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

Alice Michelot-Antalik, Nadia Michel, Jérémie Goulnik, André Blanchetête, Emile Delacroix, Patricia Faivre-Rampant, Jean-Louis Fiorelli, Jean-Noël Galliot, David Genoud, Laurent Lanore, et al.

HAL Id: hal-03315908
https://hal.inrae.fr/hal-03315908

Submitted on 2 Aug 2023

1 **Comparison of grassland plant-pollinator networks on dairy farms in three contrasting**
2 **French landscapes**
3

4 Alice Michelot-Antalik[1], Nadia Michel[1], Jérémie Goulnik[1], André Blanchetête[2], Emile

5 Delacroix[1], Patricia Faivre-Rampant[3], Jean-Louis Fiorelli[4], Jean-Noël Galliot[5], David

6 Genoud[6], Laurent Lanore[7], Isabelle Le Clainche[3], Marie Christine Le Paslier[3], Sandra Novak[8],

7 Jean-François Odoux[9], Dominique Brunel[3], Anne Farruggia[10]

8

9 [1]Université de Lorraine, INRAE, LAE, F-54000 Nancy, France

10 [2]Herbipôle, INRAE, F-63122, Saint-Genès-Champanelle, France

11 [3]Etude du Polymorphisme des Génomes Végétaux (EPGV), INRAE, Université Paris-Saclay,

12 91000 Evry, France

13 [4]ASTER, INRAE, F-88500 Mirecourt, France

14 [5]UCA, INRAE, UMR 0874 UREP, F-63000 Clermont-Ferrand, France

15 [6] DGE, F-11290 Arzens, France

16 [7]UCA, INRAE, VetAgro Sup, UMR Herbivores, F-63122 Saint-Genès-Champanelle, France

17 [8]FERLUS, INRAE, F-86600 Lusignan, France

18 [9]APIS, INRAE, Station du Magneraud, F-17700 Surgères, France

19 [10]DSLP, INRAE, F-17450 Saint Laurent de la Prée, France

20

21 *Corresponding author:

22 E-mail: alice.michelot@univ-lorraine.fr

23 Postal address:

24 Université de Lorraine

25 UMR 1121 UL-INRA Agronomie et Environnement Nancy-Colmar

26 2, avenue de la Forêt de Haye

27 54505 VANDOEUVRE LES NANCY Cedex

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 (H2') 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

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 Tscharntke, 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   (Villemey 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

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.

164

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™ tubes (to avoid pollen contamination) by an 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 Falcon^(TM) 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 m^2 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 (± 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

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

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. haemorrhoa*, *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 ± 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. haemorrhoa*: 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 (Sjödin 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. H2' 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 metaboarcoding 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 assignations, 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 assignations. 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

521

522 **Author contributions**

523 Alice Michelot-Antalik: conceptualization, data curation, formal analysis, software,

524 investigation, methodology, resources, supervision, validation, visualization, writing - original

525 draft, review & editing.

526 Nadia Michel: data curation, investigation, methodology, resources, writing - review &

527 editing

528 Jérémie Goulnik: formal analysis, software, writing - review & editing

529 André Blanchetête: data curation, investigation

530 Emile Delacroix: data curation, investigation

531 Patricia Faivre-Rampant: data curation, investigation, resources, writing - review & editing

532 Jean-Louis Fiorelli: data curation, investigation, resources

533 Jean-Noël Galliot: data curation, investigation, methodology, writing - review & editing

534 David Genoud: data curation, investigation

535 Laurent Lanore: data curation, investigation

536 Isabelle Le Clainche: data curation, investigation

537 Marie Christine Le Paslier: data curation, investigation

538 Sandra Novak: data curation, investigation, resources, writing - review & editing

539 Jean-François Odoux: data curation, investigation, writing - review & editing

540 Dominique Brunel: data curation, investigation, methodology, resources

541 Anne Farruggia: conceptualization, data curation, formal analysis, software, funding

542 acquisition, investigation, methodology, supervision, validation, visualization, project

543 administration, supervision, writing original draft, review & editing.

