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Comparison of grassland plant-pollinator networks on dairy farms in three contrasting French landscapes

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Abstract

Temperate grasslands provide both habitats and flower resources for pollinators in agricultural landscapes. Plant-pollinator networks change according to local and landscape variables, which are important to identify to help conserve pollinators in grasslands. We analysed plant-pollinator networks in 18 grasslands on experimental dairy farms located in three French regions contrasting by their climate, altitude, landscape or management. We combined visual surveys and pollen DNA barcoding. Our objectives were to determine which environmental factors influence pollinator taxa abundance and diversity and differences among the visual plant-pollinator networks in the three farming regions. Flower-visiting insects were trapped in six grasslands per farm during three sessions from mid-April to mid-July along fixed 400 m² transects. Insects were identified individually to the lowest taxonomic rank possible. Pollen carried by insects was identified using nuclear ribosomal ITS2 sequences belonging to the NCBI nucleotide database. The size and diversity of plant-pollinator networks were much larger and higher in permanent grasslands at the two farms located in lowlands (Mirecourt) and mountains (Marcenat) than those at the farm with temporary grasslands and a crop landscape (Lusignan), but the degree of specialisation (H2’) was relatively similar and low (mean of 0.46). Diptera, especially Empididae and Syrphidae, represented most plant-pollinator interactions in Mirecourt and Marcenat, while Hymenoptera were more abundant at Lusignan. The percentage of semi-natural habitats in 500 m buffers and vegetation height explained 23% of the variance in pollinator abundance. *Ranunculus* sp., *Knautia arvensis*, *Centaurea jacea* and *Trifolium repens* were key plant species in the networks. DNA metabarcoding of pollen loads identified 114 genera in addition to those identified by visual observations (+34-42 per site), reflecting insects’ floral pathways and differences in the immediate landscape among farms. This study highlighted the importance of Diptera in plant-pollinator networks and the need to conserve permanent grassland diversity to conserve pollinators.

Key words: pollination, agricultural practice, DNA bar-coding, Diptera, Hymenoptera, metagenomics

Introduction

Permanent and temporary grasslands are major ecosystems that cover 33% and 6% of utilised agricultural area in Europe, respectively (Huygues et al., 2014) and 9 and 3 million ha in France (Agreste, 2019) in different livestock farming contexts. Permanent grasslands could generally have a greater plant species diversity than temporary grasslands which are renewed within five years and are usually part of arable rotations. Permanent grasslands are suitable habitats for many flora and fauna species (Habel et al., 2013) and provide a wide range of ecosystem services (e.g. Bengtsson et al., 2019). However, they have decreased by 10 million ha over the last 50 years in Europe (Huygues et al., 2014), mainly due to conversion to arable land to produce animal feed or due to abandonment. Among grassland functions, entomophilous pollination is crucial, but pollinators have been declining for the last two decades (Potts et al., 2010), with an estimated decline of 77% in flying insect biomass from 1989-2016 in nature protection areas in Germany (Hallmann et al., 2017). In temperate ecosystems, most pollinators are insects (Hymenoptera, Diptera, Lepidoptera and Coleoptera), and 78% of plants depend on animals to ensure their sexual reproduction (Ollerton et al., 2011). Grasslands provide both habitats and flower resources for pollinators in agricultural landscapes. For instance, calcareous grasslands are major habitats for wild bees to support pollination in adjacent agricultural landscapes (Klaus et al., 2021; Steffan-Dewenter and Tscharntke, 1999). An increase in grassland plant species richness can increase pollinator diversity and the pollination of adjacent crop production (e.g. Orford et al., 2016, Woodcock et al., 2013).. In return, pollinator diversity (Fontaine et al., 2005; Fründ et al., 2013) or abundance (Lundgren et al., 2016) helps conserve the floral diversity of grasslands.

At the plot scale, local management practices (i.e. frequency of mowing or grazing, types and amounts of synthetic fertilisers) can modify the diversity of plant-pollinator communities greatly. Plant diversity decreases with early mowing (Gaujour et al., 2012) and as nitrogen fertilisation increases (Jacquemyn et al., 2003). The diversity and abundance of pollinators decreases as mowing or mineral fertilisation (Hudewenz et al., 2012) or grazing intensity increases (Lazaro et al., 2016; Sjödin, 2007) with changes in flower communities. Moreover, the composition (e.g. percentage of semi-natural habitats, SNH) and configuration of landscapes can influence pollinator diversity in grasslands, but this effect varies among studies and pollinator orders. For instance, the percentage of surrounding grasslands can increase the species richness and abundance of bumblebees in grasslands (Diaz-Forero et al., 2013; Hatfield and LeBuhn, 2007), but some studies suggest that it has no effect on bees or hoverflies (e.g. Holzschuh et al., 2016). Forest habitats near grasslands can decrease the species richness of bumblebees (Diaz-Forero et al., 2013) and increase that of butterflies (Villemey et al., 2015). Effects of both local and landscape factors on a variety of pollinator orders have been studied less, but the results highlight differences of responses among taxa. Butterflies and beetles seem to be influenced more by local factors (e.g. grassland height, management) than landscape factors, unlike hoverflies. In comparison, bees were influenced by both local and landscape factors (Kormann et al., 2015; Sjödin et al., 2008).

Plants and pollinators are organised into bipartite networks with mutualistic interactions. These networks seem highly asymmetric (i.e. “specialists tend to interact with generalist partners” (Alarcon, 2010) and nested (i.e. “specialists interact with subsets of the species with which generalists interact” (Bascompte and Jordano, 2007). These characteristics could stabilise networks in case of species extinctions, disturbance or habitat loss (Fortuna and Bascompte, 2006; Jordano, 1987; Memmott et al., 2004) but this network asymmetry could be partly due to experimental artefacts (Blüthgen, 2010). Although some studies have shown generalisation and redundancy in these networks, complementarity and specialisation between species could be important (Fründ et al., 2010). Pollinator diversity and network specialisation can be influenced by the floristic richness and total blossom cover of grasslands. Indeed, an increase in floral resource diversity can provide more ecological niches for pollinators and thus render plant-pollinator networks more specialised (Junker et al., 2015). Previous studies showed that pollinator richness or visitation frequency can increase as plant species richness increases (e.g. Fornoff et al., 2017; Fründ et al., 2010), but some studies showed that specialisation of plant-pollinator networks can remain high regardless of the plant diversity (e.g. Weiner et al., 2011). Among pollinators on grasslands, bees could have the highest specialisation and dipterans the lowest (Weiner et al., 2011). As bees are often considered to be the main pollinators in many habitats (e.g. Willmer et al., 2017), fewer studies have considered Diptera. In grasslands, Diptera can represent a large percentage of plant foragers (Rader et al., 2016; Weiner et al., 2011) and play a major role in pollination (Tiusanen et al., 2016). In particular, Diptera are more abundant when elevation increases (Kearns, 1992; Lefebvre et al., 2014).

To understand grassland pollination and improve conservation of all pollinators on farms, it is thus important to determine which local and landscape variables influence the characteristics of plant-pollinator networks. Our study objective was to analyse plant-pollinator networks in 18 grasslands on experimental farms located in three French regions with contrasting landscape composition: crop-dominated landscapes surround plots the most at Lusignan (48-91% of surface area in a 1000 m buffer), whereas SNH surround plots the most at Mirecourt (47-67%) and especially at Marcenat (81-95%). The originality was to assess plant-pollinator interactions of permanent and temporary grasslands by combining two methods: visual identification of insects and plants and identification of the pollen transported by each pollinator using DNA metabarcoding. Our research questions were: (1) which environmental factors (i.e. local plant community, local management practices and percentage of SNH around grasslands) influence the abundance and diversity of pollinator taxa? (2) how do visual plant-pollinator networks differ among the three farming regions? and (3) which contribution of metagenomics to identify pollen and track pollinator visitations in grasslands ?

Material and methods

1. Study sites and sampled grasslands

This study was conducted on three experimental farms (hereafter, “site”) of INRAE (French National Research Institute for Agriculture, Food and Environment) located at Lusignan in western France (46°25’ N, 0°07’ E, 150 m a.s.l.), Mirecourt in eastern France (48°17’ N, 6°07’ E, 300 m a.s.l.) and Marcenat in the mountains of central France (45°18’ N, 2°50’ E, 1100 m a.s.l., **Figure 1**). Lusignan was the warmest site with the lowest rainfall (mean annual temperature of 11.9°C and mean precipitation of 790 mm from 2001-2015), while Marcenat was the coldest and rainiest (7.5°C; 1154 mm) and Mirecourt was intermediate (10.3°C; 821 mm, INRAE CLIMATIK platform). Lusignan is a 91ha farm composed of 48ha of temporary grasslands and 37ha of crops. Mirecourt, an organic farm since 2006, covers 238ha composed of 133ha of permanent grasslands and 105ha of crops. Marcenat is a farm of 89.5ha composed exclusively of permanent grasslands. We selected six grassland plots per farm (N=18 plots) representative of local farm management for our study. Characteristics of each plot are detailed in table 1. Plots were selected along a gradient of grassland plant species diversity within each site. The temporary grasslands of Lusignan were sown 2 to 4 years before the insect trapping and were included in an arable rotation.

