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

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28 **Abstract**

29 Temperate grasslands provide both habitats and flower resources for pollinators in agricultural
30 landscapes. Plant-pollinator networks change according to local and landscape variables,
31 which are important to identify to help conserve pollinators in grasslands. We analysed plant-
32 pollinator networks in 18 grasslands on experimental dairy farms located in three French
33 regions contrasting by their climate, altitude, landscape or management. We combined visual
34 surveys and pollen DNA barcoding. Our objectives were to determine which environmental
35 factors influence pollinator taxa abundance and diversity and differences among the visual
36 plant-pollinator networks in the three farming regions. Flower-visiting insects were trapped in
37 six grasslands per farm during three sessions from mid-April to mid-July along fixed 400 m²
38 transects. Insects were identified individually to the lowest taxonomic rank possible. Pollen
39 carried by insects was identified using nuclear ribosomal ITS2 sequences belonging to the
40 NCBI nucleotide database. The size and diversity of plant-pollinator networks were much
41 larger and higher in permanent grasslands at the two farms located in lowlands (Mirecourt)
42 and mountains (Marcenat) than those at the farm with temporary grasslands and a crop
43 landscape (Lusignan), but the degree of specialisation (H_2') was relatively similar and low
44 (mean of 0.46). Diptera, especially Empididae and Syrphidae, represented most plant-
45 pollinator interactions in Mirecourt and Marcenat, while Hymenoptera were more abundant at
46 Lusignan. The percentage of semi-natural habitats in 500 m buffers and vegetation height
47 explained 23% of the variance in pollinator abundance. *Ranunculus* sp., *Knautia arvensis*,
48 *Centaurea jacea* and *Trifolium repens* were key plant species in the networks. DNA
49 metabarcoding of pollen loads identified 114 genera in addition to those identified by visual
50 observations (+34-42 per site), reflecting insects' floral pathways and differences in the
51 immediate landscape among farms. This study highlighted the importance of Diptera in plant-

52 pollinator networks and the need to conserve permanent grassland diversity to conserve
53 pollinators.

54
55 Key words: pollination, agricultural practice, DNA bar-coding, Diptera, Hymenoptera,
56 metagenomics
57

58 **Introduction**

59 Permanent and temporary grasslands are major ecosystems that cover 33% and 6% of utilised
60 agricultural area in Europe, respectively (Huygues et al., 2014) and 9 and 3 million ha in
61 France (Agreste, 2019) in different livestock farming contexts. Permanent grasslands could
62 generally have a greater plant species diversity than temporary grasslands which are renewed
63 within five years and are usually part of arable rotations. Permanent grasslands are suitable
64 habitats for many flora and fauna species (Habel et al., 2013) and provide a wide range of
65 ecosystem services (e.g. Bengtsson et al., 2019). However, they have decreased by 10 million
66 ha over the last 50 years in Europe (Huygues et al., 2014), mainly due to conversion to arable
67 land to produce animal feed or due to abandonment. Among grassland functions,
68 entomophilous pollination is crucial, but pollinators have been declining for the last two
69 decades (Potts et al., 2010), with an estimated decline of 77% in flying insect biomass from
70 1989-2016 in nature protection areas in Germany (Hallmann et al., 2017). In temperate
71 ecosystems, most pollinators are insects (Hymenoptera, Diptera, Lepidoptera and Coleoptera),
72 and 78% of plants depend on animals to ensure their sexual reproduction (Ollerton et al.,
73 2011). Grasslands provide both habitats and flower resources for pollinators in agricultural
74 landscapes. For instance, calcareous grasslands are major habitats for wild bees to support
75 pollination in adjacent agricultural landscapes (Klaus et al., 2021; Steffan-Dewenter and
76 Tschardtke, 1999). An increase in grassland plant species richness can increase pollinator
77 diversity and the pollination of adjacent crop production (e.g. Orford et al., 2016, Woodcock

78 et al., 2013).. In return, pollinator diversity (Fontaine et al., 2005; Fründ et al., 2013) or
79 abundance (Lundgren et al., 2016) helps conserve the floral diversity of grasslands.

80

81 At the plot scale, local management practices (i.e. frequency of mowing or grazing, types and
82 amounts of synthetic fertilisers) can modify the diversity of plant-pollinator communities
83 greatly. Plant diversity decreases with early mowing (Gaujour et al., 2012) and as nitrogen
84 fertilisation increases (Jacquemyn et al., 2003). The diversity and abundance of pollinators
85 decreases as mowing or mineral fertilisation (Hudewenz et al., 2012) or grazing intensity
86 increases (Lazaro et al., 2016; Sjödin, 2007) with changes in flower communities. Moreover,
87 the composition (e.g. percentage of semi-natural habitats, SNH) and configuration of
88 landscapes can influence pollinator diversity in grasslands, but this effect varies among
89 studies and pollinator orders. For instance, the percentage of surrounding grasslands can
90 increase the species richness and abundance of bumblebees in grasslands (Diaz-Forero et al.,
91 2013; Hatfield and LeBuhn, 2007), but some studies suggest that it has no effect on bees or
92 hoverflies (e.g. Holzschuh et al., 2016). Forest habitats near grasslands can decrease the
93 species richness of bumblebees (Diaz-Forero et al., 2013) and increase that of butterflies
94 (Villemeay et al., 2015). Effects of both local and landscape factors on a variety of pollinator
95 orders have been studied less, but the results highlight differences of responses among taxa.
96 Butterflies and beetles seem to be influenced more by local factors (e.g. grassland height,
97 management) than landscape factors, unlike hoverflies. In comparison, bees were influenced
98 by both local and landscape factors (Kormann et al., 2015; Sjödin et al., 2008).

