle possible ne prenant en compte que les facteurs intrinsèques. Nous avons comparé l'AIC obtenu avec des nombres différents de réplicats (`mcmc.burnin` et `mcmc.interval`), ce qui permet de confirmer que le modèle est toujours le plus robuste statistiquement. À partir de ce modèle, nous avons ajouté un à un les axes de l'ACP sur la phénologie, la morphologie et l'habitat optimal des plantes du Riez, jusqu'à ce que l'AIC du nouveau modèle ne baisse plus. Le meilleur modèle retenu

est fondé sur la densité du réseau (terme 'edges'), le degré des plantes (terme 'b1degree'), les cycles de tailles 4 et 6 (terme 'cycle(c(4,6))') et les variables extrinsèques « morphologie » et « habitat » (Tabl.5).

facteurs	modèle	mcmc.burnin	mcmc.interval	AIC	BIC
intrinsèque	edges+b1degree(n)+cycle(4,6)	100	10	501.5	559.5
	edges+b1degree(n)+cycle(4,6)	1000	50	501.2	559.2
intrinsèque et extrinsèque	edges+b1degree(n)+cycle(c(4,6))+b1cov(Morpho2)+b1cov(Hab1)	1000	50	493.7	560.8

Tableau 5 : Comparaison des critères AIC et BIC des meilleurs modèles trouvés lors de l'étude d'une multitude de modèles. L'AIC est inférieure pour le modèle prenant en compte les paramètres extrinsèques

Discussion

Analyse spatiale

L'analyse de redondance prenant en compte la distance géographique en plus des facteurs de phylogénie, traits et phénologie montre un très fort effet de la géographie. L'étude portant sur un gradient latitudinal de coteaux calcaires, nous pouvons considérer qu'il y a une variation significative des réseaux plantes-pollinisateurs entre les sites avec la distance géographique (Fig.5). De fait, nous nous attendons à ce que les réseaux des différents sites soient expliqués par des facteurs différents. Rejoignant les conclusions de Trøjelsgaard *et al.* (2015), la dissimilarité est également corrélée à la distance géographique puisque les sites les moins similaires sont également ceux les plus éloignés (Tabl.3), ce qui est observé à la fois pour la composition en espèces et pour les interactions au sein de la communauté. Comme attendu, les sites les plus dissimilaires correspondent aux comparaisons Nord-Sud. Bien qu'intermédiaires, les sites de Normandie sont plus proches des sites du Nord que de ceux du Sud à la fois pour la géographie, les espèces, et leurs interactions. En revanche, les sites normands sont les plus dissimilaires au sein d'une même région et ce sont aussi les sites les plus espacés.

Analyse de la communauté pour chaque site

Chaque site pris en compte dans l'analyse est expliqué significativement par au moins l'effet d'un facteur (Fig.6). Un facteur significatif signifie qu'un de ses R^2_{adj} est statistiquement différent du R^2_{adj} fondé sur 10 000 permutations de lignes du réseau. Cette statistique permet d'affirmer que le facteur a un effet sur la topologie du réseau. Lorsqu'on complète l'analyse par une comparaison du R^2_{adj} observé aux R^2_{adj} fondés sur 10 000 simulations de réseaux, on est ensuite capable d'attribuer cet effet à la seule distribution des degrés au sein du réseau (si le deuxième test n'est pas significatif) ou à un effet plus profond sur la topologie du réseau.

Nous savons donc que pour le site Bois de Fontaret, il y a un effet de la taille et de la phylogénie des syrphes sur la topologie du réseau qui n'est pas uniquement due aux degrés. Le réseau des Fourches, autre site du Sud, est expliqué par la phénologie des plantes qui n'a d'effet que sur le degré des espèces. En Normandie, il n'y a des effets que sur les degrés des espèces : dans le réseau des Falaises, l'effet n'est dû qu'au comportement des syrphes alors qu'à Château-Gaillard trois effets sont rapportés : la phylogénie des syrphes ainsi que la phénologie et la morphologie des plantes. Dans le Nord de la France, la phylogénie des syrphes du Larris et la morphologie et l'habitat optimal des plantes du Riez ont un effet sur la topologie du réseau plus profond que sur le degré. Pour les sites Riez, Larris et Bois de Fontaret, connaître l'effet qu'ont les facteurs significatifs nécessite de s'intéresser à d'autres paramètres du réseau que le degré. Entre ces trois sites, nous avons des dissimilarités dues au turnover d'espèces du même ordre de grandeur (de 23 à 29%).

L'analyse pour chaque groupe d'espèces des degrés, intermédiarités et centralités moyens permet de comparer les groupes influençant le réseau ou leurs partenaires. Pour Bois de Fontaret, les trois groupes ont des caractéristiques très variables : les *Pipizini* ont de très forts degrés, intermédiarités et centralités, alors que les autres *Eristalinae* présentent des valeurs faibles pour ces trois scores. Les *Syrphinae*, quant à eux, semblent avoir les mêmes scores que leurs partenaires. Les groupes de syrphes du Larris semblent présenter des valeurs différentes alors que leurs partenaires semblent avoir des valeurs similaires de degrés, intermédiarités et centralités. Deux groupes principaux existent pour l'habitat optimal des plantes du Riez : le groupe de plantes de pelouses ou prairies mésiques à xériques, généralistes à forts degré, centralité et intermédiarité, et les groupes de plantes de milieux divers xériques ou de prairies et pelouses mésiques à humides semblant être davantage spécialistes et dont le groupe de syrphes partenaires est généraliste. L'effet de la

morphologie des plantes est davantage expliqué par les partenaires que les groupes de plantes « plutôt zygomorphe » et « plutôt actinomorphe », par à la fois leur centralité, leur degré et l'intermédiarité.

Pour ces trois sites, il y a donc bien un effet significatif sur la topologie du réseau. Nous pouvons observer sur le réseau d'interactions que certains groupes structurent davantage le réseau et ont des interactions préférentielles avec certains groupes d'espèces.

La RDA permet de voir si les différents facteurs pris en compte ont un effet sur la topologie du réseau et si cet effet effet est uniquement le fait de la distribution des degrés des espèces. De plus, cette analyse permet d'aller plus loin en étudiant si l'effet du facteur est uniquement dû à la distribution des degrés en testant les R^2_{adj} contre des réseaux simulés conservant le degré total de chaque espèce, ce qui signifie qu'il y a, en plus de l'effet sur le degré, un effet supplémentaire sur la topologie du réseau telle une relation de certaines espèces à faire des interactions avec des partenaires préférentiels, ou agir sur la centralité ou l'intermédiarité.

Bien que les analyses effectuées, utilisant ce double critère (permutations des lignes ou randomisations du réseau), puissent paraître assez strictes pour assigner un certain effet à un facteur, elle ne prennent pas comme point de départ la structure du réseau, puisque cette discussion arrive à la toute fin de l'analyse, une fois l'effet statistique démontré, et se fait donc *a posteriori*.

Les ERGM, par rapport aux RDA, inversent l'approche utilisée en essayant de voir quelle partie de la variation peut être expliquée par la structuration au sein du réseau et ce sont les facteurs extrinsèques qui sont utilisés *a posteriori* dans un modèle déjà expliqué par les facteurs intrinsèques.

Dans le cas du réseau des Riez, le modèle intrinsèque mis en évidence prend en compte les paramètres degrés des plantes, et les chemins reliant deux nœuds en passant par 4 ou 6 nœuds (cycle(c(4,6))). Il y a donc un effet sur le degré des espèces, en l'occurrence des plantes, mais aussi sur une structuration particulière dans les interactions. Déterminer le meilleur modèle intrinsèque est cependant un compromis puisque certains cycles sont techniquement impossibles à étudier lorsqu'ils deviennent trop longs comme le cas des cycles passant par 10 nœuds ou davantage, en raison du temps de calcul trop important. Concernant les facteurs extrinsèques, nous avons pu en retenir deux, l'axe II de l'ACP de la morphologie des plantes, et l'axe I de leur habitat optimal. Il s'agit respectivement de l'axe séparant les zygomorphes et les *Campanula* des autres actinomorphes et de l'axe séparant les plantes xérophiles des plantes plus mésophiles, ce qui permet d'affirmer qu'il y a un effet de la forme de la corolle et de la xérophilie sur le réseau d'interaction du Riez. Si on utilise l'autre critère, le BIC, les modèles avec ajout de paramètres extrinsèques ne changent quasiment pas le critère, ce qui peut relativiser l'importance de l'effet observé de ces deux axes.

Données

Concernant les données, nous avons dû sélectionner seulement un nombre de vecteurs propres expliquant autour de 85 % de la variable pour les facteurs issus de l'ACP et de la SVD des phylogénies. En effet, lorsqu'on applique les critères de Bauman *et al.* (2017), beaucoup d'axes sont pris en compte, ce qui multiplie le nombre de paramètres. La probabilité de tomber sur une bonne explication de la variable augmente alors avec le nombre d'axes, même si ceux-ci n'ont pas forcément d'influence sur les données. En conséquence, la variation de la matrice d'interaction est expliquée presque entièrement et par n'importe quel facteur utilisé, d'où le besoin d'être plus strict dans la sélection du nombre de vecteurs.

Dans la phylogénie des syrphes, la position de *Microdon analis* dans la famille des *Syrphinae*, est peu probable (Young *et al*. 2016 ; Fig.A5). N'ayant qu'une séquence de COI publiée dans Genbank pour *Microdon tristis*, du même genre, nous ne pouvons comparer la position de cette espèce avec la position issue d'une autre séquence. L'erreur de position peut être due à un mauvais séquençage ou à un traitement inadéquat de la séquence ou de l'alignement, puisque le fait d'augmenter les bootstrap ou de changer d'algorithme n'ont pas d'influence sur sa position au sein de l'arbre. Le manque de matériel (une seule séquence disponible) et le temps de calcul trop important des arbres phylogénétiques ne nous permettent pas de résoudre complètement le problème, et nous préférons ne pas interpréter la position de *Microdon analis* lorsqu'un effet phylogénétique est observé.