2. Landscape characterisation around sampled plots

Landscape composition data were extracted using QGIS 3.4.3 Madeira (QGIS Development Team, 2019). The landscape surrounding each studied plot was described in 500 and 1000 m buffers around the plot's centroid, using environmental and agricultural vector data for France. The landscape was first described by agricultural land use using crop-group data from the RPG ("Registre Parcellaire Graphique") database provided by IGN (French National Geography Institute). Landscape composition was also described by SNH, which were extracted from BD TOPO® (IGN). From this GIS database of the agricultural and semi-natural landscape, we then calculated %SNH as the sum of percentages of woods, heathland, hedgerows, mountain pasture (rangelands) and permanent grasslands.

3. Pollinator and pollen trapping

To trap pollinators, one transect 100 m long and 4 m wide was delineated in the centre of each plot. Three sessions of pollinator trapping of generally 3 days each were performed on each transect in the morning, early afternoon and late afternoon. Pollinators were sampled in 2015 at Marcenat and in 2016 at Lusignan and Mirecourt. The periods of catching were 12-13-14 April; 22-23-24 June; 7-8-9 July in Lusignan, 17-20-21 May; 6-9-10 June; 27-29-30 June in Mirecourt, and 28-29-30-31 May and 2 June; 17-18-19-22 June; 7-8-9 July in Marcenat. These periods are spaced out to record the phenology changes of plant-pollinator interactions. Trapping periods ranged from 10:00-19:00. In total, 162 transect observations were performed (3 transect observations × 6 plots × 3 sessions × 3 sites). Trapping days were sunny, without precipitation or wind. All flower-visiting insects that were actively collecting pollen and/or nectar were trapped directly with Falcon^{TM} tubes (to avoid pollen contamination) by an observer walking along the transect for 15 min (stopping the stopwatch to process each insect trapped). Each flower species involved in the interaction was recorded to obtain the visual plant-pollinator network. Each insect collected was killed by injecting 10 mL of 60% ethanol into the Falcon^{TM} tube with a syringe. All tubes were stored at room temperature in the laboratories. For pollen and insect preparation methods, see Galliot et al., (2017). Briefly, pollen load was removed from the insect body by adding 30mL of 60% ethanol into the tube and shaking it firmly for 6s. The insect was then collected and pinned for visual identification to the lowest taxonomic rank possible. To identify pollen loads using DNA metabarcoding, the ethanol solution containing the pollen extracted from the insect was filtered on a 5 µm-pore nylon filter (Nitex 03–5/1, SEFAR, Heiden, CH) and the filter was then placed in a 1.5 mL microcentrifuge tube.

4. Vegetation measurements

Plant species richness was determined by walking 1-2 diagonals in each plot in May-June 2016 at Lusignan and Mirecourt. At Marcenat, floristic composition had been assessed 4-5 years previously by estimating the percentage cover of all plant species in late July in 1 m^2 quadrats distributed in each plot. The number of quadrats depended on plot size. At the three sites, total flower cover percentage (i.e. blossom cover) and flowering species richness were estimated visually in 30 m × 30 m quadrats distributed along the 400 m^2 of each observation transect according to the method of Farruggia et al. (2012). At the three sites, vegetation height was measured along each observation transect as the mean height of 90% of the total biomass.

5. DNA metabarcoding on pollen loads

We used the same metabarcoding protocol as Galliot et al. (2017) with the nuclear ribosomal universal specific primers ITS-S2F (5’-ATGCGATACTTGGTGTGAAT-3’) and ITS-4R (5’-

TCCTCCGCTTATTGATATGC-3’) (Chen et al., 2010). Sequencing was carried with the MiSeq® V3 Reagent Kit 600 cycles (Illumina Inc., San Diego, CA) but to increase the number of samples per run, we performed two libraries: 384 ITS2-F and ITS-4R and 384 to which 4 nucleotides (GATC) have been added at the 5' end. The reads of each sample were distinguished by identifying (or not) the added GATC motif in the demultiplexed reads. ITS2 sequences stored in the NCBI nucleotide database in March 2017 were retrieved with the criteria Gene name = ITS2, Organism = Plantae, and Sequence length = 100-3000 nt to construct our reference database, called “BDD_ITS2_NCBI_Plants_100-3000” constituted of 182 708 sequences belonging to 9000 genera. Tag and GATC-sorted reads were trimmed using the CLC Genomics Workbench 9.5.2 or 10.0 with the following parameters: (i) base quality Phred >20 with no ambiguity, (ii) removing 20 nt in 5’ and 3’ and (iii) read length > 130 nt. All trimmed reads were mapped using the CLC mapping algorithm with Similarity = 95%, Length = 70% against the BDD_ITS2_NCBI_Plants_100-3000 database. R2 reads were excluded from the analysis (insufficient quality). The mean length of trimmed R1 reads for the two sequencing runs were 151 nt (range: 73-212 nt) for the first run of sequencing and 176 nt (range: 87-252 nt) for the second one. Insect pollen loads were described to genus to avoid misclassification due to imprecision in the data base reference sequences. We assumed that a genus was present in a mixed pollen load if its percentage of reads in a sample exceeded 10% of all reads in the sample. This threshold was considered to be equivalent to the background noise. As suggested by Galliot et al., (2017) we retained only genera with more than three reads per genus per sample.

6. Data analysis

6.1. Spatial autocorrelation

We used R software (R Core Team, 2019) for all data analyses. The mean distance (± SD) between two sampled plots was 929 ± 611, 1242 ± 541 and 638 ± 314 m at Lusignan, Mirecourt and Marcenat, respectively. We tested the spatial autocorrelation of biodiversity data (i.e. mean blossom cover, mean flowering species richness, plant species richness and number of plant-pollinator interactions) among the six plots at each site using Mantel indices using the "ade4" package.

6.2. Visual plant-pollinator networks

Visual plant-pollinator networks were created for each site using the "bipartite" package by combining the three trapping sessions to have a sufficient data number The matrices included foraged flowers (mostly to species) and foraging insects (to the lowest taxonomic rank). The Shannon diversity index of plant-pollinator interactions, number of links per taxon, connectance, degree of specialisation H2', network asymmetry, specialisation asymmetry and weighted nestedness were calculated using the function "networklevel" of the bipartite package for each site. H2' describes the degree of "complementarity specialisation" or partitioning among two parties in the entire network (Blüthgen et al., 2006). H2' is affected less by network size or sampling intensity than connectance. H2' was also calculated for only the last two trapping sessions at each site. For each plant or pollinator taxon, we calculated strength, taxa degree and taxa specialisation d' which describes "the degree of interaction specialization" at the taxa level (Blüthgen et al., 2006). .

6.3. Statistical analysis

After checking for data normality using Shapiro-Wilk tests, Spearman's correlations were calculated between each environmental variable and the pollinator abundance for each plot.

We performed Redundancy Analysis (RDA), using the “vegan” package according to Borcard et al., (2011), to explain variation in a matrix of taxa (response variables) due to a matrix of explanatory variables. In all analyses, we used an α threshold of 0.05 and we excluded plot V12 (at Lusignan) due to under-sampling (one interaction for all sampling events performed). The response variables consisted of pollinator taxa abundance grouped by sampled plot without considering sampling dates. We transformed the pollinator matrix using a Hellinger transformation. Our explanatory variables were environmental variables known to influence pollinator community composition: mean blossom cover (i.e. total flower cover percentage, %), mean vegetation height (cm), flowering species richness and %SNH in 500 buffers around each sampled plot. We selected a %SNH in 500m buffers rather than 1000m because i) %SNH 500m and 1000m were correlated, ii) 1000m buffers overlapped between close plots in Lusignan and Marcenat, and iii) 500m buffers fitted better in our analysis. We also included variables that indicated local land-use intensification: number of mowing events of the year, grass use (livestock units-day ha^{-1}) of the year and amount of nitrogen fertilisation of the previous year (kg N ha^{-1}).

We checked for collinearity among environmental variables using a variance inflation factor (VIF) criterion. VIF<5 for all variables. We then selected a model using a step-wise procedure based on a permutation test with 10 000 iterations. For the most parsimonious model, only %SNH in a 500 m buffer and vegetation height were selected by the model. We calculated adjusted R² to assess the variance of the pollinator matrix explained by %SNH and vegetation height. We used a permutation test with 10 000 iterations to test the most parsimonious model and the significance of its canonical axes.

We then performed variance partitioning of the Hellinger-transformed pollinator community data using two environmental matrices: local variables (i.e. mean blossom cover, mean vegetation height, flowering species richness, number of mowing events, grass use and amount of nitrogen fertilisation) and a landscape variable (i.e. %SNH in a 500m buffer). We applied the same step-wise procedure as before and we kept only mean vegetation height, flowering species richness and nitrogen fertilisation for local variables. We tested the variance explained by each set of variables using permutation tests.

Finally, we compared mean d' specialisation by pollinator group (i.e. Other Apidae, Syrphidae, Diptera, Lepidoptera and Coleoptera) using ANOVA followed by Tukey's test to compare pairs of means when data were normal (Shapiro test) and homoscedastic (Bartlett test) or Kruskal-Wallis tests when data were not normal or heteroscedastic followed by the Wilcoxon test to compare pairs of means.