99 Plants and pollinators are organised into bipartite networks with mutualistic interactions.
100 These networks seem highly asymmetric (i.e. “specialists tend to interact with generalist
101 partners” (Alarcon, 2010) and nested (i.e. “specialists interact with subsets of the species with
102 which generalists interact” (Bascompte and Jordano, 2007). These characteristics could

103 stabilise networks in case of species extinctions, disturbance or habitat loss (Fortuna and
104 Bascompte, 2006; Jordano, 1987; Memmott et al., 2004) but this network asymmetry could be
105 partly due to experimental artefacts (Blüthgen, 2010). Although some studies have shown
106 generalisation and redundancy in these networks, complementarity and specialisation between
107 species could be important (Fründ et al., 2010). Pollinator diversity and network specialisation
108 can be influenced by the floristic richness and total blossom cover of grasslands. Indeed, an
109 increase in floral resource diversity can provide more ecological niches for pollinators and
110 thus render plant-pollinator networks more specialised (Junker et al., 2015). Previous studies
111 showed that pollinator richness or visitation frequency can increase as plant species richness
112 increases (e.g. Fornoff et al., 2017; Fründ et al., 2010), but some studies showed that
113 specialisation of plant-pollinator networks can remain high regardless of the plant diversity
114 (e.g. Weiner et al., 2011). Among pollinators on grasslands, bees could have the highest
115 specialisation and dipterans the lowest (Weiner et al., 2011). As bees are often considered to
116 be the main pollinators in many habitats (e.g. Willmer et al., 2017), fewer studies have
117 considered Diptera. In grasslands, Diptera can represent a large percentage of plant foragers
118 (Rader et al., 2016; Weiner et al., 2011) and play a major role in pollination (Tiusanen et al.,
119 2016). In particular, Diptera are more abundant when elevation increases (Kearns, 1992;
120 Lefebvre et al., 2014).

121 To understand grassland pollination and improve conservation of all pollinators on farms, it is
122 thus important to determine which local and landscape variables influence the characteristics
123 of plant-pollinator networks. Our study objective was to analyse plant-pollinator networks in
124 18 grasslands on experimental farms located in three French regions with contrasting
125 landscape composition: crop-dominated landscapes surround plots the most at Lusignan (48-
126 91% of surface area in a 1000 m buffer), whereas SNH surround plots the most at Mirecourt
127 (47-67%) and especially at Marcenat (81-95%). The originality was to assess plant-pollinator

128 interactions of permanent and temporary grasslands by combining two methods: visual
129 identification of insects and plants and identification of the pollen transported by each
130 pollinator using DNA metabarcoding. Our research questions were: (1) which environmental
131 factors (i.e. local plant community, local management practices and percentage of SNH
132 around grasslands) influence the abundance and diversity of pollinator taxa? (2) how do visual
133 plant-pollinator networks differ among the three farming regions? and (3) which contribution
134 of metagenomics to identify pollen and track pollinator visitations in grasslands ?

135

136 **Material and methods**

137 **1. Study sites and sampled grasslands**

138 This study was conducted on three experimental farms (hereafter, “site”) of INRAE (French
139 National Research Institute for Agriculture, Food and Environment) located at Lusignan in
140 western France (46°25’ N, 0°07’ E, 150 m a.s.l.), Mirecourt in eastern France (48°17’ N,
141 6°07’ E, 300 m a.s.l.) and Marcenat in the mountains of central France (45°18’ N, 2°50’ E,
142 1100 m a.s.l., **Figure 1**). Lusignan was the warmest site with the lowest rainfall (mean annual
143 temperature of 11.9°C and mean precipitation of 790 mm from 2001-2015), while Marcenat
144 was the coldest and rainiest (7.5°C; 1154 mm) and Mirecourt was intermediate (10.3°C; 821
145 mm, INRAE CLIMATIK platform). Lusignan is a 91ha farm composed of 48ha of temporary
146 grasslands and 37ha of crops. Mirecourt, an organic farm since 2006, covers 238ha composed
147 of 133ha of permanent grasslands and 105ha of crops. Marcenat is a farm of 89.5ha composed
148 exclusively of permanent grasslands. We selected six grassland plots per farm (N=18 plots)
149 representative of local farm management for our study. Characteristics of each plot are
150 detailed in table 1. Plots were selected along a gradient of grassland plant species diversity
151 within each site. The temporary grasslands of Lusignan were sown 2 to 4 years before the
152 insect trapping and were included in an arable rotation.

153

154 **2. Landscape characterisation around sampled plots**

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

164

165 **3. Pollinator and pollen trapping**

166 To trap pollinators, one transect 100 m long and 4 m wide was delineated in the centre of each
167 plot. Three sessions of pollinator trapping of generally 3 days each were performed on each
168 transect in the morning, early afternoon and late afternoon. Pollinators were sampled in 2015
169 at Marcenat and in 2016 at Lusignan and Mirecourt. The periods of catching were 12-13-14
170 April; 22-23-24 June; 7-8-9 July in Lusignan, 17-20-21 May; 6-9-10 June; 27-29-30 June in
171 Mirecourt, and 28-29-30-31 May and 2 June; 17-18-19-22 June; 7-8-9 July in Marcenat.
172 These periods are spaced out to record the phenology changes of plant-pollinator interactions.
173 Trapping periods ranged from 10:00-19:00. In total, 162 transect observations were performed
174 (3 transect observations × 6 plots × 3 sessions × 3 sites). Trapping days were sunny, without
175 precipitation or wind. All flower-visiting insects that were actively collecting pollen and/or
176 nectar were trapped directly with Falcon™ tubes (to avoid pollen contamination) by an
177 observer walking along the transect for 15 min (stopping the stopwatch to process each insect

178 trapped). Each flower species involved in the interaction was recorded to obtain the visual
179 plant-pollinator network. Each insect collected was killed by injecting 10 mL of 60% ethanol
180 into the FalconTM tube with a syringe. All tubes were stored at room temperature in the
181 laboratories. For pollen and insect preparation methods, see Galliot et al., (2017). Briefly,
182 pollen load was removed from the insect body by adding 30mL of 60% ethanol into the tube
183 and shaking it firmly for 6s. The insect was then collected and pinned for visual identification
184 to the lowest taxonomic rank possible. To identify pollen loads using DNA metabarcoding,
185 the ethanol solution containing the pollen extracted from the insect was filtered on a 5 µm-
186 pore nylon filter (Nitex 03-5/1, SEFAR, Heiden, CH) and the filter was then placed in a 1.5
187 mL microcentrifuge tube.

188

189 **4. Vegetation measurements**

190 Plant species richness was determined by walking 1-2 diagonals in each plot in May-June
191 2016 at Lusignan and Mirecourt. At Marcenat, floristic composition had been assessed 4-5
192 years previously by estimating the percentage cover of all plant species in late July in 1 m²
193 quadrats distributed in each plot. The number of quadrats depended on plot size. At the three
194 sites, total flower cover percentage (i.e. blossom cover) and flowering species richness were
195 estimated visually in 30 m × 30 m quadrats distributed along the 400 m² of each observation
196 transect according to the method of Farruggia et al. (2012). At the three sites, vegetation
197 height was measured along each observation transect as the mean height of 90% of the total
198 biomass.