Types de modèles utilisés

Les ERGM semblent une analyse statistique encore plus stricte vis à vis des variables extrinsèques puisque si presque toute la variation du réseau est expliquée par les seuls facteurs intrinsèques, il en sera conclu que la variable extrinsèque n'a pas d'effet significatif, ou que la part que cette variable explique est déjà expliquée par une des propriétés intrinsèques. Cette conclusion rejoint la différence entre les deux tests par randomisations effectués sur les R^2_{adj} de la RDA, mais seulement pour le degré.

Les deux types d'analyse semblent donc se compléter puisqu'elles n'utilisent pas la même approche de base. L'avantage des analyses de redondance est qu'elles permettent d'avoir un résultat assez rapidement, statistiquement robuste, mais n'expliquent pas jusqu'au bout l'effet du facteur mis en évidence s'il n'agit pas que sur le degré. En effet, lorsqu'un facteur est significatif pour les deux

tests, il est assez difficile d'interpréter sur quelle composante du réseau l'effet a lieu, bien qu'on puisse être tenté de le faire par des paramètres simples tels que la modularité, la centralité ou l'intermédiarité.

L'ERGM est également statistiquement très robuste, mais il ne permet pas de différencier si l'effet d'un facteur permet d'expliquer une même part de variation que les variables intrinsèques ou s'il n'a réellement pas d'effet significatif. En effet, dans ces deux cas, l'inclure dans le modèle ne permet pas de l'améliorer. Au contraire, si une variable extrinsèque améliore sensiblement le modèle, nous pouvons être sûr qu'elle porte un effet significatif puisqu'elle explique une partie de la variation qui n'est expliquée par aucune des variables du meilleur modèle intrinsèque possible. Le principal inconvénient de l'ERGM est surtout d'ordre technique puisque le temps de calcul va augmenter très fortement avec le nombre d'espèces et de liens dans le réseau, ainsi que par l'utilisation de motifs internes au réseau (fonction cycle). En effet, plus les motifs ont des chemins longs, plus le temps nécessaire à l'analyse devient long. De plus, plusieurs tentatives doivent être opérées pour sélectionner le nombre de réplicats (fonctions mcmc.burnin et mcmc.interval) puisqu'il faut jouer entre un nombre de réplicats suffisants pour pouvoir évaluer statistiquement le modèle et un nombre de réplicats évitant les erreurs de dégénérescence via une surexplication du modèle. Une fois la dégénerescence contournée, les paramètres d'ajustement ne semblent plus nécessaires puisque n'ayant plus d'influence sur l'amélioration du modèle.

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Figures and Tableaux Supplémentaires

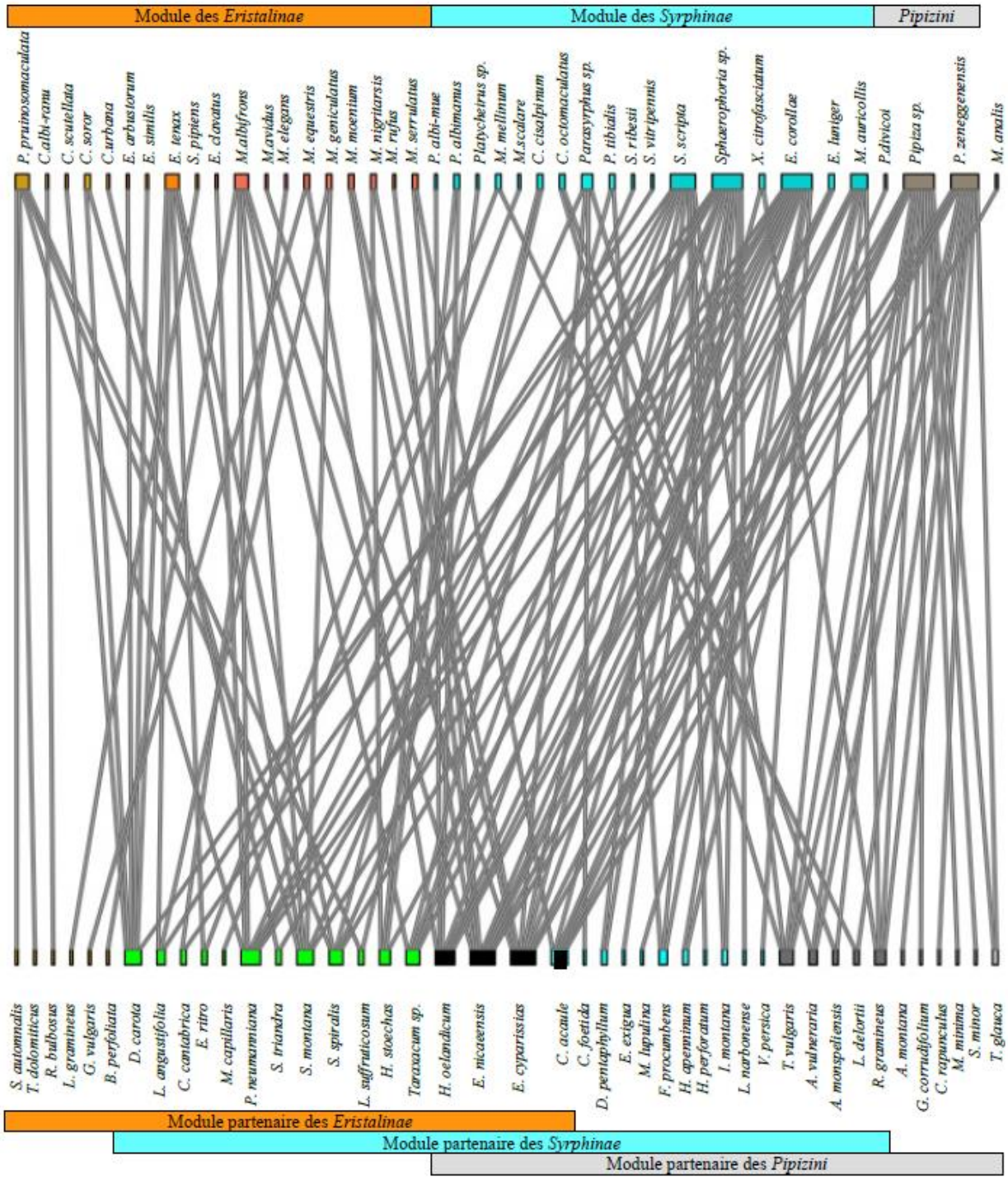

Figure A1: Réseau d'interaction du Bois de Fontaret dans lequel les espèces de syrphes ont été ordonnées selon leur taxon (famille à tribu), leur phylogénie ayant un effet significatif. Les espèces de plantes ont été ordonnées de telle sorte à être en face de leur module préférentiel partenaire

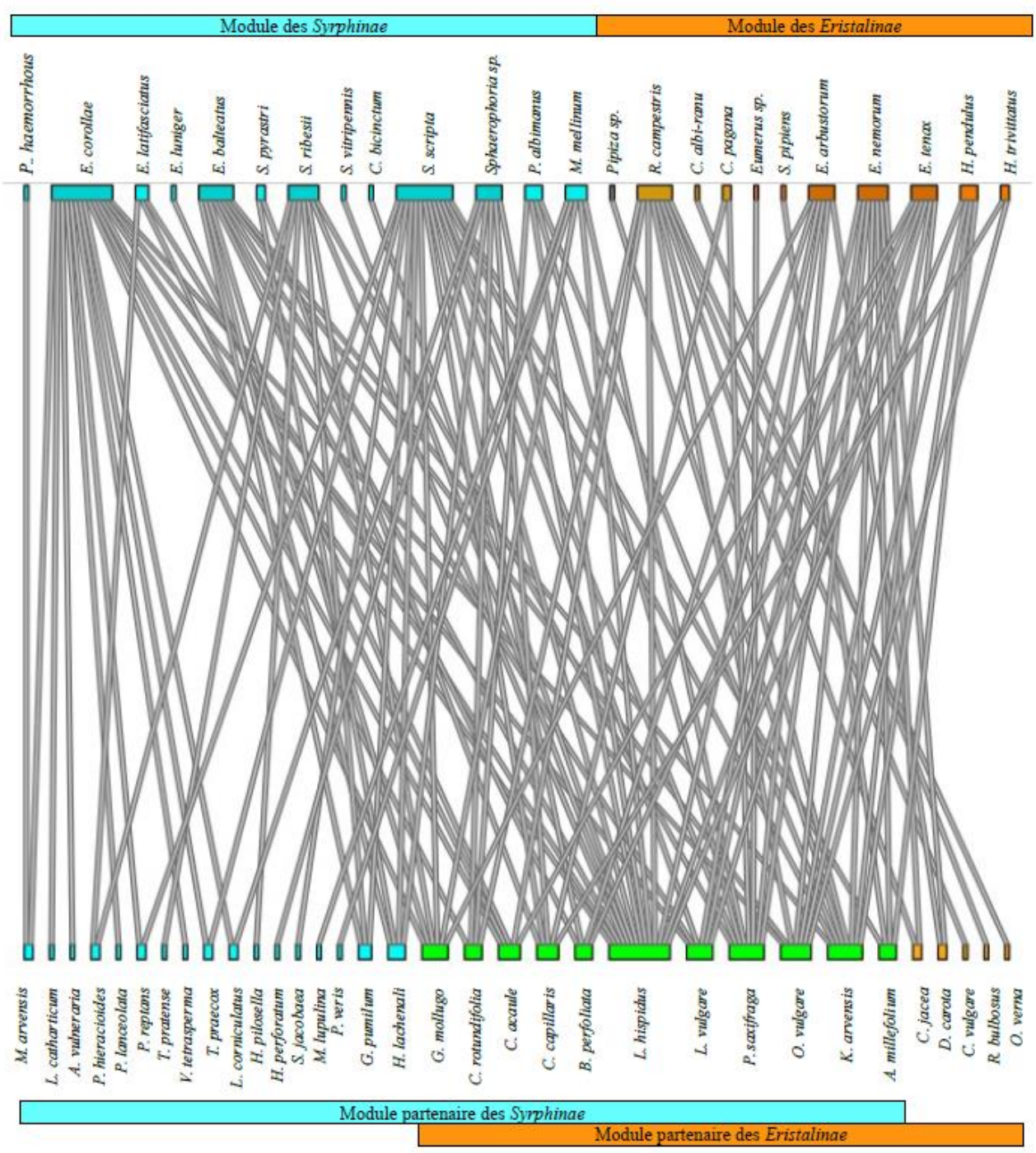

Figure A2 : Réseau d'interaction du Larris dans lequel les espèces de syrphes ont été ordonnées selon leur taxon (famille à tribu), leur phylogénie ayant un effet significatif. Les espèces de plantes ont été ordonnées de telle sorte à être en face de leur module préférentiel partenaire