Results

1. Plant and pollinator diversities

A total of 979 pollinator insects were trapped at the 3 sites during the 3 sessions on 53 plant species (**Figure 2**). The plant families of Asteraceae, Renonculaceae and Fabaceae (mainly *Trifolium repens*) represented 35%, 20% and 16% of the total interactions with pollinators, respectively. Overall, 844 insects were identified to genus, of which 583 were identified to species. The pollinator richness identified was 107 species, 78 genera and 50 families. Only 10 genera were common to the 3 sites. Most interactions were performed by Diptera (mainly Empididae and Syrphidae) at Mirecourt and Marcenat (54% and 65% of site interactions, respectively) and by Hymenoptera at Lusignan (79% of site interactions). For all sites, the interaction frequency of Diptera decreased from 242 in session 1 to 140 in session 2 and to 79 in session 3. However, that of Syrphidae increased from session 1 to 3 (18 to 61 individuals, respectively). The interaction frequency of Hymenoptera increased by a factor of 3 from session 1 to 3, with an increase for bumblebees and honey bees and constant number for wild bees.

2. Effects of environmental variables on taxa pollinator abundance

Plots of Lusignan had a low %SNH, low flowering species richness and a high grass use. Mirecourt showed mainly many mowing events, high vegetation height and high total blossom cover. Marcenat had a high %SNH, high flowering species richness and a low grass use. Hence, environmental variables clustered strongly by regional site (**table 1**). Mean blossom cover, flowering species richness, plant species richness and the number of plant-pollinator interactions were not spatially autocorrelated among the plots at each site (Mantel test, $p > 0.05$). Pollinator abundance was positively correlated with the mean blossom cover ($r_s = 0.48$, $p < 0.001$), the mean plant species richness ($r_s = 0.57$, $p = 0.017$) and the mean flowering species richness ($r_s = 0.60$, $p = 0.011$) in each plot.

The most parsimonious model, with %SNH and vegetation height as explanatory variables, showed a significant linear relationship between the pollinator taxa abundance matrix and these variables ($F = 3.348$, $p < 0.001$, **Figure 3**). The first two axes of the RDA were significant (axis 1: $F = 4.539$, $p < 0.001$; axis 2: $F = 2.158$, $p = 0.005$). %SNH and vegetation height explained 23% of the variance in the pollinator matrix (adjusted $R^2 = 0.23$). Pollinator community composition clustered strongly by regional site. Non-identified Diptera and *Melanogaster nuda* were associated with a high %SNH, while *Empis* sp. were associated with both a high %SNH and high vegetation height. *Maniola jurtina*, *Episyrphus balteatus*, *Lasioglossum pauxillum* and *Eristalis* sp. were more abundant with high vegetation height, unlike Sphaeroceridae. *Bombus terrestris* and *Bombus lapidarius*, which were more abundant with low vegetation height. *Apis mellifera* were abundant with low %SNH and high vegetation height and *Andrena haemorrhoa* with low %SNH.

The variance of pollinator taxa abundance by plot explained by both local (i.e. vegetation height, flowering plant richness and nitrogen fertilisation) and landscape (%SNH) variables differed significantly from zero ($p < 0.05$, data not shown). Local environmental variables, landscape variables and both types together explained 9% ($p < 0.001$), 9% ($p < 0.001$) and 8%, respectively, of the variance in the transformed pollinator matrix.

3. Visual plant-pollinator networks

The plant-pollinator networks differed among the three sites (**Figure 4**). The network of Mirecourt and Marcenat had much higher insect taxa richness and Shannon diversity than that of Lusignan (**Table 2**). The number of unique interactions was higher at Marcenat (158) and Mirecourt (140) than that at Lusignan (60). For all three sites, connectance was relatively low and weighted nestedness were similar. The network at Lusignan had the highest network asymmetry (0.67) and lowest specialisation asymmetry, while that at Marcenat had the lowest network asymmetry (0.27) and highest specialisation asymmetry. When all three trapping sessions at each site were considered, Lusignan had the most specialised network (H2' = 0.54). When considering only two trapping sessions (i.e. June-July), however, all three sites had a similar degree of specialisation (mean H2' = 0.46).

At the taxon level (mainly species and genus, **Table 3**), pollinators with the highest strength were observed at Marcenat (e.g. Diptera, *Empis* sp., *Lasioglossum albipes, A. mellifera*), followed by Mirecourt (e.g. *Empis* sp., *Pseudovadonia livida*, *Episyrphus balteatus*) and Lusignan (*B. lapidarius*, *A. haemorrhoa*, *A. mellifera*). The strongest plant taxa differed greatly among sites: *T. repens* and *Cirsium palustre* attracted 72% of all pollinators at Lusignan, *Centaurea jacea* (26%) and *Ranunculus acris* (21%) were the most foraged taxa at Mirecourt and *Ranunculus* sp. (20%) and *Knautia arvensis* (12%) were the most foraged taxa at Marcenat.

At Lusignan, other Diptera were more specialised than Apidae (ANOVA, F = 3.7, p = 0.038; Tukey's test, p = 0.038, **Table 4**). At Mirecourt, other Diptera were more specialised than

Lepidoptera, (ANOVA, F = 4.29, p = 0.029; Tukey's test, p = 0.040) and Apidae had low values of d' (mean ± SD: 0.25 ± 0.25). At Marcenat, other Diptera and Syrphidae tended to have lower values of d' than Apidae, but the difference was not significant (Kruskal-Wallis test, p = 0.08).

4. Pollen analysis by metagenomics

The pollen DNA metabarcoding identified 84%, 81% and 75% of the genera of plants foraged in visual interactions at Lusignan, Mirecourt and Marcenat, respectively. A total of 107 plant genera were detected on pollen loads analysed from Diptera, Hymenoptera and Lepidoptera from the three sites. Most of these pollinators transported pollen from 1-3 genera (data not shown), with a maximum of 6 genera on one individual of *Pieris brassicae* (Lepidoptera). Moreover, metabarcoding of pollen loads identified 34, 42 and 38 plant genera in addition to those identified in the visual observations at Lusignan, Mirecourt and Marcenat, respectively (**Table 5**), which corresponded to 2-3 times the genera richness found with visual observations alone. Among the plant genera found only with the metagenomics method at the three sites, Dicotyledon and grass from grasslands were the main pollen carried. At Lusignan, 26% (mainly Apidae), 15% and 13% of pollinators sampled transported pollen of trees/shrubs, Dicotyledon from grasslands, and crops, respectively. At Mirecourt, 20% and 14% of pollinators sampled (mainly Syrphidae and Nymphalidae) transported pollen of grass and Dicotyledon from grasslands, respectively. At Marcenat, 22% of pollinators sampled (mainly Empididae and Syrphidae) transported pollen of Dicotyledon from grasslands.

Discussion

Effects of environmental factors on the abundance of pollinator taxa

The three sites differed in plant-pollinator compositions, which firstly reflects different regional species pools and/or a temporal effect due to different years of trapping. Pollinator abundance of each taxa was related to both landscape (percentage of semi-natural habitats, % SNH; in 500 m buffers) and local (vegetation height) variables. We determined that communities with a high %SNH within landscape were characterised mainly by dominant non-Syrphidae Diptera and *Melanogaster nuda*. Among the few studies of the influence of landscape on non-Syrphidae Diptera, Burel et al., (2004) showed that Empididae had higher species richness in landscapes with a large percentage of permanent grasslands, woods and hedgerows than in landscapes dominated by crops. In contrast, plots with low %SNH were dominated by polylectic bee species, which are known to be associated with farmlands due to the wide range of plant species they visit (e.g. *A. haemorrhoa*: Wood et al., 2016; *B. lapidarius*: Roger et al., 2017; *A. mellifera*, which are associated only with beekeeping in most of France: Requier et al., 2015). At Lusignan, Hymenoptera probably predominated because of higher temperatures (+ 0.7°C and + 2°C in mean temperature from April to July of the pollinator trapping year compared to Mirecourt and Marcenat), the crop landscape and temporary grasslands. Temporary grasslands of Lusignan was composed of *Trifolium repens* which was the most visited species, mainly by Apidae. *T. repens* has a large amount of nectar with a high sugar concentration (ca. 804 kg of nectar productivity ha^{-1} cover year^{-1} in Baude et al., 2016) and has a pollen rich in protein for bees (e.g. Ghosh et al., 2020; Hanley et al., 2008). Perennial plants, such as *T. repens*, have an higher melliferous potential than annual plants so that temporary grasslands at Lusignan can offer continuous resources for pollinators during the growing season in annual crop-dominated landscapes (Hicks et al., 2016; Ion et al., 2018).

Among local factors, the pollinator abundance of each grassland plot at the three sites was positively correlated with mean blossom cover and flowering species richness. These results were previously obtained in other studies (e.g. Ebeling et al. 2008 ;Hudewenz et al. 2012 ; Goulnik et al. 2020).and highlighted the importance of diversified permanent grasslands for pollinator conservation. Pollinators could benefit from more resources when the blossom cover increases. An increase in flowering species richness could enhance the functional diversity of floral traits and thus increase the niche partitioning for pollinators. This process could lead to an increase in pollinator abundance as recently observed by Goulnik et al. (2020) in French permanent grasslands. Vegetation height influenced the composition of pollinator community. Vegetation was on average shorter at Marcenat than in other sites. This difference may have been due to less favourable pedo-climatic conditions for grass growth rather than more intensive management, because Marcenat had a low grass use and number of mowing events. Grassland vegetation height has been shown to influence the species composition of Lepidoptera and Syrphidae (Sjödin et al., 2008) but also of other Diptera (Milberg et al., 2016). Stratification of vegetation height has been shown to positively influence niche partitioning of pollinator species, along with flowering species richness and time of day (Venjakob et al., 2016). Moreover, an increase in mean flower height of communities could lead to higher pollinator species richness and visitations, as recently highlighted by Fornoff et al. (2017) but not find in Goulnik et al., (2020).. In addition, higher vegetation heights were associated with mown grasslands (i.e. 1-2 cuts per year), whose pollinator composition can differ from that in grazed grasslands.