544

545 **Bibliography**

546 Agreste, 2019. Statistique agricole annuelle 2016-2017. Résultats définitifs. (No. 2019–1),
547 Agreste chiffres et données. Ministère de l'Agriculture et de l'Alimentation, Paris.

548   Alarcon, R., 2010. Congruence between visitation and pollen-transport networks in a
549   California plant–pollinator community. Oikos 119, 35–44.
550   https://doi.org/10.1111/j.1600-0706.2009.17694.x
551   Baude, M., Kunin, W.E., Boatman, N.D., Conyers, S., Davies, N., Gillespie, M.A.K., Morton,
552   R.D., Smart, S.M., Memmott, J., 2016. Historical nectar assessment reveals the fall
553   and rise of floral resources in Britain. Nature 530, 85–88.
554   https://doi.org/10.1038/nature16532
555   Bengtsson, J., Bullock, J.M., Egoh, B., Everson, C., Everson, T., O'Connor, T., O'Farrell,
556   P.J., Smith, H.G., Lindborg, R., 2019. Grasslands—more important for ecosystem
557   services than you might think. Ecosphere 10, e02582.
558   https://doi.org/10.1002/ecs2.2582
559   Blüthgen, N., 2010. Why network analysis is often disconnected from community ecology: A
560   critique and an ecologist's guide. Basic and Applied Ecology 11, 185–195.
561   https://doi.org/10.1016/j.baae.2010.01.001
562   Blüthgen, N., Menzel, F., Hovestadt, T., Fiala, B., Bluethgen, N., 2007. Specialization,
563   constraints, and conflicting interests in mutualistic networks. Curr. Biol. 17, 341–346.
564   https://doi.org/10.1016/j.cub.2006.12.039
565   Blüthgen, Nico, Menzel, F., Blüthgen, Nils, 2006. Measuring specialization in species
566   interaction networks. BMC Ecology 6, 9. https://doi.org/10.1186/1472-6785-6-9
567   Borcard, D., Gillet, F., Legendre, P., 2011. Numerical Ecology with R, Use R! Springer-
568   Verlag, New York.
569   Burel, F., Butet, A., Delettre, Y.R., Millàn de la Peña, N., 2004. Differential response of
570   selected taxa to landscape context and agricultural intensification. Landscape and
571   Urban Planning, Development of European Landscapes 67, 195–204.
572   https://doi.org/10.1016/S0169-2046(03)00039-2
573   Chen, S., Yao, H., Han, J., Liu, C., Song, J., Shi, L., Zhu, Y., Ma, X., Gao, T., Pang, X., Luo,
574   K., Li, Y., Li, X., Jia, X., Lin, Y., Leon, C., 2010. Validation of the ITS2 Region as a
575   Novel DNA Barcode for Identifying Medicinal Plant Species. PLOS ONE 5, e8613.
576   https://doi.org/10.1371/journal.pone.0008613
577   Collins, R.A., Cruickshank, R.H., 2013. The seven deadly sins of DNA barcoding. Mol Ecol
578   Resour 13, 969–975. https://doi.org/10.1111/1755-0998.12046
579   de Manincor, N., Hautekeete, N., Piquot, Y., Schatz, B., Vanappelghem, C., Massol, F., 2020.
580   Does phenology explain plant-pollinator interactions at different latitudes? An
581   assessment of its explanatory power in plant-hoverfly networks in French calcareous
582   grasslands. Oikos 129, 753–765. https://doi.org/10.1111/oik.07259
583   Diaz-Forero, I., Kuusemets, V., Mänd, M., Liivamägi, A., Kaart, T., Luig, J., 2013. Influence
584   of local and landscape factors on bumblebees in semi-natural meadows: a multiple-
585   scale study in a forested landscape. J Insect Conserv 17, 113–125.
586   https://doi.org/10.1007/s10841-012-9490-3
587   Farruggia, A., Dumont, B., Scohier, A., Leroy, T., Pradel, P., Garel, J.-P., 2012. An
588   alternative rotational stocking management designed to favour butterflies in permanent
589   grasslands. Grass and Forage Science 67, 136–149. https://doi.org/10.1111/j.1365-
590   2494.2011.00829.x
591   Fišer Pečnikar, Ž., Buzan, E.V., 2014. 20 years since the introduction of DNA barcoding:
592   from theory to application. J. Appl. Genet. 55, 43–52. https://doi.org/10.1007/s13353-
593   013-0180-y
594   Fontaine, C., Dajoz, I., Meriguet, J., Loreau, M., 2005. Functional Diversity of Plant–
595   Pollinator Interaction Webs Enhances the Persistence of Plant Communities. PLoS
596   Biology 4, e1. https://doi.org/10.1371/journal.pbio.0040001