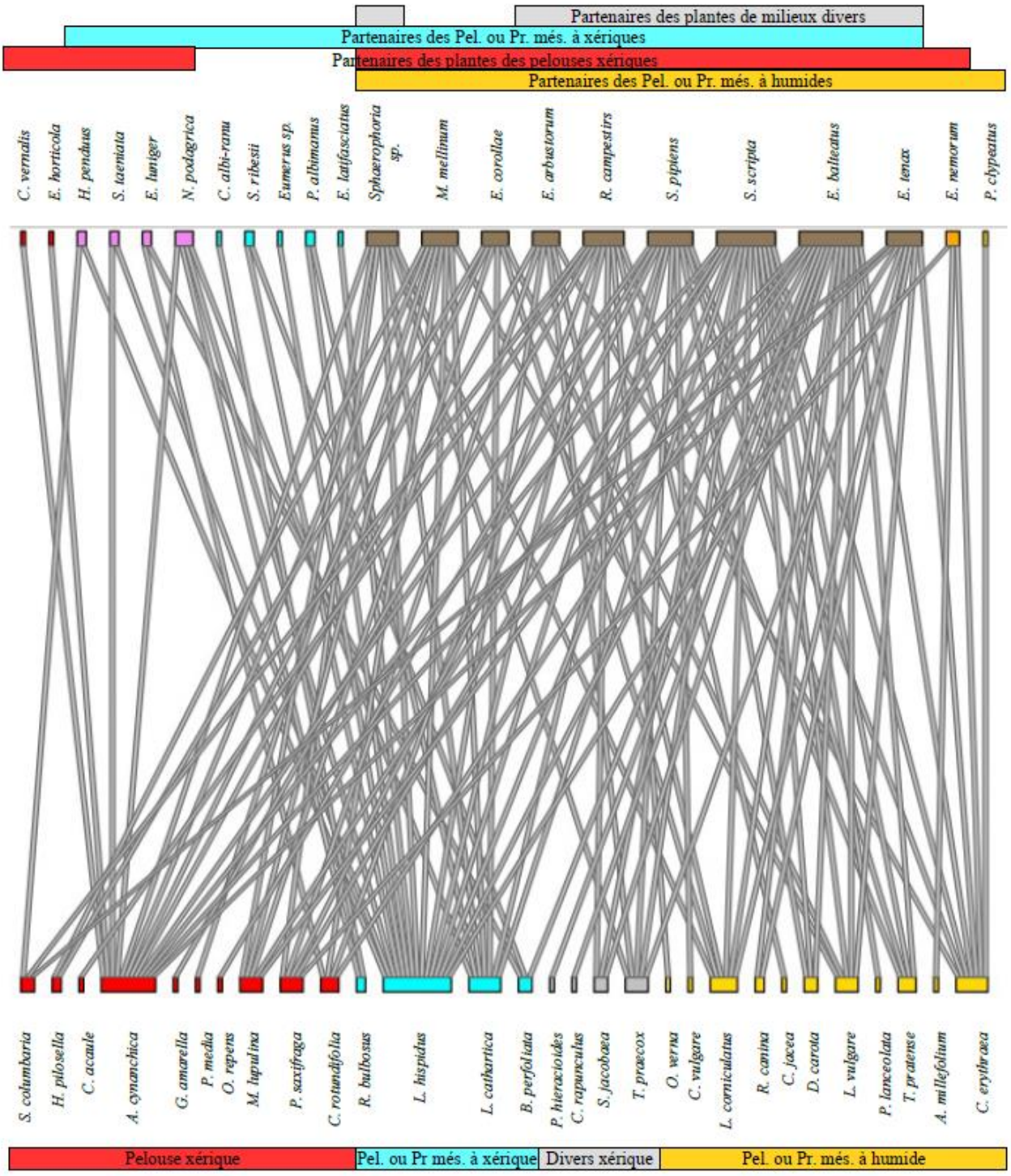

Figure A3: Réseau d'interaction des Riez dans lequel les espèces de plantes ont été ordonnées selon leur appartenance aux modules basés sur la sortie des ACP sur le facteur significatif « Habitat optimal » des plantes. Les espèces de syrphes ont été ordonnées de telle sorte à être en face de leur module préférentiel partenaire

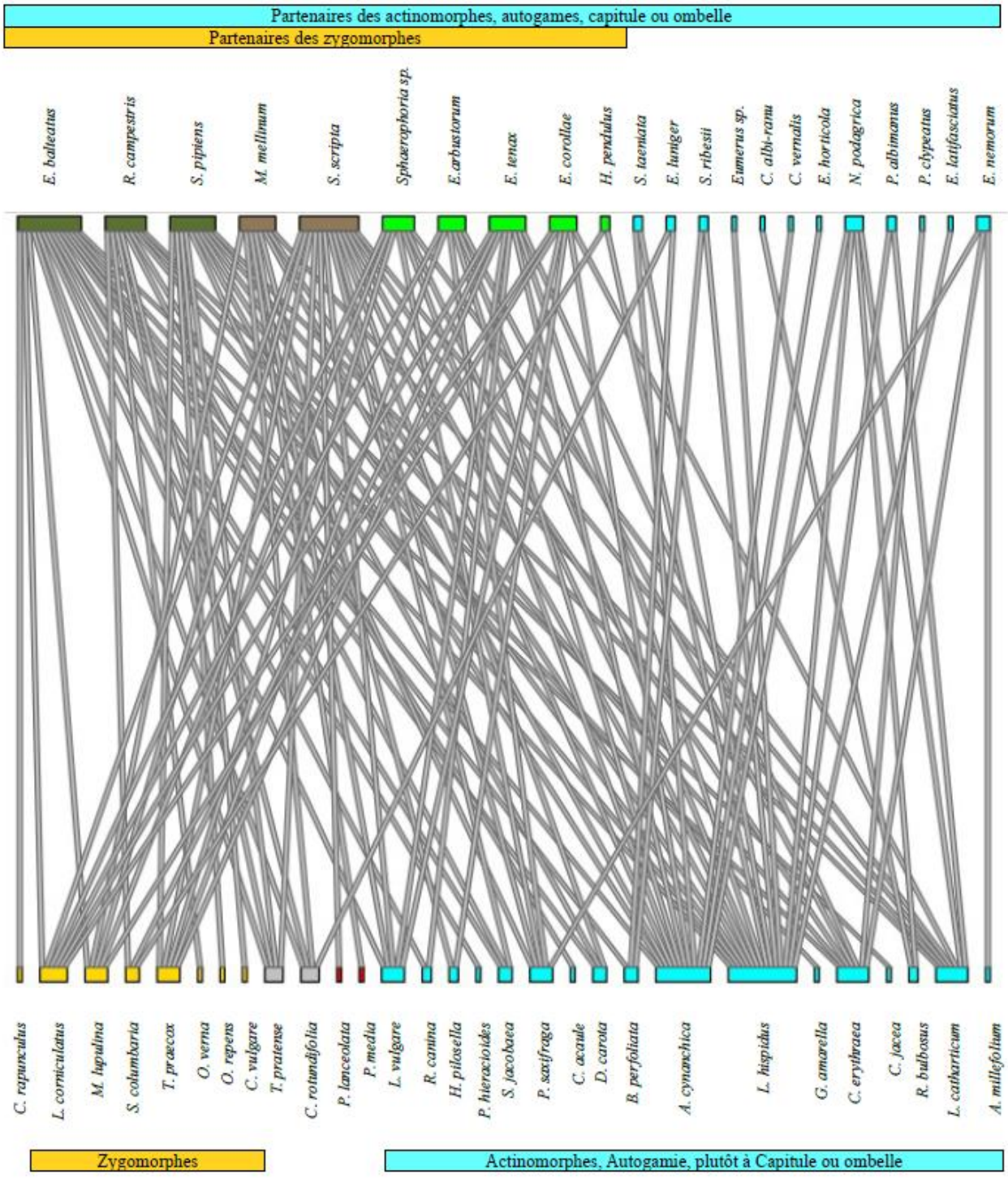

Figure A4: Réseau d'interaction des Riez dans lequel les espèces de plantes ont été ordonnées selon leur appartenance aux modules basés sur la sortie des ACP sur le facteur significatif « Morphologie » des plantes. Les espèces de syrphes ont été ordonnées de telle sorte à être en face de leur module préférentiel partenaire