Visual plant-pollinator networks in the three farming regions

Plant-pollinator networks in the three studied sites had metrics similar to those in previous studies from several continents (i.e. H2’ of 0.41-0.54, positive network asymmetry and negative specialisation asymmetry, e.g. Blüthgen et al., 2007; Fründ et al., 2010) but smaller sizes. Size and diversity of plant-pollinator networks were much larger and higher at Mirecourt and Marcenat than those at Lusignan, likely due to lower plant taxa richness, lower %SNH and a higher grass use in Lusignan. Higher network asymmetry at Lusignan also highlighted a large difference in species richness between plants and insects. Most grasslands at Lusignan were temporary, which led to low spontaneous plant enrichment and reduced the plant species richness. We observed a higher degree of network specialisation in Lusignan when all three trapping periods were considered but H2' was relatively similar given the same sampling periods (June-July) for the three sites. Two hypotheses could explain these findings. First, the network of Lusignan appeared probably more specialised due to integration over a longer period (April-July) compared to the other sites (May-July), which increased the number of missing links between plants and pollinator taxa due to differences in phenology (Vázquez et al., 2009). Secondly, the lower flower diversity at Lusignan could force pollinators to forage fewer flower types.

Concerning pollinator taxa, at Lusignan and Mirecourt, Hymenoptera had the highest strength, but Diptera were the most specialised. In contrast, at Marcenat, Diptera had the highest strength but were the least specialised, in agreement with results of Weiner et al. (2014) for 50 German grassland plots. The degree of Syrphidae specialisation on our three sites (0.40-0.63) was lower than that found by de Manincor et al. (2020) but in the same order of magnitude as that found by Weiner et al. (2014) (0.24). The few interactions for other Diptera at Lusignan (24) could partly explain the high degree of specialization d' for these taxa (see Blüthgen et al., 2006). More generally, our results highlighted the key role of Diptera in plant-pollinator networks in grasslands. Although they generally have a greater handling time (reviewed in Inouye et al., 2015) and could transport less pollen than bees (e.g.

Goulnik et al., 2020 for facial measurements; Kearns and Inouye, 1994) because of lower hairiness and smaller sizes for some taxa, , their number of interactions remained high and structured the networks observed. They play an important role in pollen transfer of open flowers such as *Ranunculus* sp. and *Anthriscus sylvestris* in our study, particularly because most flies have short mouthparts (Woodcock et al., 2014). Some plant species were important to the network structure, particularly due to flower rewards (i.e. nectar and pollen). At both Mirecourt and Marcenat, *Ranunculus* sp. and *K. arvensis* had high strength. *Ranunculus* sp. contains a large amount of pollen and an average amount of nectar (http://www.floraweb.de). They bloomed quite early in the season, which benefited foragers such as *Empis* sp. *K. arvensis* is a well-known melliferous species that offers an average amount of nectar (Baude et al., 2016). At Mirecourt, *C. jacea* appeared as a key species that produced an average amount of nectar and was associated with extensive management of grasslands (Jacot et al., 2007) and wild crop-pollinating bees (Sutter et al., 2017). At Lusignan, *T. repens* was visited frequently by Hymenoptera such as *Bombus* sp. and *A. mellifera*, which confirms previous results (e.g. Goulson et al., 2005) and the important ability of *Trifolium* sp. to produce large amounts of nectar and pollen for foragers.

Contributions and limits of pollen DNA barcoding

The pollinators sampled carried pollen from an average of 1-3 genera at the three sites, with no difference among insect orders (i.e. Diptera, Hymenopteran and Lepidoptera). DNA metaboarcoding data highlighted that more plant genera than visual assessed were probably visited by pollinators (+34-42), which was 2-3 times the genera richness found with visual observations only. Pornon et al., (2017) also found an increase in plant visitations with pollen DNA metabarcoding compared to visual networks (+26-68 plant species), unlike previous results (e.g. Popic et al., 2013). Metabarcoding allowed us to reveal the flowers foraged by the insects at the plot scale. Moreover, our results suggest that pollen DNA barcoding can reflect differences in the surrounding landscape among sites. At Lusignan DNA barcoding showed that they were mainly Apidae but also Halictidae and Andrenidae which transported pollens of trees or shrubs, dicotyledons from grasslands and crops. Ligneous vegetation is known to play a key role for wild bee community (Rollin et al., 2019) and honeybee colonies development as well as large pollen supply for honeybees in spring in Europe (e.g. Requier , 2015) but also in America (Sponsler et al., 2020). On the other sites, DNA barcoding reflected pollen transports of grassland's plants (i.e. grass and dicotyledons) by Syrphidae and Nymphalidae (mainly *Maniola jurtina*) at Mirecourt and by Empididae and Syrphidae at Marcenat. Diptera and Lepidoptera can need different habitats and host plants for their larvae and adult development. However, characterising the landscape and metabarcoding to genus instead of species limited metagenomic analysis of the surrounding landscape.

We performed metabarcoding using ITS2 sequences from the NCBI nucleotide database to identify the mixed pollen loads. Using this database, we had to identify pollen to genus and not to species to minimise the risk of misclassifying species due to imprecise reference sequences. Indeed, despite the advantages of the NCBI library, one drawback of its open-accessibility remains a lack of homogeneity of taxonomic assignations, incomplete data, changes in taxonomic nomenclature, variability in the quality of the sequences provided and the frequent shortage of data traceability (Collins and Cruickshank, 2013; Fišer Pečnikar and Buzan, 2014; Galliot et al., 2017; Rimet et al., 2016). Consequently, as many studies, we stress the urgent need for a curated barcode database that researchers can use without uncertainty about taxonomic assignations. Moreover, 20% of pollen identified by visual observation was not retrieved by metabarcoding which could have been due to failure of DNA extraction from pollen loads, low quality of sequences (below the trimming threshold) or that some insects did not carry pollen even though they visited a flower. The insects could also have foraged quickly on the flower and not picked up pollen, or some plants could have had small amounts of pollen.

Conclusion

Plant-pollinator networks of grasslands situated in three different French regions were contrasted. The pollinator taxa abundance of each plot was influenced by vegetation height and %SNH in 500 m buffers but was also related to plant species richness. Diptera were abundant in permanent grasslands with landscapes with a high percentage of SNH, whereas Hymenoptera, particularly polylectic bees, dominated in temporary grasslands surrounded by crops. Metabarcoding supplemented the plant genera identified by visual networks well and allowed us to reflect insects' floral pathways as a function of the plot and landscape species pools. Even though metabarcoding still has strong limitations, it offers new perspectives for plant-pollinator network analysis. Pollination could thus be integrated more into multi-function assessment of agroecosystems.

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Table 1. Mean site and plot characteristics: plot name, grassland type, successive management events during the studied year (M: mowing; P: pasture), grass use calculated in livestock units (LU) day ha^{-1}, sum of organic and mineral nitrogen fertilisation (for the year before pollinator trapping/current year of trapping), herbaceous plant species richness, total blossom cover and mean richness of flowering species.

Site	Plot	Grassland type	Plot size (ha)	Management	Grass use (LU day ha^{-1})[b]	Nitrogen fertilisation (kg ha^{-1})	Species richness	Total blossom cover (%)	Richness of flowering species	Vegetation height (cm)	%SNH
Lusignan	G12	Temporary	3.1	MP	504	0/41	14	3.4	1.4	16.7	13.7
	G14	Temporary	0.8	MMM	552	0/0	10	9.6	2.3	40.0	15.2
	G8	Permanent	0.6	M	0	0/0	33	6.7	2.1	51.7	15.4
	M5	Temporary	3.3	P	540	0/140	13	5.3	2.0	21.7	17.8
	M7	Temporary	1.6	P	904	0/0	10	12.7	2.7	10.0	17.2
	V12	Temporary	2.9	MMM	457	0/38	11	<0.1	0.1	25.0	49.2
Mirecourt	Fr12	Permanent	2.3	MP	781	115/19	18	0.8	4.0	55.0	90.5
	HdV2	Permanent	1.8	MP	678	50/37	27	10.7	7.3	48.9	71.4
	Jo15	Permanent	1.9	MM	343	80/0	34	35.7	10.0	43.9	60.8
	Jo89	Permanent	1.6	MP[a]	1117	0/0	26	7.5	5.2	37.2	61.7
	Ma11	Permanent	1.7	MP	899	0/100	38	15	12.7	43.9	74.5
	Mu42	Permanent	2.6	MP	937	0/11	25	9.6	5.7	42.8	56.8
Marcenat	10Bis	Permanent	1.6	MP	153	70/70	47	3.2	10.1	20.0	99.1
	MFH	Permanent	8.6	P	112	0/0	86	7.2	18.4	10.9	99.3
	SF	Permanent	6.1	MP	153	57/31	34	0.1	3.6	18.9	94.6
	Tri	Permanent	1.8	MMP	214	91/218	24	0.1	4.3	17.3	100.0
	V1	Permanent	2.8	P	368	0/67	17	2.1	2.4	22.2	78.8
	V8P	Permanent	1.4	P	355	0/67	26	1.4	7.3	10.2	87.5

[a] Plot management was only pasture for the 8 previous years.