Fornoff, F., Klein, A.-M., Hartig, F., Benadi, G., Venjakob, C., Schaefer, H.M., Ebeling, A., 2017. Functional flower traits and their diversity drive pollinator visitation. Oikos.

Fortuna, M.A., Bascompte, J., 2006. Habitat loss and the structure of plant-animal mutualistic networks. Ecol. Lett. 9, 278–283. https://doi.org/10.1111/j.1461-0248.2005.00868.x

Fründ, J., Dormann, C.F., Holzschuh, A., Tscharntke, T., 2013. Bee diversity effects on pollination depend on functional complementarity and niche shifts. Ecology 94, 2042–2054. https://doi.org/10.1890/12-1620.1

Fründ, J., Linsenmair, K.E., Blüthgen, N., 2010. Pollinator diversity and specialization in relation to flower diversity. Oikos 119, 1581–1590. https://doi.org/10.1111/j.1600-0706.2010.18450.x

Galliot, J.-N., Brunel, D., Bérard, A., Chauveau, A., Blanchetête, A., Lanore, L., Farruggia, A., 2017. Investigating a flower-insect forager network in a mountain grassland community using pollen DNA barcoding. J Insect Conserv 21, 827–837. https://doi.org/10.1007/s10841-017-0022-z

Gaujour, E., Amiaud, B., Mignolet, C., Plantureux, S., 2012. Factors and processes affecting plant biodiversity in permanent grasslands. A review. Agron. Sustain. Dev. 32, 133–160. https://doi.org/10.1007/s13593-011-0015-3

Ghosh, S., Jeon, H., Jung, C., 2020. Foraging behaviour and preference of pollen sources by honey bee (Apis mellifera) relative to protein contents. Journal of Ecology and Environment 44, 4. https://doi.org/10.1186/s41610-020-0149-9

Goulnik, J., Plantureux, S., Thery, M., Baude, M., Delattre, M., van Reeth, C., Villerd, J., Michelot-Antalik, A., 2020a. Floral trait functional diversity is related to soil characteristics and positively influences pollination function in semi-natural grasslands. Agric. Ecosyst. Environ. 301, 107033. https://doi.org/10.1016/j.agee.2020.107033

Goulnik, J., Plantureux, S., Van Reeth, C., Baude, M., Mesbahi, G., Michelot-Antalik, A., 2020b. Facial area and hairiness of pollinators visiting semi-natural grassland wild plants predict their facial pollen load. Ecol. Entomol. https://doi.org/10.1111/een.12913

Goulson, D., Hanley, M.E., Darvill, B., Ellis, J.S., Knight, M.E., 2005. Causes of rarity in bumblebees. Biol. Conserv. 122, 1–8. https://doi.org/10.1016/j.biocon.2004.06.017

Habel, J.C., Dengler, J., Janišová, M., Török, P., Wellstein, C., Wiezik, M., 2013. European grassland ecosystems: threatened hotspots of biodiversity. Biodiversity and Conservation 22, 2131–2138. https://doi.org/10.1007/s10531-013-0537-x

Hallmann, C.A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., Stenmans, W., Müller, A., Sumser, H., Hörren, T., Goulson, D., Kroon, H. de, 2017. More than 75 percent decline over 27 years in total flying insect biomass in protected areas. PLOS ONE 12, e0185809. https://doi.org/10.1371/journal.pone.0185809

Hanley, M.E., Franco, M., Pichon, S., Darvill, B., Goulson, D., 2008. Breeding System, Pollinator Choice and Variation in Pollen Quality in British Herbaceous Plants. Functional Ecology 22, 592–598.