Espèce	N°accession	Espèce	N°accession	Espèce	N°accession
Chalarus spurius	KU687412	Graptomyza sp.	KR260218	Platypeza sp.	KR260237
Allograpta obliqua	KR260202	Helophilus fasciatus	KR260219	Rhingia coerulescens	JN992024
Baccha elongata	KR260206	Helophilus hybridus	KR521604	Rhingia nasica	JN992026
Betasyrphus serarius	KR260207	Helophilus hybridus	KY844010	Rhingia nasica	KR260238
Brachypalpus oarus	KR260208	Helophilus hybridus	KY844010	Rhingia nasica	MG165144
Callicera montensis	KR260209	Heringia calcarata	KR260220	Rhingia nasica	MG166638
Cheilosia albitarsis	LT707496	Leucozona americana	KR260224	Scaeva dignota	KR260239
Cheilosia albitarsis	LT707496	Melanostoma mellinum	KR260227	Scaeva dignota	KR260239
Cheilosia soror	KR260210	Melanostoma mellinum	KR260227	Scaeva pyrastri	KR429784
Cheilosia soror	KR260210	Melanostoma scalare	JN992005	Sericomyia chrysotoxoides	MG169101
Chrysotoxum pubescens	KR669545	Melanostoma scalare	KF919075	Sericomyia nigra	KU876180
Chrysotoxum pubescens	KR671894	Meliscaeva auricollis	JN992006	Sphaerophoria scripta	JN992033
Chrysotoxum sp.	MG169494	Merodon aberrans	KR260228	Sphaerophoria scripta	KC900480
Chrysotoxum sp.	MG170911	Merodon albifrons	FM206510	Sphaerophoria scripta	KR260241
Citrogramma circumdatus	KR260211	Merodon avidus	FM206518	Sphaerophoria scripta	KR260241
Copestylum caudatum	KR260212	Merodon equestris	MG169358	Sphaerophoria sp.	KY834690
Dasysyrphus albostriatus	KF939555	Merodon equestris	MG170543	Sphaerophoria sp.	MG163897
Epalpus signifer	KR260213	Merodon nigritarsis	FM206494	Sphaerophoria sp.	MG164378
Epistrophe grossulariae	KR260214	Merodon serrulatus	FM206493	Sphegina rufiventris	KR260242
Episyrphus balteatus	KR260215	Microdon tristis	KR260229	Syritta pipiens	MG169778
Episyrphus balteatus	KR260215	Neoascia podagrica	JN992017	Syritta sp.	KX054854
Eristalis arbustorum	JN991982	Ocyptamus fuscipennis	KR260230	Syrphus ribesii	KU876292
Eristalis arbustorum	KC900447	Paragus azureus	KR831131	Syrphus ribesii	MG165935
Eristalis tenax	KR520640	Paragus azureus	KR831134	Syrphus torvus	JN992043
Eristalis tenax	KR694524	Paragus haemorrhous	KR260231	Syrphus torvus	KR662493
Eumerus sp.	KR260216	Paragus haemorrhous	KR260231	Syrphus vitripennis	KR660667
Eupeodes corollae	KY831535	Paragus serratus	KY838632	Syrphus vitripennis	KR670342
Eupeodes corollae	KY847463	Parasyrphus annulatus	KR260232	Themira nigricornis	KR260243
Eupeodes latifasciatus	KR678230	Pipiza crassipes	KR260233	Volucella bombylans	KC900470
Eupeodes latifasciatus	MG163414	Pipiza nigripilosa	KR260234	Volucella bombylans	KU876446
Eupeodes latifasciatus	MG163414	Pipizella nigriana	KF919074	Volucella sp.	KR656124
Eupeodes luniger	JN991995	Pipunculus sp.	KR260235	Volucella zonaria	HE614015
Eupeodes luniger	KU875039	Platycheirus clypeatus	JN992018	Xanthogramma flavipes	KR672643
Eupeodes luniger	KU875039	Platycheirus clypeatus	KT601616	Xanthogramma flavipes	MG170232
Ferdinandea buccata	KR661965	Platycheirus sp.	KR260236	Xylota bicolor	KR260244
Ferdinandea croesus	KM945277	Platycheirus sp.	KR260236		
Ferdinandea ruficornis	LT707518				

Tableau A1 : Numéros d'accession des séquences des gènes de la cytochrome oxydase I (COI) importées de Genbank et utilisées pour réaliser un arbre global de phylogénie des syrphes

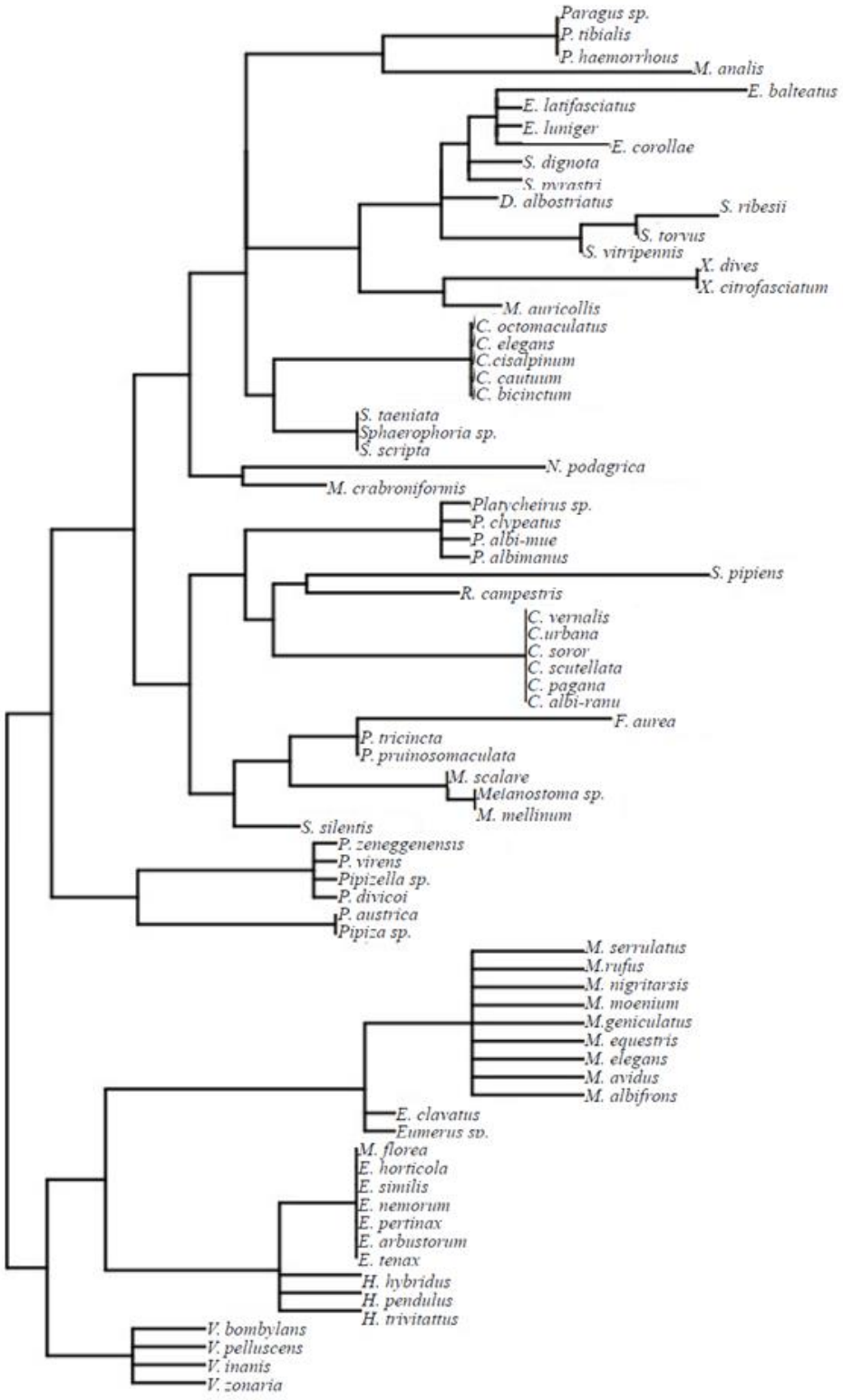

Figure A5: Arbre phylogénétique des espèces de syrphes utilisées dans l'analyse

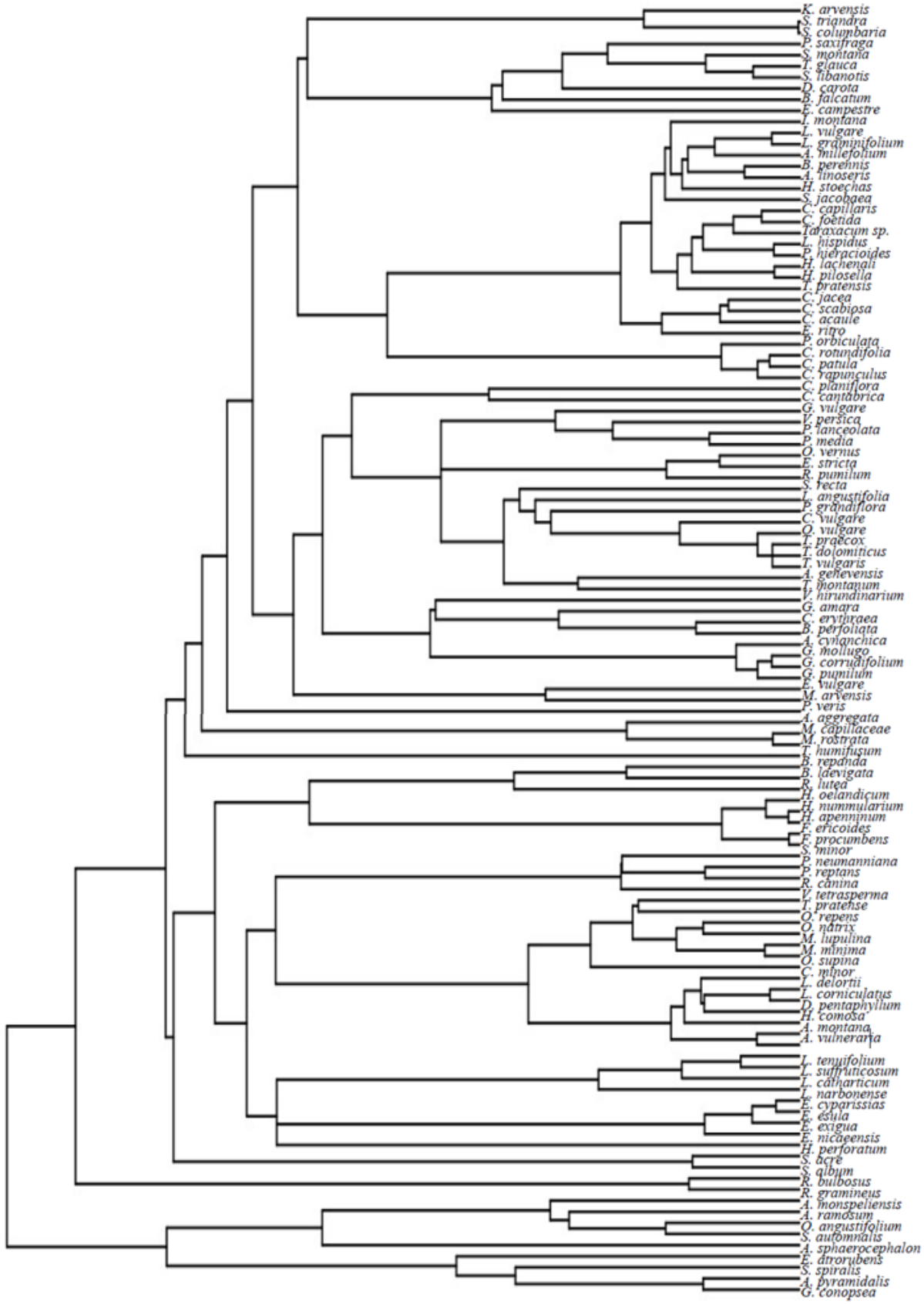

Figure A5: Arbre phylogénétique des espèces de plantes utilisées dans l'analyse. Il s'agit de l'arbre Daphne (Durka et Michalski, 2012) dans lequel ont été sélectionnées les espèces d'intérêt