199

200 **5. DNA metabarcoding on pollen loads**

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

203 TCCTCCGCTTATTGATATGC-3') (Chen et al., 2010). Sequencing was carried with the
204 MiSeq® V3 Reagent Kit 600 cycles (Illumina Inc., San Diego, CA) but to increase the
205 number of samples per run, we performed two libraries: 384 ITS2-F and ITS-4R and 384 to
206 which 4 nucleotides (GATC) have been added at the 5' end. The reads of each sample were
207 distinguished by identifying (or not) the added GATC motif in the demultiplexed reads. ITS2
208 sequences stored in the NCBI nucleotide database in March 2017 were retrieved with the
209 criteria Gene name = ITS2, Organism = Plantae, and Sequence length = 100-3000 nt to
210 construct our reference database, called "BDD_ITS2_NCBI_Plants_100-3000" constituted of
211 182 708 sequences belonging to 9000 genera. Tag and GATC-sorted reads were trimmed
212 using the CLC Genomics Workbench 9.5.2 or 10.0 with the following parameters: (i) base
213 quality Phred >20 with no ambiguity, (ii) removing 20 nt in 5' and 3' and (iii) read length >
214 130 nt. All trimmed reads were mapped using the CLC mapping algorithm with Similarity =
215 95%, Length = 70% against the BDD_ITS2_NCBI_Plants_100-3000 database. R2 reads were
216 excluded from the analysis (insufficient quality). The mean length of trimmed R1 reads for
217 the two sequencing runs were 151 nt (range: 73-212 nt) for the first run of sequencing and 176
218 nt (range: 87-252 nt) for the second one. Insect pollen loads were described to genus to avoid
219 misclassification due to imprecision in the data base reference sequences. We assumed that a
220 genus was present in a mixed pollen load if its percentage of reads in a sample exceeded 10%
221 of all reads in the sample. This threshold was considered to be equivalent to the background
222 noise. As suggested by Galliot et al., (2017) we retained only genera with more than three
223 reads per genus per sample.

224

225 **6. Data analysis**

226 **6.1. Spatial autocorrelation**

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

233

234 **6.2. Visual plant-pollinator networks**

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

247

248 **6.3. Statistical analysis**

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

251 We performed Redundancy Analysis (RDA), using the “vegan” package according to Borcard
252 et al., (2011), to explain variation in a matrix of taxa (response variables) due to a matrix of
253 explanatory variables. In all analyses, we used an α threshold of 0.05 and we excluded plot
254 V12 (at Lusignan) due to under-sampling (one interaction for all sampling events performed).
255 The response variables consisted of pollinator taxa abundance grouped by sampled plot
256 without considering sampling dates. We transformed the pollinator matrix using a Hellinger
257 transformation. Our explanatory variables were environmental variables known to influence
258 pollinator community composition: mean blossom cover (i.e. total flower cover percentage,
259 %), mean vegetation height (cm), flowering species richness and %SNH in 500 buffers
260 around each sampled plot. We selected a %SNH in 500m buffers rather than 1000m because
261 i) %SNH 500m and 1000m were correlated, ii) 1000m buffers overlapped between close plots
262 in Lusignan and Marcenat, and iii) 500m buffers fitted better in our analysis. We also
263 included variables that indicated local land-use intensification: number of mowing events of
264 the year, grass use (livestock units-day ha⁻¹) of the year and amount of nitrogen fertilisation of
265 the previous year (kg N ha⁻¹).

266 We checked for collinearity among environmental variables using a variance inflation factor
267 (VIF) criterion. VIF<5 for all variables. We then selected a model using a step-wise procedure
268 based on a permutation test with 10 000 iterations. For the most parsimonious model, only
269 %SNH in a 500 m buffer and vegetation height were selected by the model. We calculated
270 adjusted R² to assess the variance of the pollinator matrix explained by %SNH and vegetation
271 height. We used a permutation test with 10 000 iterations to test the most parsimonious model
272 and the significance of its canonical axes.

273 We then performed variance partitioning of the Hellinger-transformed pollinator community
274 data using two environmental matrices: local variables (i.e. mean blossom cover, mean
275 vegetation height, flowering species richness, number of mowing events, grass use and

276 amount of nitrogen fertilisation) and a landscape variable (i.e. %SNH in a 500m buffer). We
277 applied the same step-wise procedure as before and we kept only mean vegetation height,
278 flowering species richness and nitrogen fertilisation for local variables. We tested the variance
279 explained by each set of variables using permutation tests.

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

285

286 **Results**

287 **1. Plant and pollinator diversities**

288 A total of 979 pollinator insects were trapped at the 3 sites during the 3 sessions on 53 plant
289 species (**Figure 2**). The plant families of Asteraceae, Renonculaceae and Fabaceae (mainly
290 *Trifolium repens*) represented 35%, 20% and 16% of the total interactions with pollinators,
291 respectively. Overall, 844 insects were identified to genus, of which 583 were identified to
292 species. The pollinator richness identified was 107 species, 78 genera and 50 families. Only
293 10 genera were common to the 3 sites. Most interactions were performed by Diptera (mainly
294 Empididae and Syrphidae) at Mirecourt and Marcenat (54% and 65% of site interactions,
295 respectively) and by Hymenoptera at Lusignan (79% of site interactions). For all sites, the
296 interaction frequency of Diptera decreased from 242 in session 1 to 140 in session 2 and to 79
297 in session 3. However, that of Syrphidae increased from session 1 to 3 (18 to 61 individuals,
298 respectively). The interaction frequency of Hymenoptera increased by a factor of 3 from
299 session 1 to 3, with an increase for bumblebees and honey bees and constant number for wild
300 bees.

301

302 2. Effects of environmental variables on taxa pollinator abundance

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

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

324 The variance of pollinator taxa abundance by plot explained by both local (i.e. vegetation
325 height, flowering plant richness and nitrogen fertilisation) and landscape (%SNH) variables

326 differed significantly from zero ($p < 0.05$, data not shown). Local environmental variables,
327 landscape variables and both types together explained 9% ($p < 0.001$), 9% ($p < 0.001$) and
328 8%, respectively, of the variance in the transformed pollinator matrix.