[b] Forage production from mowing was converted into LU day ha^{-1} assuming ingestion of 15 kg dry matter day^{-1} LU^{-1} and summed with the grazing use to estimate total grass use.

Table 2. Main characteristics of plant-pollinator networks for each site: insect taxa richness, plant taxa richness, number of unique interactions, Shannon diversity index of plant-pollinator interactions, mean number of links per taxon, connectance, degree of specialisation H2’ for all three trapping sessions, degree of specialisation H2’ of two last trapping sessions, network asymmetry, specialisation asymmetry and weighted nestedness.

Characteristic	**Lusignan**	**Mirecourt**	**Marcenat**
Insect taxa richness	45	74	73
Plant taxa richness	9	20	42
Number of unique interactions	61	140	158
Shannon diversity	2.97	4.28	4.69
Links per taxon	1.13	1.49	1.37
Connectance	0.15	0.09	0.05
H2’ of all three trapping sessions	0.54	0.44	0.41
H2’ of last two trapping sessions	0.46	0.48	0.44
Network asymmetry	0.67	0.57	0.27
Specialisation asymmetry	-0.23	-0.17	-0.05
Weighted nestedness	0.56	0.53	0.51

Table 3. Taxa-level descriptors for the 10 pollinator and 5 plant taxa with the highest strength (S) for each site. D: taxa degree, d’: taxa specialisation. n.i. not identified to lower taxon.

Lusignan				**Mirecourt**				**Marcenat**			
	S	**D**	**d'**		**S**	**D**	**d'**		**S**	**D**	**d'**
Pollinator											
Bombus lapidarius	2.66	6	0.36	*Empis* sp.	2.66	6	0.42	*Diptera n.i*	7.76	18	0.43
Andrena haemorrhoa	1.22	2	0.63	*Pseudovadonia livida*	2.00	2	1.00	*Empis* sp.	6.69	14	0.42
Apis mellifera	0.73	3	0.19	*Episyrphus balteatus*	1.63	8	0.15	*Lasioglossum albipes*	3.03	9	0.35
Andrena flavipes	0.52	2	0.46	*Sphaerophoria scripta*	1.48	6	0.28	*Apis mellifera*	2.24	3	0.84
Sicus ferrugineus	0.50	1	0.85	*Maniola jurtina*	1.39	4	0.35	*Bombus sylvarum*	1.57	3	0.69
Syrphidae n.i	0.44	3	0.33	*Scathophaga stercoraria*	1.13	4	0.38	*Sphaeroceridae n.i*	1.36	6	0.46
Diptera n.i	0.37	2	0.50	*Lasioglossum lativentre*	1.05	3	0.40	*Phyllopertha horticola*	1.29	4	0.42
Scathophaga stercoraria	0.37	1	0.68	Cratosilis sp.	1.00	1	1.00	*Sphaerophoria scripta*	1.27	3	0.47
Andrena ranunculi	0.33	1	0.77	*Eucera nigrescens*	1.00	1	1.00	*Bombus lapidarius*	1.21	3	0.56
Bombus terrestris	0.26	3	0.13	*Diptera n.i*	0.69	4	0.39	*Cryptocephalus sericeus*	1.06	5	0.53
Plant											
Cirsium palustre	16.74	20	0.57	*Centaurea jacea*	18.98	27	0.53	*Knautia arvensis*	10.99	14	0.71
Trifolium repens	10.39	12	0.42	*Ranunculus acris*	14.11	25	0.34	*Ranunculus* sp.	9.82	16	0.44
Taraxacum sp.	7.56	10	0.79	*Knautia arvensis*	8.77	17	0.25	*Anthriscus sylvestris*	6.86	11	0.43

Cichorium intybus	6.02	8	0.67	*Crepis biennis*	6.44	14	0.28	*Conopodium majus*	5.69	12	0.34
Ranunculus sp.	1.83	3	0.85	*Heracleum spondylium*	5.44	8	0.74	*Rhinanthus minor*	4.53	6	0.86

Table 4. Mean (±SD) specialisation (d') of six pollinator groups at each site.

Group	Lusignan	Mirecourt	Marcenat
Apis mellifera	0.19	0.18	0.84
Other Apidae	0.31 ± 0.21	0.25 ± 0.25	0.49 ± 0.25
Syrphidae	0.28 ± 0.21	0.23 ± 0.12	0.31 ± 0.28
Diptera	0.61 ± 0.20	0.44 ± 0.20	0.32 ± 0.23
Lepidoptera	0.06 ± 0.09	0.21 ± 0.16	0.44 ± 0.20
Coleoptera	0.27 ± 0.15	0.33 ± 0.30	0.35 ± 0.18

Table 5. Number of plant genera found with the metagenomics method on pollen loads of Diptera, Hymenoptera and Lepidoptera and percentage of sampled pollinators that transported pollen of each vegetation type for the three sites. Percentages higher than 10% are indicated in bold. The insect families that transported the more pollen of each type (> 20% of insects that transported genera found only with metagenomics) were indicated in brackets for each site.

	Lusignan	Mirecourt	Marcenat
Total number of genera	34	42	38
Dicotyledon from grassland	16/**15.3**% (Apidae, Halictidae)	17/**13.9%** (Syrphidae, Nymphalidae)	23/**21.9**% (Empididae, Syrphidae)
Grass	5/5.1%	12/**19.9**% (Syrphidae, Nymphalidae)	4/1.2%
Crop	3/**12.8**% (Apidae, Andrenidae)	2/0.9%	0
Tree/shrub	5/**26.0**% (Apidae)	5/2.9%	8/4.7%
Crop weed	2/5.1%	1/1.2%	1/0.3%
Vegetable	2/2.0%	1/0.3%	0
Ornamental	1/4.1%	4/1.2%	2/0.9%