Espèce	Abbréviation		Espèce	Abbréviation
Cheilosia albitarsis (Meigen), 1822	*C. albi-ranu*		*Paragus absidatus* Goeldlin, 1971	*Paragus sp.*
Cheilosia ranunculi Doczkal, 2000	*C. albi-ranu*		*Paragus albifrons* (Fallen), 1817	
Cheilosia pagana (Meigen), 1822	*C. pagana*		*Paragus atlasi* Claussen, 1989	
Cheilosia scutellata (Fallen), 1817	*C. scutellata*		*Paragus bicolor* (Fabricius), 1794	
Cheilosia soror (Zetterstedt), 1843	*C. soror*		*Paragus bradescui* Stanescu, 1981	
Cheilosia urbana (Meigen), 1822	*C. urbana*		*Paragus cinctus* Schiner & Egger, 1853	
Cheilosia vernalis (Fallen), 1817	*C. vernalis*		*Paragus flammeus* Goeldlin, 1971	
Chrysotoxum bicinctum (L.), 1758	*C. bicinctum*		*Paragus pecchiolii* Rondani, 1857	
Chrysotoxum cautum (Harris), 1776	*C. cautum*		*Paragus punctulatus* Zetterstedt, 1838	
Chrysotoxum cisalpinum Rondani, 1845	*C. cisalpinum*		*Paragus quadrifasciatus* Meigen, 1822	
Chrysotoxum elegans Loew, 1841	*C. elegans*		*Paragus romanicus* Stanescu, 1992	
Chrysotoxum octomaculatum Curtis, 1837	*C. octomaculatum*		*Paragus strigatus* Meigen, 1822	
Dasysyrphus albostriatus (Fallen), 1817	*D. albostriatus*		*Pelecocera pruinosomaculata* Strobl, 1906	*P. pruinosomaculata*
Episyrphus balteatus (De Geer), 1776	*E. balteatus*		*Pelecocera tricincta* Meigen, 1822	*P. tricincta*
Eristalis arbustorum (L.), 1758	*E. arbustorum*		*Pipiza austriaca* Meigen, 1822	*P. austriaca*
Eristalis horticola (De Geer), 1776	*E. horticola*		*Pipizella divicoi* (Goeldlin), 1974	*P. divicoi*
Eristalis nemorum (L.), 1758	*E. nemorum*		*Pipizella virens* (Fabricius), 1805	*P. virens*
Eristalis pertinax (Scopoli), 1763	*E. pertinax*		*Pipizella zeneggenensis* (Goeldlin), 1974	*P. zeneggenensis*
Eristalis similis (Fallen), 1817	*E. similis*		*Pipizella annulata* (Macquart), 1829	*Pipizella sp.*
Eristalis tenax (L.), 1758	*E. tenax*		*Pipizella brevis* Lucas, 1977	
Eumerus clavatus Becker, 1923	*E. clavatus*		*Pipizella maculipennis* (Meigen), 1822	
Eumerus amoenus Loew, 1848	*Eumerus sp.*		*Pipizella viduata* (L.), 1758	
Eumerus consimilis Simic & Vujic, 1996			*Platycheirus albimanus* (Fabricius), 1781	*P. albimanus*
Eumerus elaverensis Seguy, 1961			*Platycheirus muelleri* Marcuzzi, 1941	*P. albi-mue*
Eumerus flavitarsis Zetterstedt, 1843			*Platycheirus clypeatus* (Meigen), 1822	*P. clypeatus*
Eumerus funeralis Meigen, 1822			*Platycheirus ambiguus* (Fallen), 1817	*Platycheirus sp.*
Eumerus ornatus Meigen, 1822			*Platycheirus angustatus* (Zetterstedt), 1843	
Eumerus pulchellus Loew, 1848			*Platycheirus angustipes* Goeldlin, 1974	
Eumerus ruficornis Meigen, 1822			*Platycheirus europaeus* Goeldlin, Maibach & Speight, 1990	
Eumerus sabulonum (Fallen), 1817			*Platycheirus fulviventris* (Macquart), 1829	
Eumerus sinuatus Loew, 1855			*Platycheirus manicatus* (Meigen), 1822	
Eumerus sogdianus Stackelberg, 1952			*Platycheirus melanopsis* Loew, 1856	
Eumerus strigatus (Fallen), 1817			*Platycheirus occultus* Goeldlin, Maibach & Speight, 1990	
Eumerus tricolor (Fabricius), 1798			*Platycheirus parmatus* Rondani, 1857	
Eupeodes corollae (Fabricius), 1794	*E. corollae*		*Platycheirus scambus* (Staeger), 1843	
Eupeodes latifasciatus (Macquart), 1829	*E. latifasciatus*		*Platycheirus scutatus* (Meigen), 1822	
Eupeodes luniger (Meigen), 1822	*E. luniger*		*Platycheirus splendidus* Rotheray, 1998	
Ferdinandea aurea Rondani, 1844	*F. aurea*		*Platycheirus sticticus* (Meigen), 1822	
Helophilus hybridus Loew, 1846	*H. hybridus*		*Platycheirus transfugus* (Zetterstedt), 1838	
Helophilus pendulus (L.), 1758	*H. pendulus*		*Rhingia campestris* Meigen, 1822	*R. campestris*
Helophilus trivittatus (Fabricius), 1805	*H. trivittatus*		*Scaeva dignota* (Rondani, 1857)	*S. dignota*
Melanostoma mellinum (L.), 1758	*M. mellinum*		*Scaeva pyrastri* (L.), 1758	*S. pyrastri*
Melanostoma scalare (Fabricius), 1794	*M. scalare*		*Sericomyia silentis* (Harris), 1776	*S. silentis*
Meliscaeva auricollis (Meigen), 1822	*M. auricollis*		*Sphaerophoria scripta* (L.), 1758	*S. scripta*
Merodon albifrons Meigen, 1822	*M. albifrons*		*Sphaerophoria taeniata* (Meigen), 1822	*S. taeniata*
Merodon avidus (Rossi), 1790	*M. avidus*		*Sphaerophoria bankowskae* Goeldlin, 1989	*Sphaerophoria sp.*
Merodon elegans Hurkmans, 1993	*M. elegans*		*Sphaerophoria batava* Goeldlin, 1974	
Merodon equestris (Fabricius), 1794	*M. equestris*		*Sphaerophoria chongjini* Bankowska, 1964	
Merodon geniculatus Strobl, 1909	*M. geniculatus*		*Sphaerophoria estebani* Goeldlin, 1991	
Merodon moenium (Wiedemann), 1822	*M. moenium*		*Sphaerophoria infuscata* Goeldlin, 1974	
Merodon nigritarsis Rondani, 1845	*M. nigritarsis*		*Sphaerophoria interrupta* (Fabricius), 1805	
Merodon rufus Meigen, 1838	*M. rufus*		*Sphaerophoria loewi* Zetterstedt, 1843	
Merodon serrulatus Wiedemann in Meigen, 1822	*M. serrulatus*		*Sphaerophoria philanthus* (Meigen), 1822	
Microdon analis (Macquart), 1842	*M. analis*		*Sphaerophoria potentillae* Claussen, 1984	
Milesia crabroniformis (Fabricius), 1775	*M. crabroniformis*		*Sphaerophoria rueppelli* (Wiedemann), 1830	
Myathropa florea (L.), 1758	*M. florea*		*Sphaerophoria virgata* Goeldlin, 1974	
Neoascia podagrica (Fabricius), 1775	*N. podagrica*		*Syritta pipiens* (L.), 1758	*S. pipiens*
Paragus haemorrhous Meigen, 1822	*P. haemorrhous*		*Syrphus ribesii* (L.), 1758	*S. ribesii*
Paragus tibialis (Fallen), 1817	*P. tibialis*		*Syrphus torvus* Osten-Sacken, 1875	*S. torvus*
			Syrphus vitripennis Meigen, 1822	*S. vitripennis*
			Volucella bombylans (L.), 1758	*V. bombylans*
			Volucella inanis (L.), 1758	*V. inanis*
			Volucella pellucens (L.), 1758	*V. pellucens*
			Volucella zonaria (Poda), 1761	*V. zonaria*
			Xanthogramma citrofasciatum (De Geer), 1776	*X. citrofasciatum*
			Xanthogramma dives (Rondani), 1857	*X. dives*

Tableau A2 : Espèces de syrphes étudiées dans l'analyse et abréviations utilisées