329

330 **3. Visual plant-pollinator networks**

331 The plant-pollinator networks differed among the three sites (**Figure 4**). The network of
332 Mirecourt and Marcenat had much higher insect taxa richness and Shannon diversity than that
333 of Lusignan (**Table 2**). The number of unique interactions was higher at Marcenat (158) and
334 Mirecourt (140) than that at Lusignan (60). For all three sites, connectance was relatively low
335 and weighted nestedness were similar. The network at Lusignan had the highest network
336 asymmetry (0.67) and lowest specialisation asymmetry, while that at Marcenat had the lowest
337 network asymmetry (0.27) and highest specialisation asymmetry. When all three trapping
338 sessions at each site were considered, Lusignan had the most specialised network ($H2' =$
339 0.54). When considering only two trapping sessions (i.e. June-July), however, all three sites
340 had a similar degree of specialisation (mean $H2' = 0.46$).

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

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

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

355

356 **4. Pollen analysis by metagenomics**

357 The pollen DNA metabarcoding identified 84%, 81% and 75% of the genera of plants foraged
358 in visual interactions at Lusignan, Mirecourt and Marcenat, respectively. A total of 107 plant
359 genera were detected on pollen loads analysed from Diptera, Hymenoptera and Lepidoptera
360 from the three sites. Most of these pollinators transported pollen from 1-3 genera (data not
361 shown), with a maximum of 6 genera on one individual of *Pieris brassicae* (Lepidoptera).

362 Moreover, metabarcoding of pollen loads identified 34, 42 and 38 plant genera in addition to
363 those identified in the visual observations at Lusignan, Mirecourt and Marcenat, respectively
364 (**Table 5**), which corresponded to 2-3 times the genera richness found with visual
365 observations alone. Among the plant genera found only with the metagenomics method at the
366 three sites, Dicotyledon and grass from grasslands were the main pollen carried. At Lusignan,
367 26% (mainly Apidae), 15% and 13% of pollinators sampled transported pollen of
368 trees/shrubs, Dicotyledon from grasslands, and crops, respectively. At Mirecourt, 20% and
369 14% of pollinators sampled (mainly Syrphidae and Nymphalidae) transported pollen of grass
370 and Dicotyledon from grasslands, respectively. At Marcenat, 22% of pollinators sampled
371 (mainly Empididae and Syrphidae) transported pollen of Dicotyledon from grasslands.

372

373 **Discussion**

374

375 **Effects of environmental factors on the abundance of pollinator taxa**

376 The three sites differed in plant-pollinator compositions, which firstly reflects different
377 regional species pools and/or a temporal effect due to different years of trapping. Pollinator
378 abundance of each taxa was related to both landscape (percentage of semi-natural habitats, %
379 SNH; in 500 m buffers) and local (vegetation height) variables. We determined that
380 communities with a high %SNH within landscape were characterised mainly by dominant
381 non-Syrphidae Diptera and *Melanogaster nuda*. Among the few studies of the influence of
382 landscape on non-Syrphidae Diptera, Burel et al., (2004) showed that Empididae had higher
383 species richness in landscapes with a large percentage of permanent grasslands, woods and
384 hedgerows than in landscapes dominated by crops. In contrast, plots with low %SNH were
385 dominated by polylectic bee species, which are known to be associated with farmlands due to
386 the wide range of plant species they visit (e.g. *A. haemorrhua*: Wood et al., 2016; *B.*
387 *lapidarius*: Roger et al., 2017; *A. mellifera*, which are associated only with beekeeping in
388 most of France: Requier et al., 2015). At Lusignan, Hymenoptera probably predominated
389 because of higher temperatures (+ 0.7°C and + 2°C in mean temperature from April to July of
390 the pollinator trapping year compared to Mirecourt and Marcenat), the crop landscape and
391 temporary grasslands. Temporary grasslands of Lusignan was composed of *Trifolium repens*
392 which was the most visited species, mainly by Apidae. *T. repens* has a large amount of nectar
393 with a high sugar concentration (ca. 804 kg of nectar productivity ha⁻¹ cover year⁻¹ in Baude et
394 al., 2016) and has a pollen rich in protein for bees (e.g. Ghosh et al., 2020; Hanley et al.,
395 2008). Perennial plants, such as *T. repens*, have an higher melliferous potential than annual
396 plants so that temporary grasslands at Lusignan can offer continuous resources for pollinators
397 during the growing season in annual crop-dominated landscapes (Hicks et al., 2016; Ion et al.,
398 2018).

399

400 Among local factors, the pollinator abundance of each grassland plot at the three sites was
401 positively correlated with mean blossom cover and flowering species richness. These results
402 were previously obtained in other studies (e.g. Ebeling et al. 2008 ;Hudewenz et al. 2012 ;
403 Goulnik et al. 2020).and highlighted the importance of diversified permanent grasslands for
404 pollinator conservation. Pollinators could benefit from more resources when the blossom
405 cover increases. An increase in flowering species richness could enhance the functional
406 diversity of floral traits and thus increase the niche partitioning for pollinators. This process
407 could lead to an increase in pollinator abundance as recently observed by Goulnik et al.
408 (2020) in French permanent grasslands. Vegetation height influenced the composition of
409 pollinator community. Vegetation was on average shorter at Marcenat than in other sites. This
410 difference may have been due to less favourable pedo-climatic conditions for grass growth
411 rather than more intensive management, because Marcenat had a low grass use and number of
412 mowing events. Grassland vegetation height has been shown to influence the species
413 composition of Lepidoptera and Syrphidae (Sjödín et al., 2008) but also of other Diptera
414 (Milberg et al., 2016). Stratification of vegetation height has been shown to positively
415 influence niche partitioning of pollinator species, along with flowering species richness and
416 time of day (Venjakob et al., 2016). Moreover, an increase in mean flower height of
417 communities could lead to higher pollinator species richness and visitations, as recently
418 highlighted by Fornoff et al. (2017) but not find in Goulnik et al., (2020).. In addition, higher
419 vegetation heights were associated with mown grasslands (i.e. 1-2 cuts per year), whose
420 pollinator composition can differ from that in grazed grasslands.

421

422 **Visual plant-pollinator networks in the three farming regions**

423 Plant-pollinator networks in the three studied sites had metrics similar to those in previous
424 studies from several continents (i.e. H_2' of 0.41-0.54, positive network asymmetry and

425 negative specialisation asymmetry, e.g. Blüthgen et al., 2007; Fründ et al., 2010) but smaller
426 sizes. Size and diversity of plant-pollinator networks were much larger and higher at
427 Mirecourt and Marcenat than those at Lusignan, likely due to lower plant taxa richness, lower
428 %SNH and a higher grass use in Lusignan. Higher network asymmetry at Lusignan also
429 highlighted a large difference in species richness between plants and insects. Most grasslands
430 at Lusignan were temporary, which led to low spontaneous plant enrichment and reduced the
431 plant species richness. We observed a higher degree of network specialisation in Lusignan
432 when all three trapping periods were considered but H2' was relatively similar given the same
433 sampling periods (June-July) for the three sites. Two hypotheses could explain these findings.
434 First, the network of Lusignan appeared probably more specialised due to integration over a
435 longer period (April-July) compared to the other sites (May-July), which increased the
436 number of missing links between plants and pollinator taxa due to differences in phenology
437 (Vázquez et al., 2009). Secondly, the lower flower diversity at Lusignan could force
438 pollinators to forage fewer flower types.