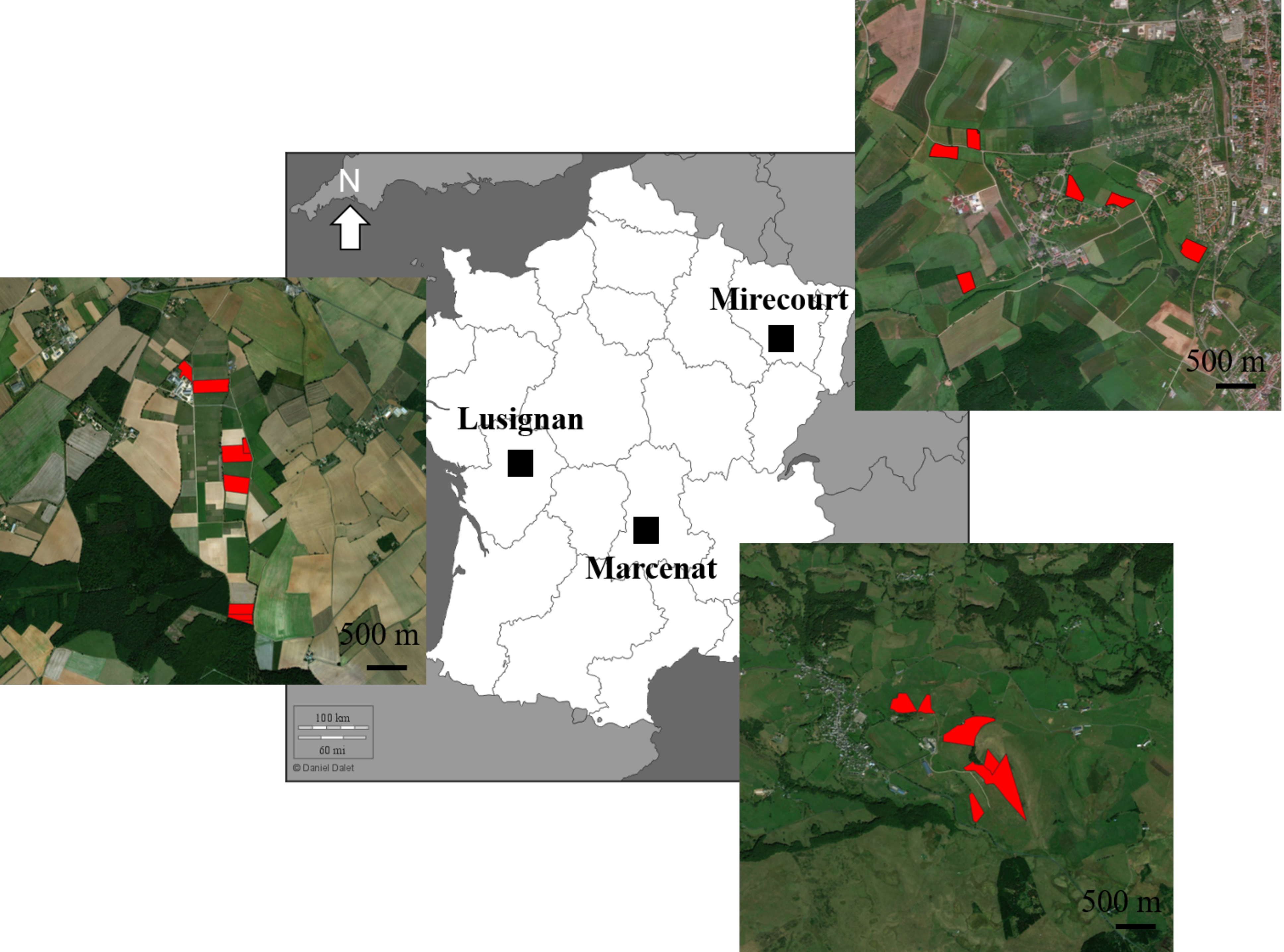
Figure captions

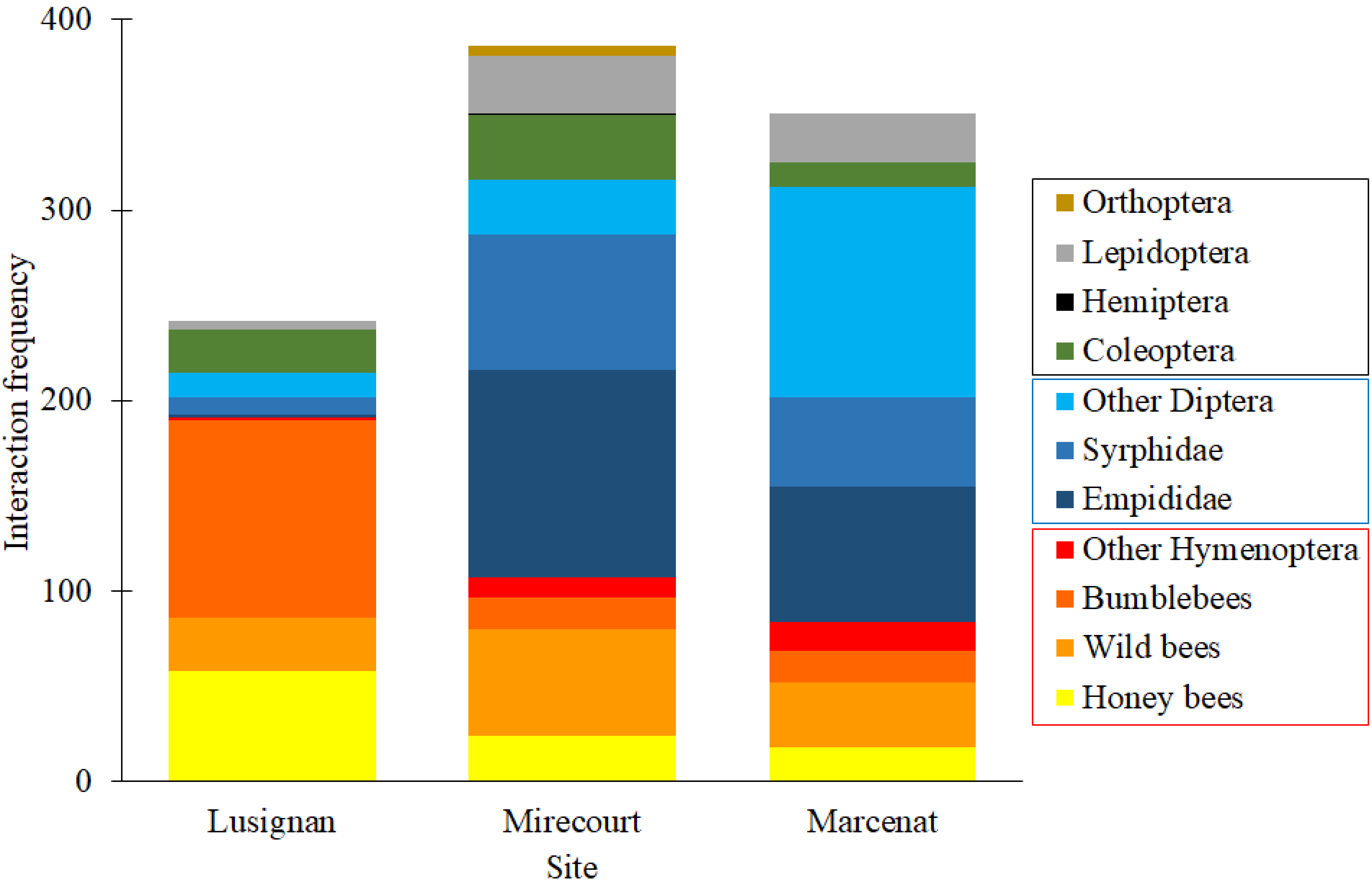
Figure 1. Map of site locations. The 18 sampled plots are indicated in red.

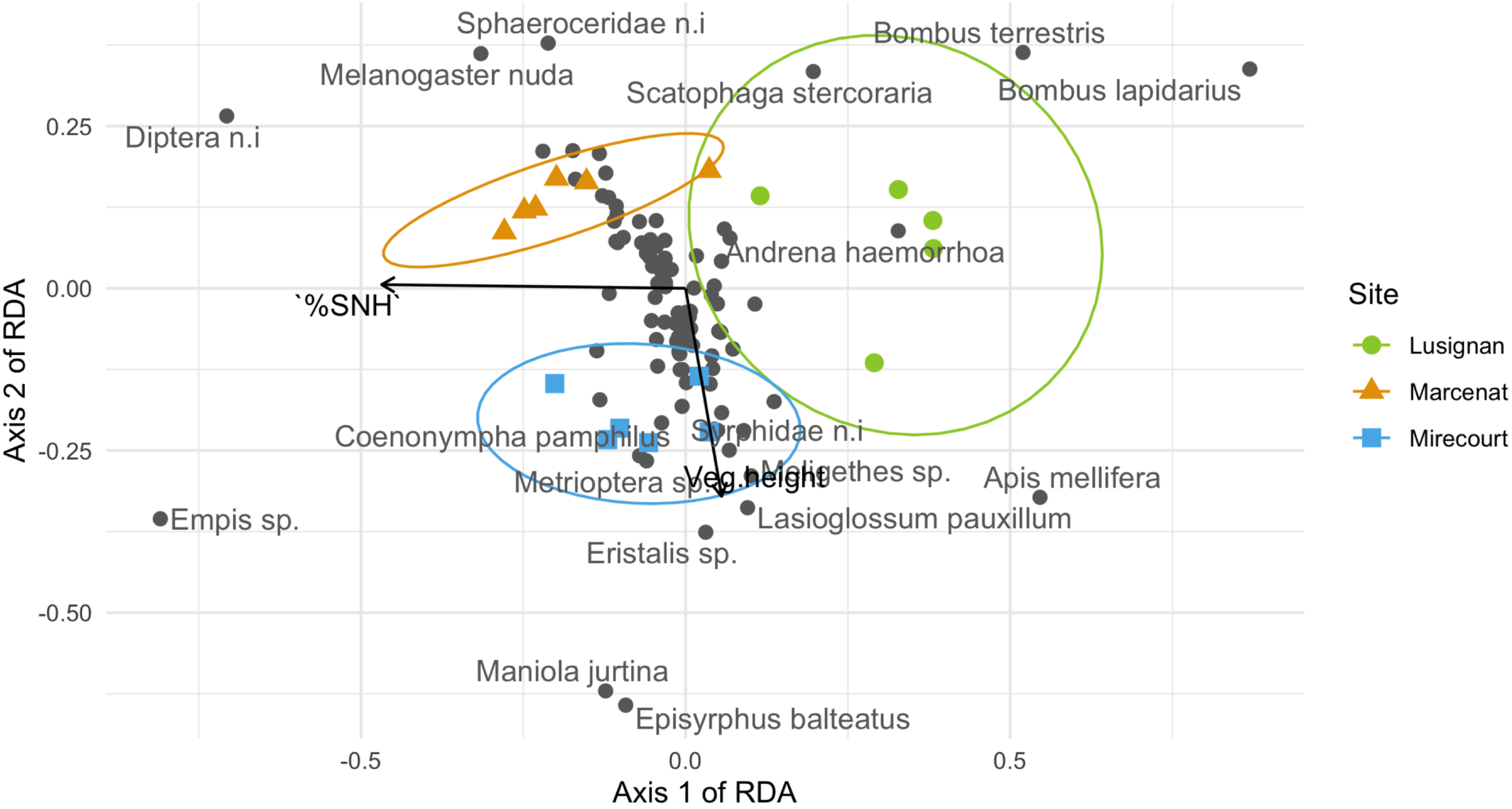
Figure 2. Interaction frequency of plant pollinators by insect taxon at the three study sites.