Espèce	Abbréviation		Espèce	Abbréviation
Achillea millefolium	*A. millefolium*		*Lavandula angustifolia*	*L. angustifolia*
Ajuga genevensis	*A. genevensis*		*Leontodon hispidus*	*L. hispidus*
Allium sphaerocephalon	*A. sphaerocephalon*		*Leucanthemum graminifolium*	*L. graminifolium*
Anacamptis pyramidalis	*A. pyramidalis*		*Leucanthemum vulgare*	*L. vulgare*
Anthericum ramosum	*A. ramosum*		*Linum catharticum*	*L. catharticum*
Anthyllis montana	*A. montana*		*Linum narbonense*	*L. narbonense*
Anthyllis vulneraria	*A. vulneraria*		*Linum suffruticosum*	*L. suffruticosum*
Aphyllanthes monspeliensis	*A. monspeliensis*		*Linum tenuifolium*	*L. tenuifolium*
Arenaria aggregata	*A. aggregata*		*Lotus corniculatus*	*L. corniculatus*
Asperula cynanchica	*A. cynanchica*		*Lotus delortii*	*L. delortii*
Aster linosyris	*A. linosyris*		*Medicago lupulina*	*M. lupulina*
Bellis perennis	*B. perennis*		*Medicago minima*	*M. minima*
Biscutella laevigata	*B. laevigata*		*Minuartia capillacea*	*M. capillacea*
Blackstonia perfoliata	*B. perfoliata*		*Minuartia rostrata*	*M. rostrata*
Brassica repanda	*B. repanda*		*Myosotis arvensis*	*M. arvensis*
Bupleurum falcatum	*B. falcatum*		*Odontites verna*	*O. verna*
Campanula patula	*C. patula*		*Onobrychis supina*	*O. supina*
Campanula rapunculus	*C. rapunculus*		*Ononis natrix*	*O. natrix*
Campanula rotundifolia	*C. rotundifolia*		*Ononis repens*	*O. repens*
Centaurea jacea	*C. jacea*		*Origanum vulgare*	*O. vulgare*
Centaurea scabiosa	*C. scabiosa*		*Ornithogalum angustifolium*	*O. angustifolium*
Centaurium erythraea	*C. erythraea*		*Phyteuma orbiculare*	*P. orbiculare*
Cirsium acaule	*C. acaule*		*Picris hieracioides*	*P. hieracioides*
Clinopodium vulgare	*C. vulgare*		*Pimpinella saxifraga*	*P. saxifraga*
Convolvulus cantabrica	*C. cantabrica*		*Plantago lanceolata*	*P. lanceolata*
Coronilla minima	*C. minima*		*Plantago media*	*P. media*
Crepis capillaris	*C. capillaris*		*Potentilla neumanniana*	*P. neumanniana*
Crepis foetida	*C. foetida*		*Potentilla reptans*	*P. reptans*
Cuscuta planiflora var. godronii	*C. planiflora*		*Primula veris*	*P. veris*
Daucus carota	*D. carota*		*Prunella grandiflora*	*P. grandiflora*
Dorycnium pentaphyllum	*D. pentaphyllum*		*Ranunculus bulbosus*	*R. bulbosus*
Echinops ritro	*E. ritro*		*Ranunculus gramineus*	*R. gramineus*
Echium vulgare	*E. vulgare*		*Reseda lutea*	*R. lutea*
Epipactis atrorubens	*E. atrorubens*		*Rhinanthus pumilus*	*R. pumilus*
Eryngium campestre	*E. campestre*		*Rosa canina*	*R. canina*
Euphorbia cyparissias	*E. cyparissias*		*Sanguisorba minor*	*S. minor*
Euphorbia esula	*E. esula*		*Scabiosa columbaria*	*S. columbaria*
Euphorbia exigua	*E. exigua*		*Scabiosa triandra*	*S. triandra*
Euphorbia nicaeensis	*E. nicaeensis*		*Scilla autumnalis*	*S. autumnalis*
Euphrasia stricta	*E. stricta*		*Sedum acre*	*S. acre*
Fumana ericoides	*F. ericoides*		*Sedum album subsp micranthum*	*S. album*
Fumana procumbens	*F. procumbens*		*Senecio jacobaea*	*S. jacobaea*
Galium corrudifolium	*G. corrudifolium*		*Seseli libanotis*	*S. libanotis*
Galium mollugo	*G. mollugo*		*Seseli montanum*	*S. montanum*
Galium pumilum	*G. pumilum*		*Spiranthes spiralis*	*S. spiralis*
Gentianella amarella	*G. amarella*		*Stachys recta*	*S. recta*
Globularia vulgaris	*G. vulgaris*		*Taraxacum sp.*	*Taraxacum sp.*
Gymnadenia conopsea	*G. conopsea*		*Teucrium montanum*	*T. montanum*
Helianthemum apenninum	*H. apenninum*		*Thesium humifusum*	*T. humifusum*
Helianthemum nummularium	*H. nummularium*		*Thymus dolomiticus*	*T. dolomiticus*
Helianthemum oelandicum	*H. oelandicum*		*Thymus praecox*	*T. praecox*
Helichrysum stoechas	*H. stoechas*		*Thymus vulgaris*	*T. vulgaris*
Hieracium lachenalii	*H. lachenalii*		*Tragopogon pratensis*	*T. pratensis*
Hieracium pilosella	*H. pilosella*		*Trifolium pratense*	*T. pratense*
Hippocrepis comosa	*H. comosa*		*Trinia glauca*	*T. glauca*
Hypericum perforatum	*H. perforatum*		*Veronica persica*	*V. persica*
Inula montana	*I. montana*		*Vicia tetrasperma*	*V. tetrasperma*
Knautia arvensis	*K. arvensis*		*Vincetoxicum hirundinaria*	*V. hirundinaria*

Tableau A3 : Espèces de plantes déterminées sur l'ensemble des sites et leur abréviation

Annex III

Morphological analysis of *Globularia vulgaris* and *Ranunculus bulbosus*

Extract from Hineiti Lou Chao's Master Dissertation

Matériels et méthodes

Sites géographiques et espèces étudiées :

Dans le cadre de mon stage, deux espèces entomophiles de prairies calcicoles ont été choisies : Globularia vulgaris *(Plantaginacée)*, la globulaire commune et Ranunculus bulbosus *(Ranunculacée)*, la renoncule bulbeuse (voir Figures 1 et 2). Leur aire de répartition respective était assez étendue et assurait de trouver la même espèce dans au moins deux des régions étudiées. Ainsi, la variabilité des traits floraux pouvait être étudiée pour les populations de G. vulgaris d'Occitanie et de Haute-Normandie, ainsi que pour celles de R. bulbosus d'Occitanie et des Hauts-de-France.

Figure 1 : Fleur de *Globularia vulgaris* (d'après tela-botanica.org)

Figure 2 : Fleur de *Ranunculus bulbosus* (d'après tela-botanica.org)

G. vulgaris (anciennement *Globularia bisnagarica*) est une herbacée pérenne haute de 10 à 30 centimètres. Ses fleurs sont bleues avec un capitule globuleux de 1 à 1,5 cm de diamètre. Cette espèce thermophile est retrouvée en France métropolitaine hormis dans la zone la plus septentrionale. *La floraison de la globulaire se déroule de mai à juillet, période pendant laquelle chaque plante produit 1 à 10 tiges portant chacune une fleur. D'après* Honnay et al. *(2007), les pollinisateurs principaux de cette herbacée sont les papillons, les Apoïdés à langue longue (Apidés et Mégachilidés) et les Syrphes (Diptères).*

R. bulbosus (sous-espèce bulbosus) est aussi une herbacée pérenne qui elle, est caractérisée par un bulbe. Ses fleurs sont jaunes assez brillantes avec 5 pétales (voire 7 pour certains individus). Cette espèce est présente dans toute la France métropolitaine. R. bulbosus *fleurit entre avril et juillet où elle développe 1 à 10 tiges portant chacune jusqu'à 5 fleurs. D'après Matter* et al. *(2012), cette espèce est visitée par plusieurs pollinisateurs généralistes tels que les Diptères (Syrphidés, Muscidae, Anthomyiidae), les Coléoptères et les petites abeilles sauvages (Halictidae). Certaines visites d'*Apis melifera *ont également été observées bien que les Apidés sont plus rares sur* R. bulbosus.

Les sites géographiques prospectés pour l'étude de la variabilité des traits floraux sont ceux choisis dans le cadre du projet ARSENIC pour l'étude des interactions plantes-pollinisateurs. En effet, le choix de ces sites a été dirigé par la volonté de comparer les réseaux plantes-pollinisateurs sur un gradient de diversité, symbolisé par un gradient latitudinal (voir Figure 3). Les six sites sélectionnés se répartissent donc sur trois zones géographiques en France :

dans les Hauts-de-France pour le Larris et le Riez,

en Haute-Normandie pour Château Gaillard et les Falaises de Giverny,

et en Occitanie dans les Causses de Blandas pour le Bois de Fontaret et les Fourches.

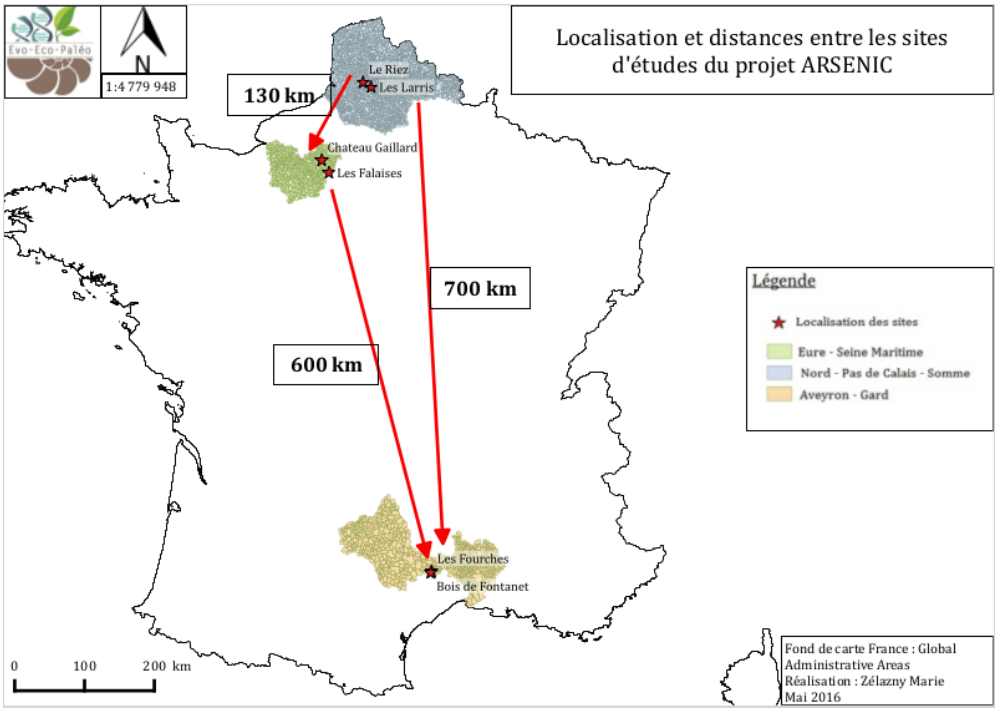

Figure 3 : Carte des six sites étudiés dans le cadre du projet ARSENIC

Récolte et analyse des données morphologiques :

1) Mesures morphologiques :

En parallèle des extractions d'odeurs de 2017, j'ai relevé plusieurs caractéristiques morphologiques des plantes échantillonnées : le diamètre sur pied des fleurs échantillonnées, le nombre de fleurs en bon état (non flétries et avec du pollen), le nombre de boutons et le nombre total de fleurs sur la rosette, ainsi que les plus grands et plus petits diamètres de la rosette qui ont servi à calculer sa surface. Pour le diamètre de la rosette de *R. bulbosus*, seul le diamètre maximal a été conservé pour les

analyses car certaines rosettes n'étaient pas encore assez développées pour pouvoir prendre les deux diamètres.