439

440 Concerning pollinator taxa, at Lusignan and Mirecourt, Hymenoptera had the highest
441 strength, but Diptera were the most specialised. In contrast, at Marcenat, Diptera had the
442 highest strength but were the least specialised, in agreement with results of Weiner et al.
443 (2014) for 50 German grassland plots. The degree of Syrphidae specialisation on our three
444 sites (0.40-0.63) was lower than that found by de Manincor et al. (2020) but in the same order
445 of magnitude as that found by Weiner et al. (2014) (0.24). The few interactions for other
446 Diptera at Lusignan (24) could partly explain the high degree of specialization d' for these
447 taxa (see Blüthgen et al., 2006). More generally, our results highlighted the key role of
448 Diptera in plant-pollinator networks in grasslands. Although they generally have a greater
449 handling time (reviewed in Inouye et al., 2015) and could transport less pollen than bees (e.g.

450 Goulnik et al., 2020 for facial measurements; Kearns and Inouye, 1994) because of lower
451 hairiness and smaller sizes for some taxa, , their number of interactions remained high and
452 structured the networks observed. They play an important role in pollen transfer of open
453 flowers such as *Ranunculus* sp. and *Anthriscus sylvestris* in our study, particularly because
454 most flies have short mouthparts (Woodcock et al., 2014). Some plant species were important
455 to the network structure, particularly due to flower rewards (i.e. nectar and pollen). At both
456 Mirecourt and Marcenat, *Ranunculus* sp. and *K. arvensis* had high strength. *Ranunculus* sp.
457 contains a large amount of pollen and an average amount of nectar (<http://www.floraweb.de>).
458 They bloomed quite early in the season, which benefited foragers such as *Empis* sp. *K.*
459 *arvensis* is a well-known melliferous species that offers an average amount of nectar (Baude
460 et al., 2016). At Mirecourt, *C. jacea* appeared as a key species that produced an average
461 amount of nectar and was associated with extensive management of grasslands (Jacot et al.,
462 2007) and wild crop-pollinating bees (Sutter et al., 2017). At Lusignan, *T. repens* was visited
463 frequently by Hymenoptera such as *Bombus* sp. and *A. mellifera*, which confirms previous
464 results (e.g. Goulson et al., 2005) and the important ability of *Trifolium* sp. to produce large
465 amounts of nectar and pollen for foragers.

466

467 **Contributions and limits of pollen DNA barcoding**

468 The pollinators sampled carried pollen from an average of 1-3 genera at the three sites, with
469 no difference among insect orders (i.e. Diptera, Hymenopteran and Lepidoptera). DNA
470 metabarcoding data highlighted that more plant genera than visual assessed were probably
471 visited by pollinators (+34-42), which was 2-3 times the genera richness found with visual
472 observations only. Pornon et al., (2017) also found an increase in plant visitations with pollen
473 DNA metabarcoding compared to visual networks (+26-68 plant species), unlike previous
474 results (e.g. Popic et al., 2013). Metabarcoding allowed us to reveal the flowers foraged by the

475 insects at the plot scale. Moreover, our results suggest that pollen DNA barcoding can reflect
476 differences in the surrounding landscape among sites. At Lusignan DNA barcoding showed
477 that they were mainly Apidae but also Halictidae and Andrenidae which transported pollens
478 of trees or shrubs, dicotyledons from grasslands and crops. Ligneous vegetation is known to
479 play a key role for wild bee community (Rollin et al., 2019) and honeybee colonies
480 development as well as large pollen supply for honeybees in spring in Europe (e.g. Requier ,
481 2015) but also in America (Sponsler et al., 2020). On the other sites, DNA barcoding reflected
482 pollen transports of grassland's plants (i.e. grass and dicotyledons) by Syrphidae and
483 Nymphalidae (mainly *Maniola jurtina*) at Mirecourt and by Empididae and Syrphidae at
484 Marcenat. Diptera and Lepidoptera can need different habitats and host plants for their larvae
485 and adult development. However, characterising the landscape and metabarcoding to genus
486 instead of species limited metagenomic analysis of the surrounding landscape.

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

500 have foraged quickly on the flower and not picked up pollen, or some plants could have had
501 small amounts of pollen.

502

503 **Conclusion**

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

514

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521

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791 **Table 1.** Mean site and plot characteristics: plot name, grassland type, successive management events during the studied year (M: mowing; P:
792 pasture), grass use calculated in livestock units (LU) day ha⁻¹, sum of organic and mineral nitrogen fertilisation (for the year before pollinator
793 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 |

794 ^aPlot management was only pasture for the 8 previous years.

795 ^bForage 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
796 grazing use to estimate total grass use.

797 **Table 2.** Main characteristics of plant-pollinator networks for each site: insect taxa richness,
798 plant taxa richness, number of unique interactions, Shannon diversity index of plant-pollinator
799 interactions, mean number of links per taxon, connectance, degree of specialisation H2' for all
800 three trapping sessions, degree of specialisation H2' of two last trapping sessions, network
801 asymmetry, specialisation asymmetry and weighted nestedness.
802