Hatfield, R.G., LeBuhn, G., 2007. Patch and landscape factors shape community assemblage of bumble bees, Bombus spp. (Hymenoptera: Apidae), in montane meadows. Biological Conservation 139, 150–158. https://doi.org/10.1016/j.biocon.2007.06.019

Hicks, D.M., Ouvrard, P., Baldock, K.C.R., Baude, M., Goddard, M.A., Kunin, W.E., Mitschunas, N., Memmott, J., Morse, H., Nikolitsi, M., Osgathorpe, L.M., Potts, S.G., Robertson, K.M., Scott, A.V., Sinclair, F., Westbury, D.B., Stone, G.N., 2016. Food for Pollinators: Quantifying the Nectar and Pollen Resources of Urban Flower Meadows. PLOS ONE 11, e0158117. https://doi.org/10.1371/journal.pone.0158117

646 Holzschuh, A., Dainese, M., González-Varo, J.P., Mudri-Stojnić, S., Riedinger, V., Rundlöf,
647   M., Scheper, J., Wickens, J.B., Wickens, V.J., Bommarco, R., Kleijn, D., Potts, S.G.,
648   Roberts, S.P.M., Smith, H.G., Vilà, M., Vujić, A., Steffan-Dewenter, I., 2016. Mass-
649   flowering crops dilute pollinator abundance in agricultural landscapes across Europe.
650   Ecology Letters 19, 1228–1236. https://doi.org/10.1111/ele.12657
651 Hudewenz, A., Klein, A.-M., Scherber, C., Stanke, L., Tscharntke, T., Vogel, A., Weigelt, A.,
652   Weisser, W.W., Ebeling, A., 2012. Herbivore and pollinator responses to grassland
653   management intensity along experimental changes in plant species richness.
654   Biological Conservation 150, 42–52. https://doi.org/10.1016/j.biocon.2012.02.024
655 Inouye, D.W., Larson, B.M.H., Ssymank, A., Kevan, P.G., 2015. Flies and Flowers III:
656   Ecology of foraging and pollination. Journal of Pollination Ecology 16.
657   https://doi.org/10.26786/1920-7603(2015)15
658 Ion, N., Odoux, J.-F., Vaissière, B.E., 2018. Melliferous Potential of Weedy Herbaceous
659   Plants in Crop Fields of Romania from 1949 to 2012. Journal of Apicultural Science
660   62, 149–165. https://doi.org/10.2478/jas-2018-0017
661 Jacot, K., Beerli, C., Eggenschwiler, L., 2007. Improved field margins and the effects on
662   voles and moles. Agrarforschung 14, 214–219.
663 Jacquemyn, H., Brys, R., Hermy, M., 2003. Short-term effects of different management
664   regimes on the response of calcareous grassland vegetation to increased nitrogen.
665   Biological Conservation 111, 137–147. https://doi.org/10.1016/S0006-
666   3207(02)00256-2
667 Jordano, P., 1987. Patterns of Mutualistic Interactions in Pollination and Seed Dispersal:
668   Connectance, Dependence Asymmetries, and Coevolution. The American Naturalist
669   129, 657–677.
670 Junker, R.R., Blüthgen, N., Keller, A., 2015. Functional and phylogenetic diversity of plant
671   communities differently affect the structure of flower-visitor interactions and reveal
672   convergences in floral traits. Evol Ecol 29, 437–450. https://doi.org/10.1007/s10682-
673   014-9747-2
674 Kearns, C., Inouye, D., 1994. Fly Pollination of Linum-Lewisii (linaceae). Am. J. Bot. 81,
675   1091–1095. https://doi.org/10.2307/2445470
676 Kearns, C.A., 1992. Anthophilous Fly Distribution Across an Elevation Gradient. The
677   American Midland Naturalist 127, 172–182. https://doi.org/10.2307/2426332
678 Klaus, F., Tscharntke, T., Uhler, J., Grass, I., 2021. Calcareous grassland fragments as sources
679   of bee pollinators for the surrounding agricultural landscape. Global Ecology and
680   Conservation 26, e01474. https://doi.org/10.1016/j.gecco.2021.e01474
681 Kormann, U., Rösch, V., Batáry, P., Tscharntke, T., Orci, K.M., Samu, F., Scherber, C., 2015.
682   Local and landscape management drive trait-mediated biodiversity of nine taxa on
683   small grassland fragments. Diversity and Distributions 21, 1204–1217.
684   https://doi.org/10.1111/ddi.12324
685 Lazaro, A., Tscheulin, T., Devalez, J., Nakas, G., Petanidou, T., 2016. Effects of grazing
686   intensity on pollinator abundance and diversity, and on pollination services. Ecol.
687   Entomol. 41, 400–412. https://doi.org/10.1111/een.12310
688 Lefebvre, V., Fontaine, C., Villemant, C., Daugeron, C., 2014. Are empidine dance flies
689   major flower visitors in alpine environments? A case study in the Alps, France. Biol
690   Lett 10. https://doi.org/10.1098/rsbl.2014.0742
691 Lundgren, R., Totland, Ø., Lázaro, A., 2016. Experimental simulation of pollinator decline
692   causes community-wide reductions in seedling diversity and abundance. Ecology 97,
693   1420–1430.
694 Memmott, J., Waser, N.M., Price, M.V., 2004. Tolerance of pollination networks to species
695   extinctions. Proc Biol Sci 271, 2605–2611. https://doi.org/10.1098/rspb.2004.2909