Pour avoir une idée plus globale de la variabilité des traits morphologiques, j'ai aussi récolté d'autres fleurs que celles dont les odeurs ont été extraites. Par cet échantillonnage, nous voulions estimer la taille des fleurs à l'échelle de la population en choisissant des fleurs au hasard sur le site. Pour ce faire, après chaque session de travail sur le terrain, ces fleurs ont été scotchées sur une feuille de papier afin de mesurer le diamètre maximal des deux espèces étudiées. Ce travail a été effectué pour 10 fleurs par espèce et par site géographique.

2) Analyses statistiques des traits morphologiques :

À partir des données morphologiques que j'ai récoltées, j'ai conduit des analyses statistiques de type non paramétrique en fonction de la normalité des données. Ces analyses ont été effectuées uniquement sur les données d'avril 2017 afin que je puisse finaliser mon rapport de stage dans le temps imparti. Toutes les analyses statistiques effectuées ont été réalisées sous R.

Pour l'ensemble des données morphologiques récoltées en avril 2017, je disposais des données récoltées dans deux régions différentes pour *G. vulgaris* alors que pour *R. bulbosus*, cela n'était pas le cas. En effet, pour *G. vulgaris*, les traits avaient été mesurés en Occitanie, sur Bois de Fontaret et les Fourches, ainsi qu'en Haute-Normandie, sur Château Gaillard et les Falaises de Giverny. Par contre, pour *R. bulbosus*, les analyses que j'ai faites n'ont concerné que les traits relevés dans les Hauts-de-France, sur le Riez et le Larris, étant donné que la floraison y a été plus tardive. À noter que pour *G. vulgaris*, les traits morphologiques à l'échelle de la population n'ont pas été récolté sur les Fourches, ce qui explique pour cette catégorie de traits morphologiques, il n'y a que 3 sites prospectés au sein de deux régions différentes. Ainsi, je ne pouvais pas m'intéresser à l'effet du facteur 'Région' en conduisant mes analyses statistiques mais uniquement à celui du facteur 'Site'.

Afin de déterminer s'il existait des différences significatives entre les sites étudiés, j'ai, dans un premier temps, effectué des tests de normalité de Shapiro sur les données. La normalité des données brutes n'étant pas vérifiée pour la majorité des données (voir Annexe 3), j'ai décidé d'effectuer des tests non paramétriques pour échantillons indépendants sur l'ensemble des données morphologiques : des tests de Wilcoxon-Mann-Whitney lorsque je devais comparer les populations de deux sites et des tests Kruskal-Wallis lorsque je devais comparer au moins 3 populations différentes. Pour les tests de Kruskal-Wallis qui montraient qu'il existait bien une différence significative entre les sites, j'ai appliqué un test post-hoc prenant en compte la présence de nombreux ex-aequo parmi les données en appliquant la fonction posthoc.kruskal.nemeyi.test disponible sous le package PMCMR.

Variabilité de la morphologie chez *G. vulgaris* :

Le tableau 4 reprend les résultats des tests statistiques de Kruskal-Wallis effectués sur les traits morphologiques pris sur *G. vulgaris*.

Tableau 4: Résultats des tests de Kruskal-Wallis sur les données morphologiques prises sur G. vulgaris

Ce tableau contient la valeur de la variable statistique de Kruskal-Wallis 'χ²', le degré de liberté 'dF' et la p-value 'p-value' correspondante. La colonne de droite indique si la différence entre au moins un échantillon et les autres est significative (*), très significative (**), hautement significative (***) ou non significative (x).

Trait considéré	Caractère considéré	χ²	dF	p – value	Significativité
Morphologie des individus choisis pour l'extraction des odeurs	Diamètre de la fleur sur pied	24.409	3	2.052e-05	***
	Surface de la rosette	2.3166	3	0.5094	x
	Nombre de fleurs total	13.879	3	0.003075	**
	Nombre de fleurs avec du pollen	14.86	3	0.001941	**
	Nombre de boutons	10.067	3	0.01801	*
Morphologie des individus pris au hasard sur le site	Hauteur de la fleur	23.18	2	9.257e-06	***
	Largeur de la fleur	23.259	2	8.902e-06	***
	Surface de la fleur	23.272	2	8.841e-06	***

D'après le tableau 4, on peut constater qu'il existe des différences significatives entre les populations de *G. vulgaris* pour l'ensemble des traits morphologiques relevés hormis pour la surface de la rosette. Par exemple, pour le nombre total de fleurs sur la rosette et le nombre de fleurs en bon état, la différence entre les 4 sites étudiés est très significative avec un nombre de fleurs nettement plus important pour la population du Bois de Fontaret (voir Figure 13).

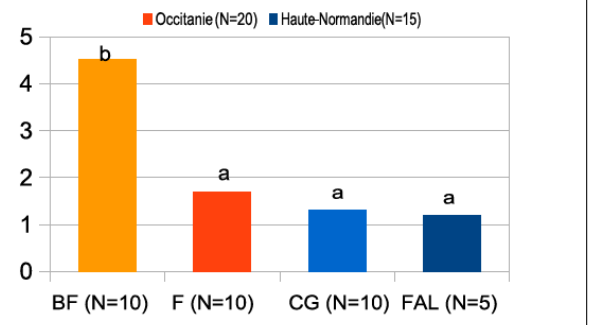

Figure 13 : Nombre moyen total de fleurs sur rosette pour G. vulgaris

Les lettres placées sur le graphique symbolisent les résultats obtenus aux tests posthoc de Kruskal-Wallis : « a » et « b » représentent respectivement des valeurs significativement inférieures et supérieures aux autres.

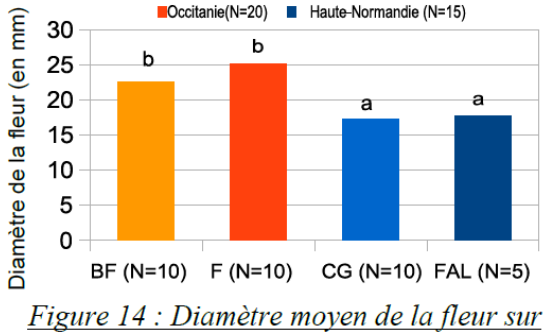

Figure 14 : Diamètre moyen de la fleur sur pied pour G. vulgaris

Les lettres placées sur le graphique symbolisent les résultats obtenus aux tests posthoc de Kruskal- Wallis : « a » et « b » représentent respectivement des valeurs significativement inférieures et supérieures aux autres.

Pour d'autres variables comme le diamètre moyen de la fleur sur pied, la différence entre les sites étudiés s'avère être une différence entre régions (voir Figure 14). Ainsi, les populations de Haute-Normandie auraient des fleurs moins grandes mais également moins de boutons sur leur rosette que les populations d'Occitanie. Pour les traits morphologiques des individus choisis au hasard sur les

sites, il existe également une variabilité intraspécifique significative (p-values inférieures à 9,3e-06). Ainsi, la population du Bois de Fontaret présente des fleurs de taille et de surface plus importantes que celles des sites de Haute-Normandie (voir Figures 15 et 16).

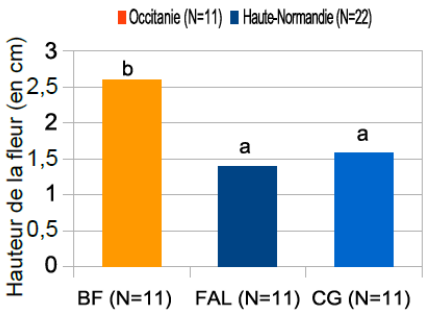

Figure 15 : Largeur moyenne de la fleur pour G. vulgaris

Les lettres placées sur le graphique symbolisent les résultats obtenus aux tests posthoc de Kruskal-Wallis : « a » et « b » représentent respectivement des valeurs significativement inférieures et supérieures aux autres.

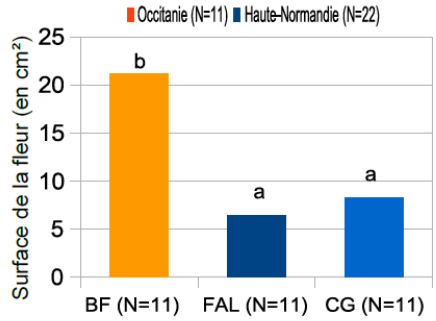

Figure 16 : Surface moyenne de la fleur pour G. vulgaris

Les lettres placées sur le graphique symbolisent les résultats obtenus aux tests posthoc de Kruskal- Wallis : « a » et « b » représentent respectivement des valeurs significativement inférieures et supérieures aux autres.

Variabilité de la morphologie chez *R. bulbosus* :

Le tableau 7 regroupe les résultats des tests de Wilcoxon-Mann-Whitney pour 2 échantillons indépendants, appliqués sur l'ensemble des variables mesurées sur *R. bulbosus*.

Tableau 7: Résultats des tests de Wilcoxon-Mann-Whitney pour les variables morphologiques relevéees sur R. bulbosus

Ce tableau contient la valeur de la variable statistique de Wilcoxon-Mann-Whitney 'W- stat' et la p-value 'p-value' correspondante. La colonne de droite indique si la différence entre les deux échantillons est significative (*), très significative (**), hautement significative (***) ou non significative (x).