| 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 |

803
804 **Table 3.** Taxa-level descriptors for the 10 pollinator and 5 plant taxa with the highest strength
805 (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 | | | | | | | | | | | |
| <i>Bombus lapidarius</i> | 2.66 | 6 | 0.36 | <i>Empis</i> sp. | 2.66 | 6 | 0.42 | <i>Diptera</i> n.i | 7.76 | 18 | 0.43 |
| <i>Andrena haemorrhoa</i> | 1.22 | 2 | 0.63 | <i>Pseudovadonia livida</i> | 2.00 | 2 | 1.00 | <i>Empis</i> sp. | 6.69 | 14 | 0.42 |
| <i>Apis mellifera</i> | 0.73 | 3 | 0.19 | <i>Episyrphus balteatus</i> | 1.63 | 8 | 0.15 | <i>Lasioglossum albipes</i> | 3.03 | 9 | 0.35 |
| <i>Andrena flavipes</i> | 0.52 | 2 | 0.46 | <i>Sphaerophoria scripta</i> | 1.48 | 6 | 0.28 | <i>Apis mellifera</i> | 2.24 | 3 | 0.84 |
| <i>Sicus ferrugineus</i> | 0.50 | 1 | 0.85 | <i>Maniola jurtina</i> | 1.39 | 4 | 0.35 | <i>Bombus sylvarum</i> | 1.57 | 3 | 0.69 |
| <i>Syrphidae</i> n.i | 0.44 | 3 | 0.33 | <i>Scathophaga stercoraria</i> | 1.13 | 4 | 0.38 | <i>Sphaeroceridae</i> n.i | 1.36 | 6 | 0.46 |
| <i>Diptera</i> n.i | 0.37 | 2 | 0.50 | <i>Lasioglossum lativentre</i> | 1.05 | 3 | 0.40 | <i>Phyllopertha horticola</i> | 1.29 | 4 | 0.42 |
| <i>Scathophaga stercoraria</i> | 0.37 | 1 | 0.68 | <i>Cratosilis</i> sp. | 1.00 | 1 | 1.00 | <i>Sphaerophoria scripta</i> | 1.27 | 3 | 0.47 |
| <i>Andrena ranunculi</i> | 0.33 | 1 | 0.77 | <i>Eucera nigrescens</i> | 1.00 | 1 | 1.00 | <i>Bombus lapidarius</i> | 1.21 | 3 | 0.56 |
| <i>Bombus terrestris</i> | 0.26 | 3 | 0.13 | <i>Diptera</i> n.i | 0.69 | 4 | 0.39 | <i>Cryptocephalus sericeus</i> | 1.06 | 5 | 0.53 |
| Plant | | | | | | | | | | | |
| <i>Cirsium palustre</i> | 16.74 | 20 | 0.57 | <i>Centaurea jacea</i> | 18.98 | 27 | 0.53 | <i>Knautia arvensis</i> | 10.99 | 14 | 0.71 |
| <i>Trifolium repens</i> | 10.39 | 12 | 0.42 | <i>Ranunculus acris</i> | 14.11 | 25 | 0.34 | <i>Ranunculus</i> sp. | 9.82 | 16 | 0.44 |
| <i>Taraxacum</i> sp. | 7.56 | 10 | 0.79 | <i>Knautia arvensis</i> | 8.77 | 17 | 0.25 | <i>Anthriscus sylvestris</i> | 6.86 | 11 | 0.43 |

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

806

807 **Table 4.** Mean (\pm SD) specialisation (d') of six pollinator groups at each site.

| Group | Lusignan | Mirecourt | Marcenat |
|-----------------------|-----------------|-----------------|-----------------|
| <i>Apis mellifera</i> | 0.19 | 0.18 | 0.84 |
| Other Apidae | 0.31 \pm 0.21 | 0.25 \pm 0.25 | 0.49 \pm 0.25 |
| Syrphidae | 0.28 \pm 0.21 | 0.23 \pm 0.12 | 0.31 \pm 0.28 |
| Diptera | 0.61 \pm 0.20 | 0.44 \pm 0.20 | 0.32 \pm 0.23 |
| Lepidoptera | 0.06 \pm 0.09 | 0.21 \pm 0.16 | 0.44 \pm 0.20 |
| Coleoptera | 0.27 \pm 0.15 | 0.33 \pm 0.30 | 0.35 \pm 0.18 |

808

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

814

| | 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% |

815

816

817 **Figure captions**

818

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

820

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

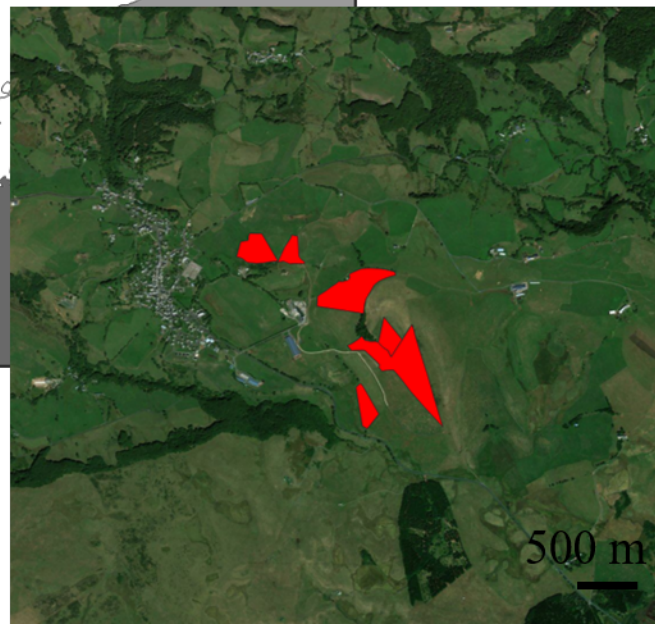
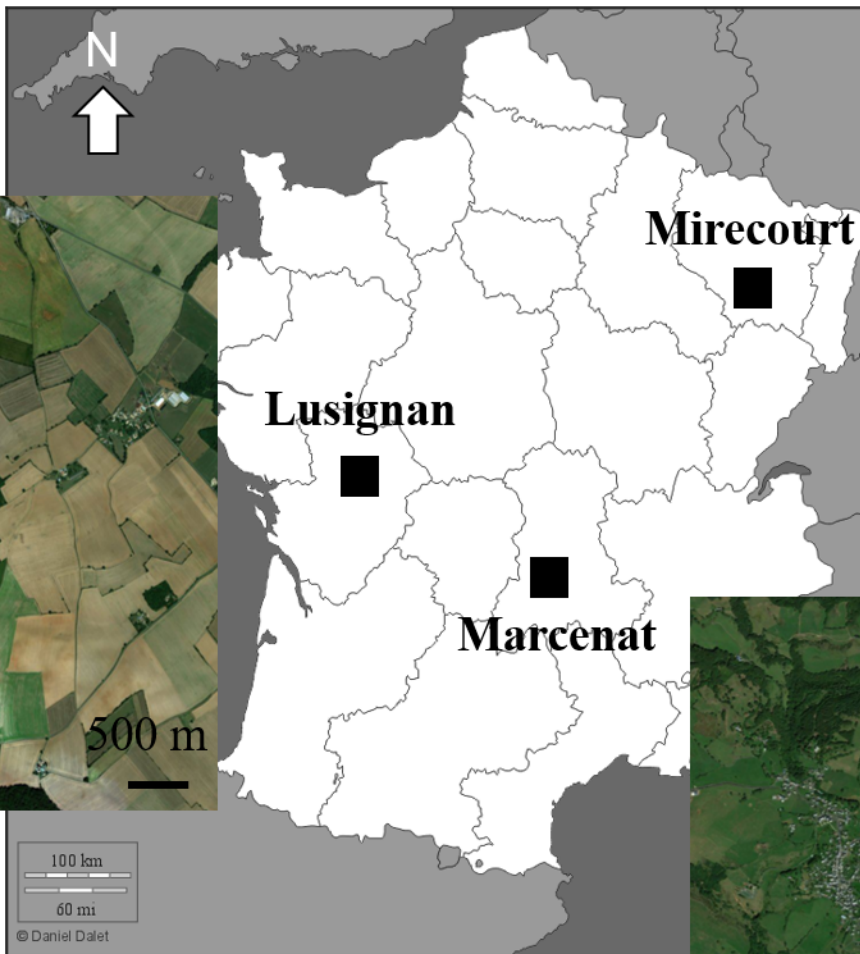
822