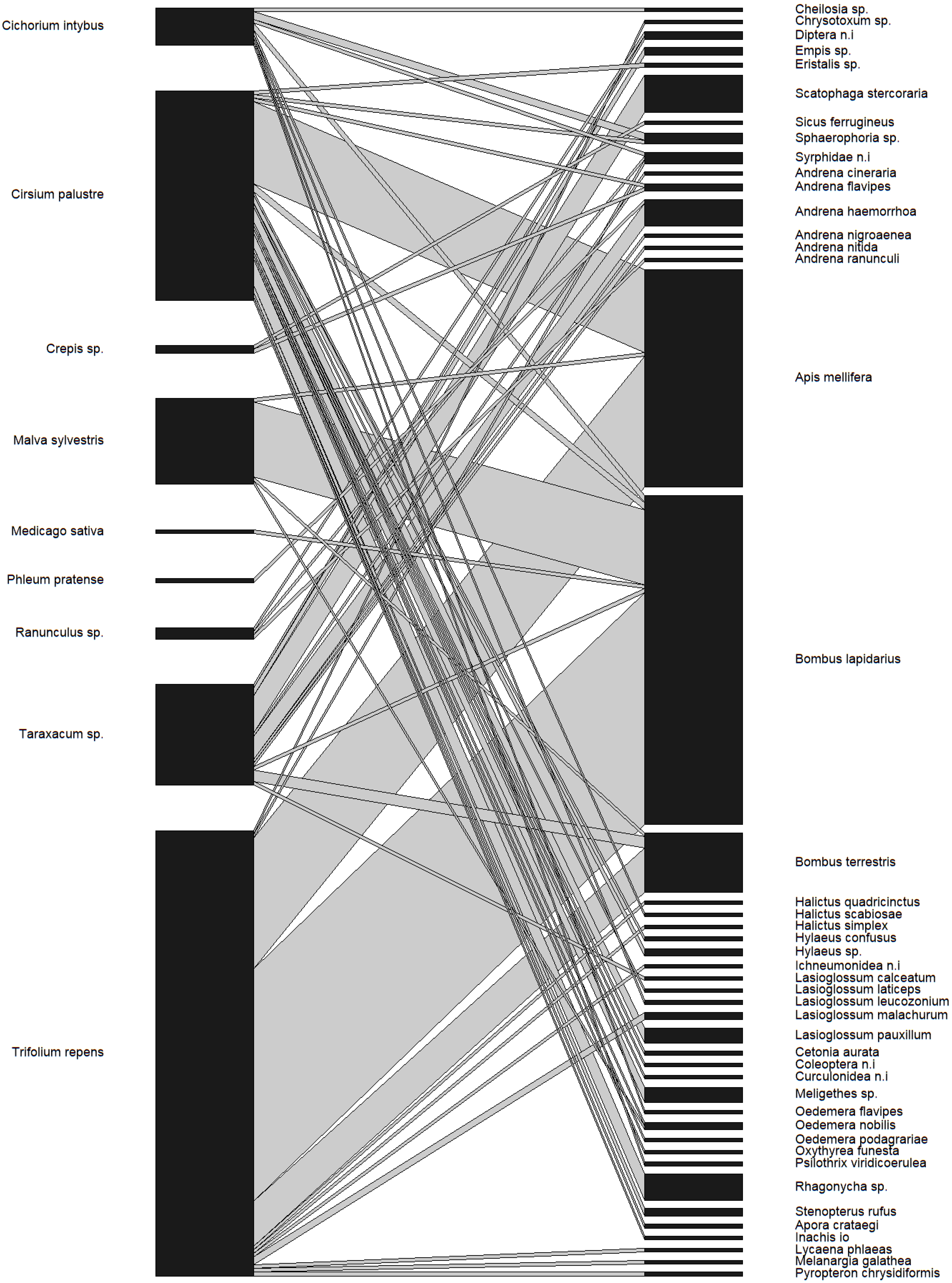
Figure 3. Distance triplot of the two significant axes of the redundancy analysis. Black arrow: explanatory variables. %SNH: percentage of semi-natural habitats in a 500 m buffer around each plot; Veg.height: mean vegetation height (cm). Ellipses represent normal 95% confidence intervals for plots at each site. Grey circles indicate insect taxa. Only most abundant insect taxa have their name displayed.

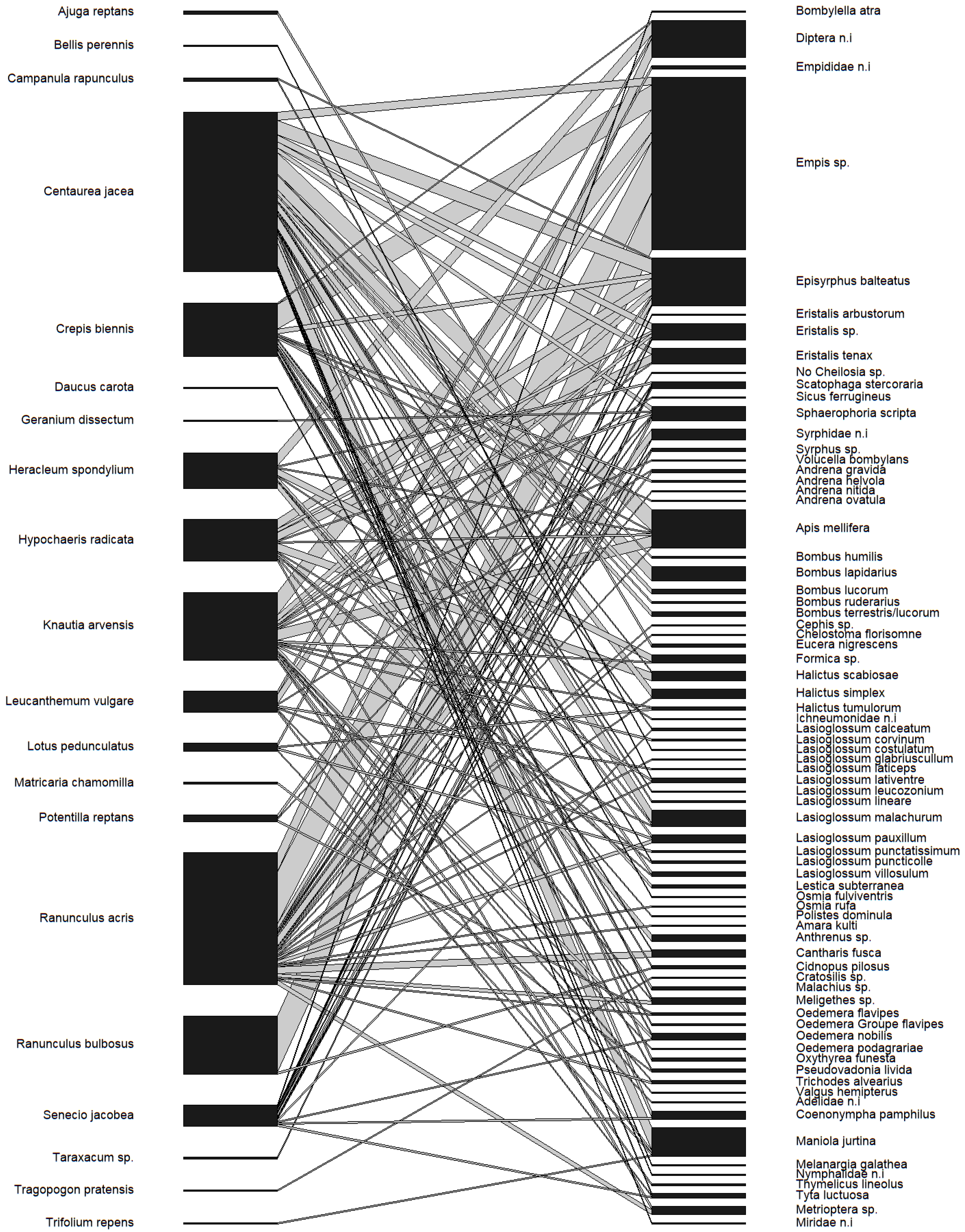
Figure 4. Plant-pollinator networks obtained from visual surveys in (a) Lusignan, (b) Mirecourt and (c) Marcenat for the three trapping sessions. Rectangles represent insect taxa (above) and plant taxa (below), in which rectangle widths are proportional to the sum of the interactions that involve them.


N
Mirecourt
Lusignan
Marcenat
500 m
500 m
500 m
100 km
60 mi
© Daniel Dalet


400
300
200
100
0
Interaction frequency
Lusignan
Mirecourt
Marcenat
Site
Orthoptera
Lepidoptera
Hemiptera
Coleoptera
Other Diptera
Syrphidae
Empididae
Other Hymenoptera
Bumblebees
Wild bees
Honey bees


Sphaeroceridae n.i
Bombus terrestris
Melanogaster nuda
Scatophaga stercoraria
Bombus lapidarius
Diptera n.i
Andrena haemorrhoa
`%SNH`
Coenonympha pamphilus
Syrphidae n.i
Metrioptera sp.
Veg.height
Meligethes sp.
Apis mellifera
Empis sp.
Lasioglossum pauxillum
Eristalis sp.
Maniola jurtina
Episyrphus balteatus
0.25
0.00
-0.25
-0.50
-0.5
0.0
0.5
Axis 1 of RDA
Axis 2 of RDA
Site
Lusignan
Marcenat
Mirecourt


Cichorium intybus
Cirsium palustre
Crepis sp.
Malva sylvestris
Medicago sativa
Phleum pratense
Ranunculus sp.
Taraxacum sp.
Trifolium repens
Cheilosia sp.
Chrysotoxum sp.
Diptera n.i
Empis sp.
Eristalis sp.
Scatophaga stercoraria
Sicus ferrugineus
Sphaerophoria sp.
Syrphidae n.i
Andrena cineraria
Andrena flavipes
Andrena haemorrhoa
Andrena nigroaenea
Andrena nitida
Andrena ranunculi
Apis mellifera
Bombus lapidarius
Bombus terrestris
Halictus quadricinctus
Halictus scabiosae
Halictus simplex
Hylaeus confusus
Hylaeus sp.
Ichneumonidea n.i
Lasioglossum calceatum
Lasioglossum laticeps
Lasioglossum leucozonium
Lasioglossum malachurum
Lasioglossum pauxillum
Cetonia aurata
Coleoptera n.i
Curculonidea n.i
Meligethes sp.
Oedemera flavipes
Oedemera nobilis
Oedemera podagrariae
Oxythyrea funesta
Psilothrix viridicoerulea
Rhagonycha sp.
Stenopterus rufus
Apora crataegi
Inachis io
Lycaena phlaeas
Melanargia galathea
Pyropteron chrysidiformis