Trait considéré	Caractère considéré	W - stat	p - value	Significativité
Morphologie des individus choisis pour l'extraction des odeurs	Diamètre de la fleur sur pied	400	4.199e-08	***
	Diamètre maximal de la rosette	1089	2.412e-12	***
	Nombre de fleurs total	280	0.02117	*
	Nombre de fleurs avec du pollen	120	0.006759	**
	Nombre de boutons	160	0.2572	x
Morphologie des fleurs choisies auhasardsurles sites	Hauteur de la fleur	245	0.2169	x
	Largeur de la fleur	255	0.1328	x
	Surface de la fleur	400	4.18e-08	***

Hormis le nombre de boutons, tous les traits morphologiques des individus choisis pour l'extraction d'odeurs présentent des différences au moins significatives entre les deux populations échantillonnées (voir Tableau 7). D'après la figure 19, on peut constater que la population du Riez présente

effectivement des rosettes plus grandes mais également un nombre de fleurs avec et sans pollen plus important que la population du Larris. Cependant, la population du Larris est celle qui présente des fleurs de taille plus importante (voir Figure 20).

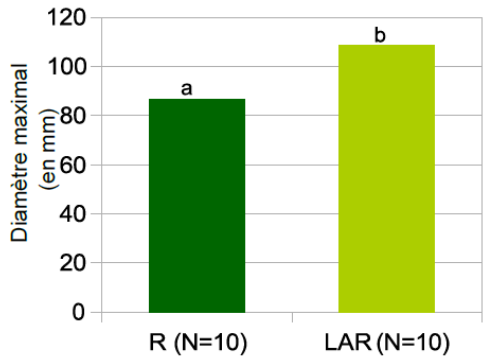

Figure 19 : Diamètre maximal moyen de la rosette pour R. bulbosus

Les lettres placées sur le graphique symbolisent les résultats obtenus aux tests de Wilcoxon-Mann-Whitney qui indiquent que la variable mesurée est significativement différente sur les deux sites. « a » et « b » représentent respectivement une valeur significativement inférieure ou supérieure à l'autre.

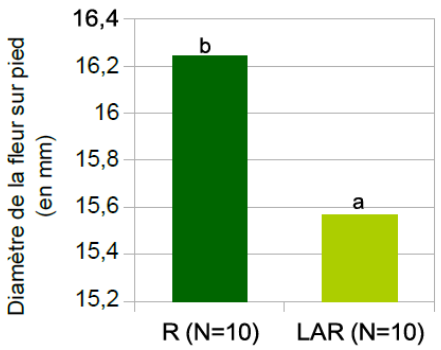

Figure 20 : Diamètre moyen de la fleur sur pied pour R. bulbosus

Les lettres placées sur le graphique symbolisent les résultats obtenus aux tests de Wilcoxon-Mann-Whitney qui indiquent que la variable mesurée est significativement différente sur les deux sites. « a » et « b » représentent respectivement une valeur significativement inférieure ou supérieure à l'autre.

Pour les traits morphologiques des fleurs prises au hasard sur les sites, seule la surface de la fleur est significativement différente entre les populations du Riez et du Larris (voir Figure 21).

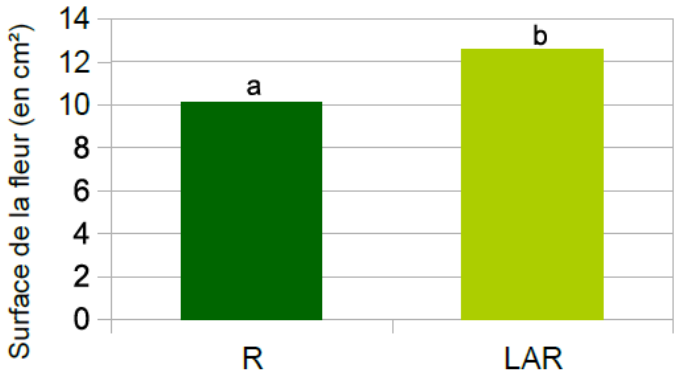

Figure 21 : Surface moyenne de la fleur chez R. bulbosus

Les lettres placées sur le graphique symbolisent les résultats obtenus aux tests de Wilcoxon-Mann-Whitney qui indiquent que la variable mesurée est significativement différente sur les deux sites. « a » et « b » représentent respectivement une valeur significativement inférieure ou supérieure à l'autre.

Abstract

In the current context of biodiversity crisis and the associated risks of ecosystem service failure, plant-pollinator networks are among the most studied mutualistic networks. Without pollinators, many plants could not reproduce and set seed, and 70% of agricultural production directly depends on them. However, pollinating insects constitute some of the terrestrial taxa most affected by global changes. As such, understanding plant-pollinator networks is of particular relevance if we are to prevent catastrophic disruption of pollination interactions and associated ecosystem services. In plant-pollinator networks, species need to be present in the same site and at the same moment for interactions to occur. In France, plant and pollinator abundance, richness and presence differ along the latitudinal gradient, which correspond to natural variations in biodiversity, and these variations could potentially affect network structure. Moreover, interaction networks are often reported based on temporally aggregated data, but in truth pollination interactions are not static and vary in time, since different plant and pollinator species display different phenologies. Large datasets on plant-pollinator interactions which comprise the entire flowering season or multiple years and allow relevant comparisons among networks along environmental gradient are rare. Due to their complexity and variation among years, most studies of mutualistic networks have focused on predicting and comparing classic network metrics which are all influenced by network size, *i.e.* the number of plant and insect species. Furthermore, most of these networks are based on interactions observed in the field, and thus some existing links between species remain unobserved. As such, visit-based networks represent a subsample of possible interactions, which call for the development of new methodological approaches to better explore the ecological processes determining species interactions. The general aim of this study is to understand and help predict the effects of environmental changes on plant and pollinator communities by studying plant-pollinator associations along an environmental gradient. Here, I provide and analyse a new database made of geo-localized data characterizing plant-pollinator associations at the species level, spatial variation in community structure and trait assemblage, focusing on six different calcareous grasslands along a latitudinal gradient in France. I first compared the taxonomical diversity variation in space (between and within region) and time (along the season). Then, I used a new methodological approach to compare networks of different size and to study the consequences of environmental gradients on plant-pollinator interaction probability. To understand how much distorted is our vision of plant-pollinator networks sampled following classic methods, I built more complete interaction networks using the pollen found on insects. Finally, I studied the mechanism behind geographical variation of floral scents and among-populations differences linked to the variation in the pollinator community. Because of the complexity and variation of plant-pollinator interactions, our study highlighted the importance to consider the entire flowering season and insects flying period, using species-level identifications, to disentangle the ecological species' role and the network variations. The use of new methodological approach allowed us to make networks comparison along the environmental and diversity gradient avoiding data circularity. The high amount of data provided in this thesis permitted to make comparisons at different level, from the entire network to species-specific variation among-populations.

Résumé

Dans le contexte actuel de crise de la biodiversité et des risques associés de dégradation des services écosystémiques, les réseaux plantes-pollinisateurs sont parmi les réseaux mutualistes les plus étudiés. Sans pollinisateurs, de nombreuses plantes sauvages et cultivées ne pourraient pas se reproduire. Il est important de comprendre les réseaux plantes-pollinisateurs si nous souhaitons empêcher la destruction des interactions de pollinisation et des services écosystémiques connexes. Dans ces réseaux, les espèces doivent être présentes au même endroit et au même moment pour interagir. En France, l'abondance, la richesse des plantes et des pollinisateurs diffèrent le long du gradient latitudinal, qui correspond aux variations naturelles de la biodiversité. Ces variations pourraient potentiellement affecter la structure du réseau. En outre, les réseaux d'interactions sont souvent décrits sur la base de données agrégées dans le temps, mais en réalité les interactions varient dans le temps. Les grands ensembles de données sur les interactions plantes-pollinisateurs, couvrant toute la saison de floraison ou plusieurs années et permettant des comparaisons pertinentes entre les réseaux le long du gradient environnemental, sont rares. En raison de leur complexité et de leur variation interannuelle, la plupart des études sur les réseaux mutualistes se sont concentrées sur la comparaison des mesures classiques des réseaux qui sont toutes influencées par la taille des réseaux (le nombre d'espèces). De plus, la plupart de ces réseaux sont fondés sur des interactions observées sur le terrain, ce qui fait que certains liens ne sont pas observés. Ainsi, ces réseaux représentent un sous-échantillon d'interactions possibles, ce qui nécessite l'élaboration de nouvelles approches méthodologiques pour mieux explorer les processus écologiques qui déterminent les interactions entre les espèces. L'objectif général de cette étude est de comprendre et d'aider à prédire les effets des changements environnementaux sur les communautés de plantes et de pollinisateurs en étudiant les associations plantes-pollinisateurs selon un gradient environnemental. Je fournis et analyse ici une nouvelle base de données constituée de données géolocalisées caractérisant les associations plantes-pollinisateurs au niveau de l'espèce, la variation spatiale de la structure des communautés et l'assemblage des traits, en me concentrant sur six prairies calcaires le long d'un gradient latitudinal en France. J'ai d'abord comparé la variation de la diversité taxonomique dans l'espace (inter et intra-région) et dans le temps (tout au long de la saison). Ensuite, j'ai utilisé une nouvelle approche méthodologique pour comparer des réseaux de tailles différentes et pour étudier les conséquences des gradients environnementaux sur la probabilité d'interaction plante-pollinisateur. Pour comprendre à quel point notre vision des réseaux échantillonnés selon des méthodes classiques est déformée, j'ai construit des réseaux d'interaction plus complets en utilisant le pollen trouvé sur les insectes. Enfin, j'ai étudié le mécanisme de variation géographique des odeurs florales et les différences entre les populations liées à la variation de la communauté des pollinisateurs. En raison de la complexité et variabilité des interactions plantes-pollinisateurs, notre étude a souligné l'importance de tenir compte de toute la saison de floraison et de la période de vol des insectes, en utilisant des identifications au niveau de l'espèce, afin de démêler le rôle écologique des espèces et les variations du réseau. L'utilisation d'une nouvelle approche méthodologique nous a permis de faire des comparaisons de réseaux le long du gradient environnemental et de diversité en évitant des problèmes de circularité. La grande quantité de données fournies dans cette thèse a permis d'effectuer des comparaisons à différents niveaux, du réseau dans son ensemble aux variations entre populations d'une même espèce.