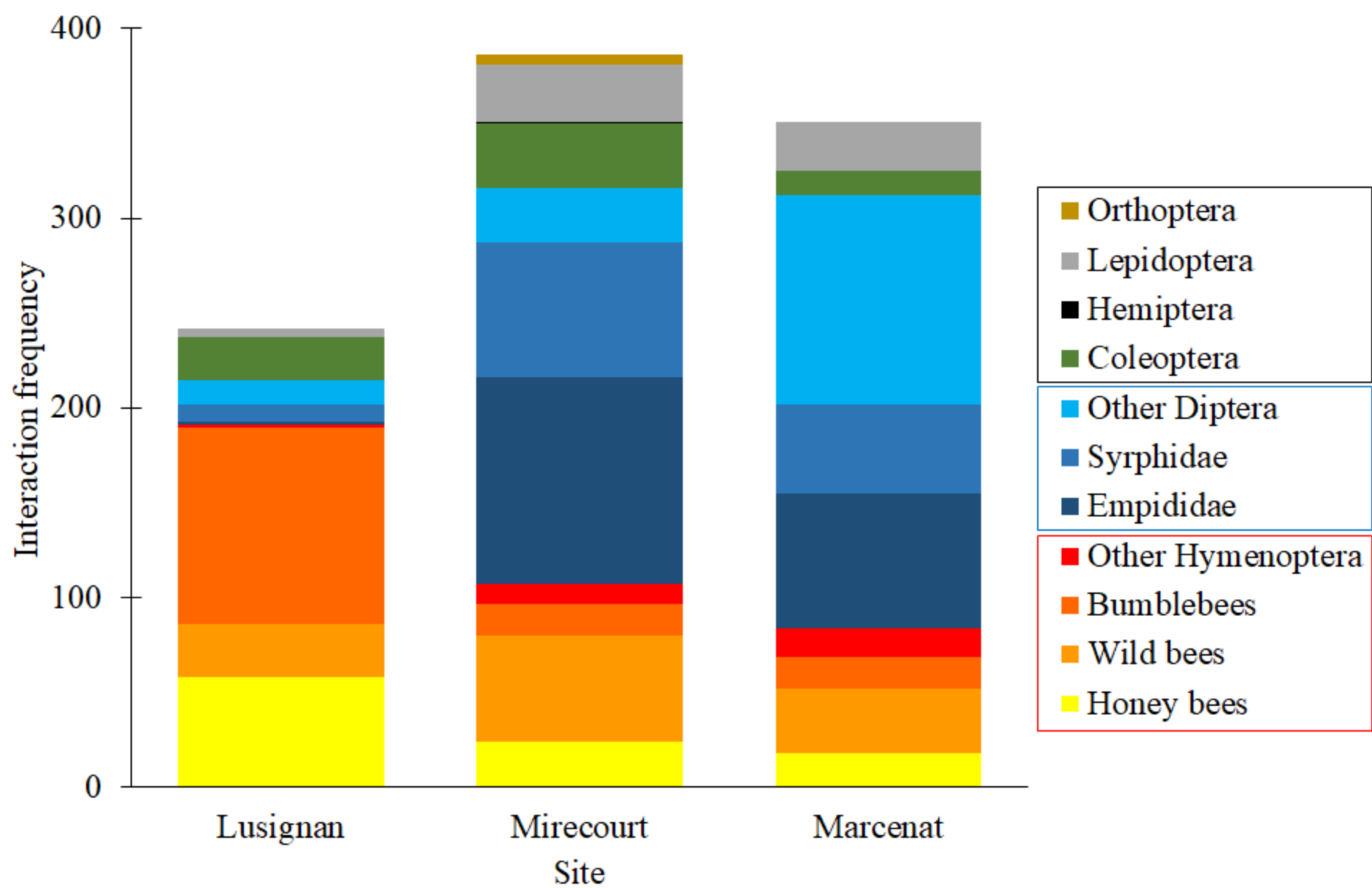
823 **Figure 3.** Distance triplot of the two significant axes of the redundancy analysis. Black arrow:
824 explanatory variables. %SNH: percentage of semi-natural habitats in a 500 m buffer around each plot;
825 Veg.height: mean vegetation height (cm). Ellipses represent normal 95% confidence intervals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