Ajuga reptans
Bellis perennis
Campanula rapunculus
Centaurea jacea
Crepis biennis
Daucus carota
Geranium dissectum
Heracleum spondylium
Hypochaeris radicata
Knautia arvensis
Leucanthemum vulgare
Lotus pedunculatus
Matricaria chamomilla
Potentilla reptans
Ranunculus acris
Ranunculus bulbosus
Senecio jacobea
Taraxacum sp.
Tragopogon pratensis
Trifolium repens
Bombylella atra
Diptera n.i
Empididae n.i
Empis sp.
Episyrphus balteatus
Eristalis arbustorum
Eristalis sp.
Eristalis tenax
No Cheilosia sp.
Scatophaga stercoraria
Sicus ferrugineus
Sphaerophoria scripta
Syrphidae n.i
Syrphus sp.
Volucella bombylans
Andrena gravida
Andrena helvola
Andrena nitida
Andrena ovatula
Apis mellifera
Bombus humilis
Bombus lapidarius
Bombus lucorum
Bombus ruderarius
Bombus terrestris/lucorum
Cephis sp.
Chelostoma florisomne
Eucera nigrescens
Formica sp.
Halictus scabiosae
Halictus simplex
Halictus tumulorum
Ichneumonidae n.i
Lasioglossum calceatum
Lasioglossum corvinum
Lasioglossum costulatum
Lasioglossum glabriuscullum
Lasioglossum laticeps
Lasioglossum lativentre
Lasioglossum leucozonium
Lasioglossum lineare
Lasioglossum malachurum
Lasioglossum pauxillum
Lasioglossum punctatissimum
Lasioglossum puncticolle
Lasioglossum villosulum
Lestica subterranea
Osmia fulviventris
Osmia rufa
Polistes dominula
Amara kulti
Anthrenus sp.
Cantharis fusca
Cidnopus pilosus
Cratosilis sp.
Malachius sp.
Meligethes sp.
Oedemera flavipes
Oedemera Groupe flavipes
Oedemera nobilis
Oedemera podagrariae
Oxythyrea funesta
Pseudovadonia livida
Trichodes alvearius
Valgus hemipterus
Adelidae n.i
Coenonympha pamphilus
Maniola jurtina
Melanargia galathea
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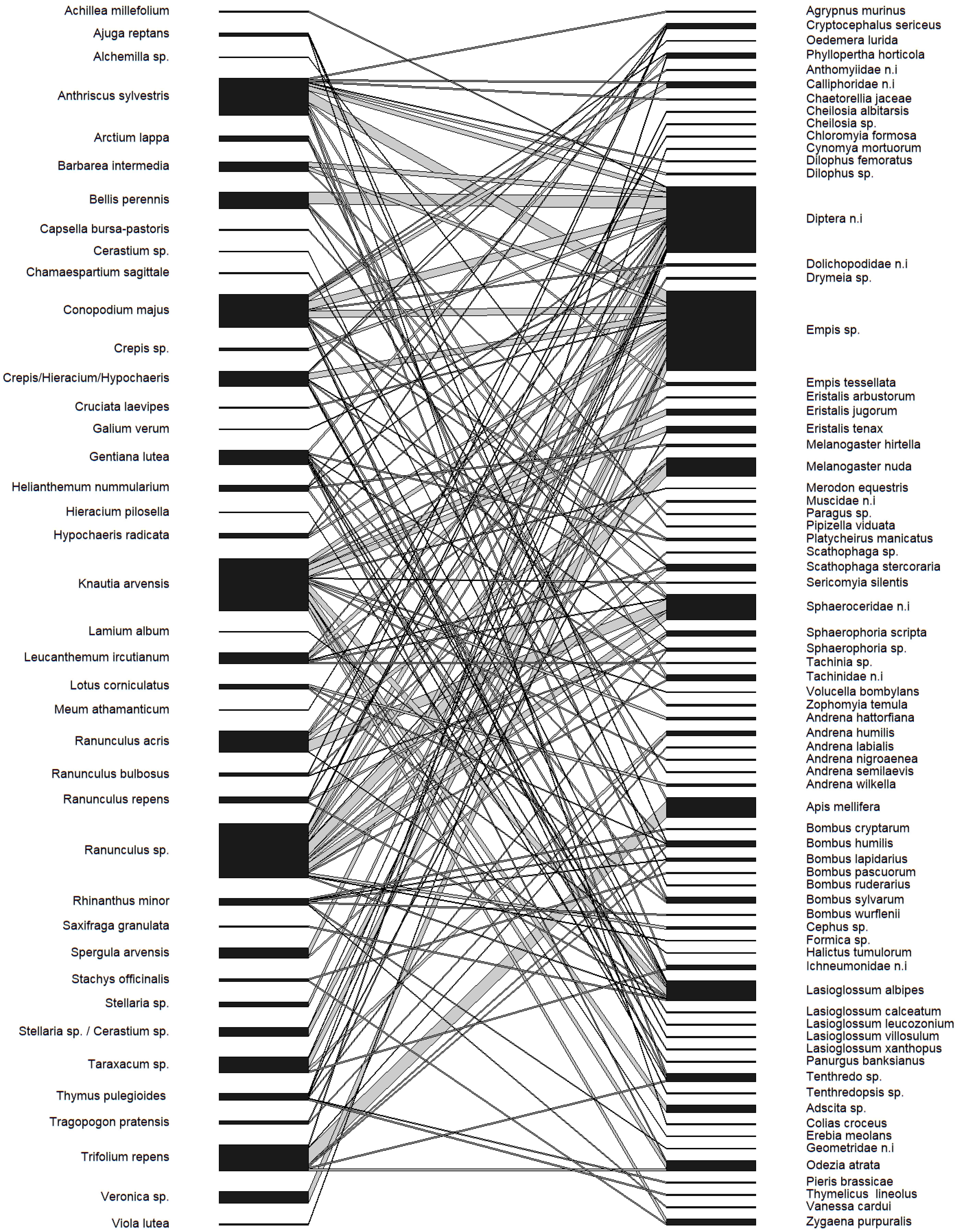
Thymelicus lineolus
Tyta luctuosa
Metrioptera sp.
Miridae n.i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Ajuga reptans
Alchemilla sp.
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Bellis perennis
Capsella bursa-pastoris
Cerastium sp.
Chamaespartium sagittale
Conopodium majus
Crepis sp.
Crepis/Hieracium/Hypochaeris
Cruciata laevipes
Galium verum
Gentiana lutea
Helianthemum nummularium
Hieracium pilosella
Hypochaeris radicata
Knautia arvensis
Lamium album
Leucanthemum ircutianum
Lotus corniculatus
Meum athamanticum
Ranunculus acris
Ranunculus bulbosus
Ranunculus repens
Ranunculus sp.
Rhinanthus minor
Saxifraga granulata
Spergula arvensis
Stachys officinalis
Stellaria sp.
Stellaria sp. / Cerastium sp.
Taraxacum sp.
Thymus pulegioides
Tragopogon pratensis
Trifolium repens
Veronica sp.
Viola lutea
Agrypnus murinus
Cryptocephalus sericeus
Oedemera lurida
Phyllopertha horticola
Anthomyiidae n.i
Calliphoridae n.i
Chaetorellia jaceae
Cheilosia albitarsis
Cheilosia sp.
Chloromyia formosa
Cynomya mortuorum
Dilophus femoratus
Dilophus sp.
Diptera n.i
Dolichopodidae n.i
Drymeia sp.
Empis sp.
Empis tessellata
Eristalis arbustorum
Eristalis jugorum
Eristalis tenax
Melanogaster hirtella
Melanogaster nuda
Merodon equestris
Muscidae n.i
Paragus sp.
Pipizella viduata
Platycheirus manicatus
Scathophaga sp.
Scathophaga stercoraria
Sericomyia silentis
Sphaeroceridae n.i
Sphaerophoria scripta
Sphaerophoria sp.
Tachinia sp.
Tachinidae n.i
Volucella bombylans
Zophomyia temula
Andrena hattorfiana
Andrena humilis
Andrena labialis
Andrena nigroaenea
Andrena semilaevis
Andrena wilkella
Apis mellifera
Bombus cryptarum
Bombus humilis
Bombus lapidarius
Bombus pascuorum
Bombus ruderarius
Bombus sylvarum
Bombus wurflenii
Cephus sp.
Formica sp.
Halictus tumulorum
Ichneumonidae n.i
Lasioglossum albipes
Lasioglossum calceatum
Lasioglossum leucozonium
Lasioglossum villosulum
Lasioglossum xanthopus
Panurgus banksianus
Tenthredo sp.
Tenthredopsis sp.
Adscita sp.
Colias croceus
Erebia meolans
Geometridae n.i
Odezia atrata
Pieris brassicae
Thymelicus lineolus
Vanessa cardui
Zygaena purpuralis