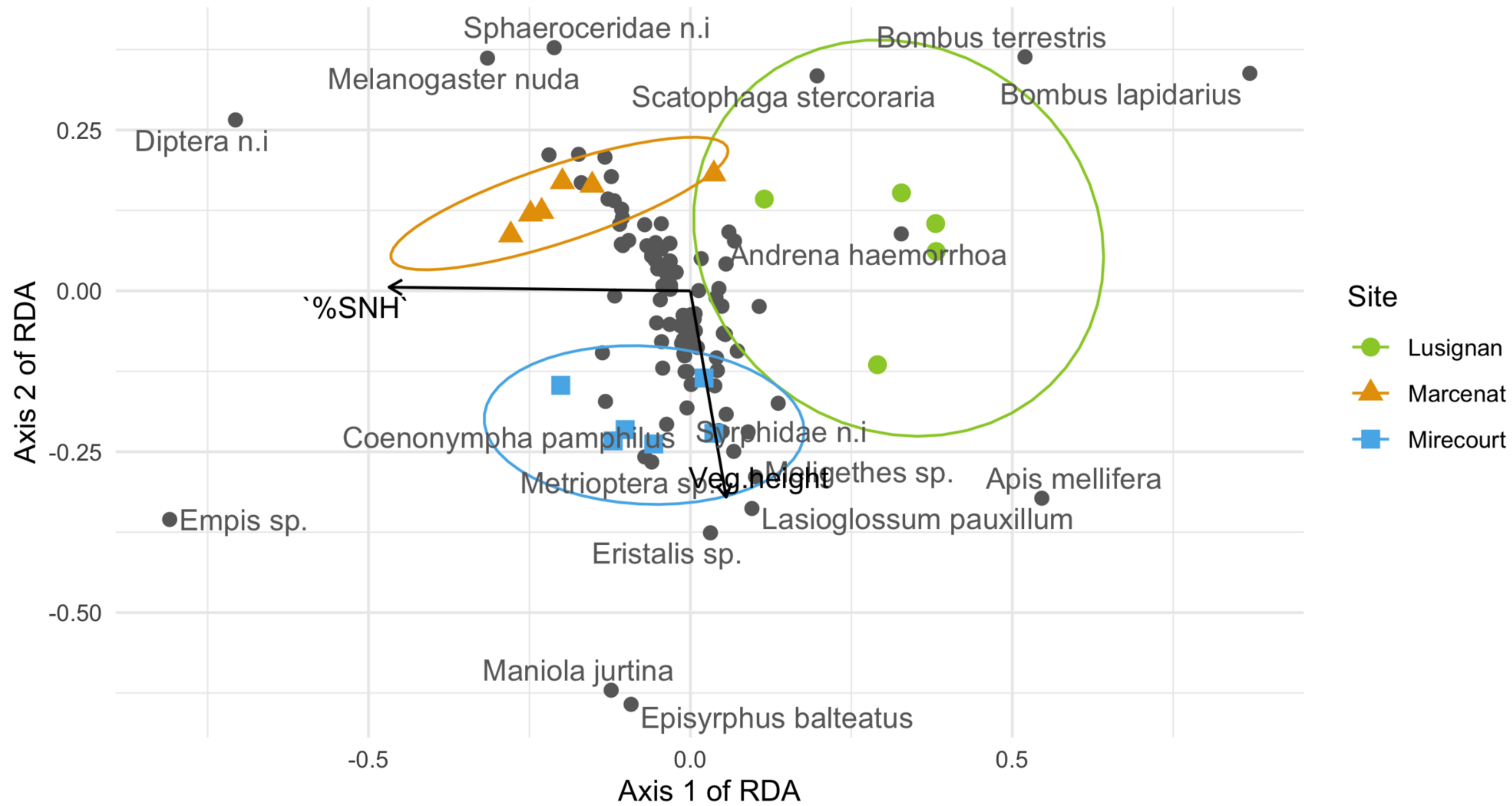
828

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

833







Cichorium intybus

Cirsium palustre

Crepis sp.

Malva sylvestris

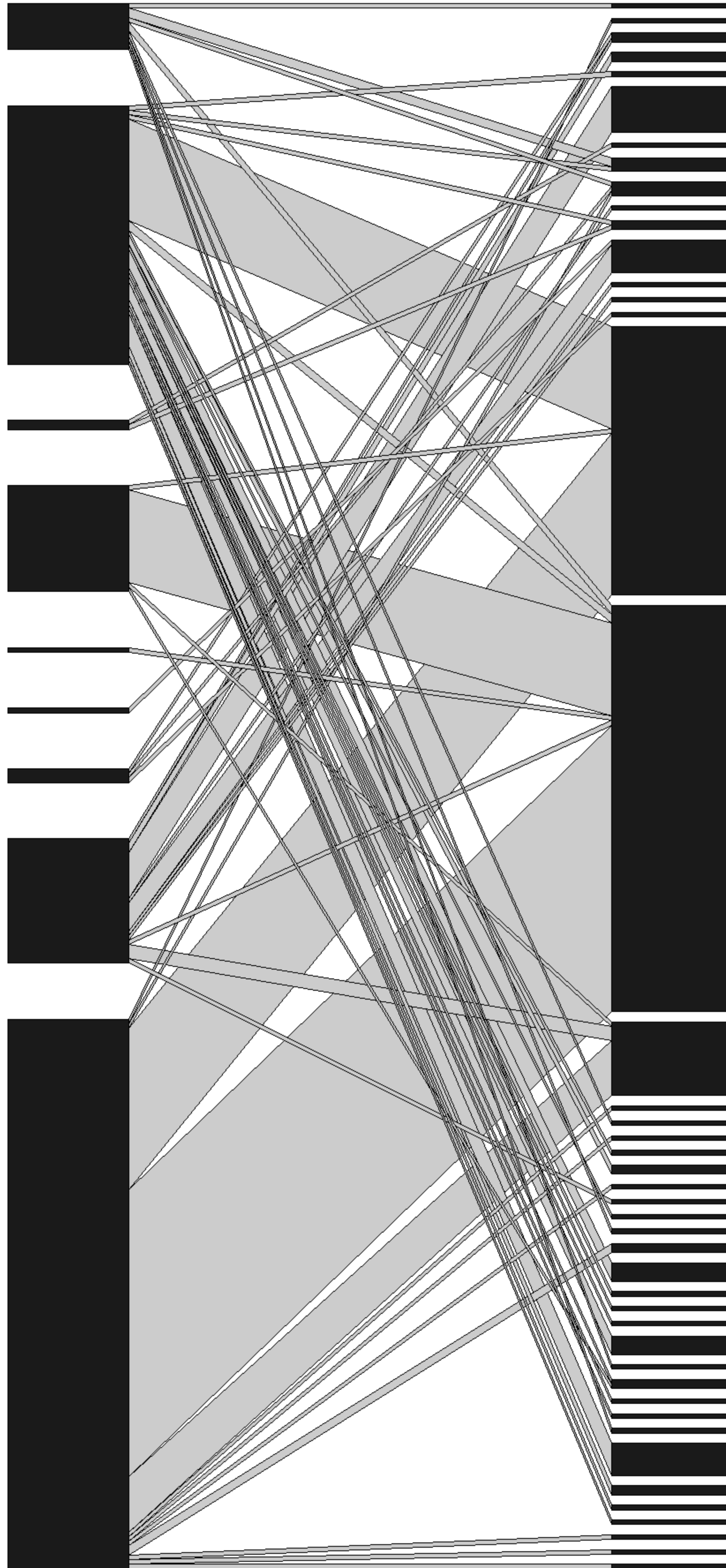
Medicago sativa

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 haemorrhoea

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