696   Milberg, P., Bergman, K.-O., Cronvall, E., Eriksson, Å.I., Glimskär, A., Islamovic, A.,
697   Jonason, D., Löfqvist, Z., Westerberg, L., 2016. Flower abundance and vegetation
698   height as predictors for nectar-feeding insect occurrence in Swedish semi-natural
699   grasslands. Agriculture, Ecosystems & Environment 230, 47–54.
700   https://doi.org/10.1016/j.agee.2016.05.029
701   Ollerton, J., Winfree, R., Tarrant, S., 2011. How many flowering plants are pollinated by
702   animals? Oikos 120, 321–326. https://doi.org/10.1111/j.1600-0706.2010.18644.x
703   Orford, K.A., Murray, P.J., Vaughan, I.P., Memmott, J., 2016. Modest enhancements to
704   conventional grassland diversity improve the provision of pollination services. J Appl
705   Ecol 53, 906–915. https://doi.org/10.1111/1365-2664.12608
706   Popic, T.J., Wardle, G.M., Davila, Y.C., 2013. Flower-visitor networks only partially predict
707   the function of pollen transport by bees. Austral Ecology 38, 76–86.
708   https://doi.org/10.1111/j.1442-9993.2012.02377.x
709   Pornon, A., Andalo, C., Burrus, M., Escaravage, N., 2017. DNA metabarcoding data unveils
710   invisible pollination networks, in: Scientific Reports.
711   https://doi.org/10.1038/srep27282
712   Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O., Kunin, W.E., 2010.
713   Global pollinator declines: trends, impacts and drivers. Trends in Ecology & Evolution
714   25, 345–353. https://doi.org/10.1016/j.tree.2010.01.007
715   QGIS Development Team, 2019. QGIS Geographic Information System. Open Source
716   Geospatial Foundation Project.
717   Rader, R., Bartomeus, I., Garibaldi, L.A., Garratt, M.P.D., Howlett, B.G., Winfree, R.,
718   Cunningham, S.A., Mayfield, M.M., Arthur, A.D., Andersson, G.K.S., Bommarco, R.,
719   Brittain, C., Carvalheiro, L.G., Chacoff, N.P., Entling, M.H., Foully, B., Freitas, B.M.,
720   Gemmill-Herren, B., Ghazoul, J., Griffin, S.R., Gross, C.L., Herbertsson, L., Herzog,
721   F., Hipólito, J., Jaggar, S., Jauker, F., Klein, A.-M., Kleijn, D., Krishnan, S., Lemos,
722   C.Q., Lindström, S.A.M., Mandelik, Y., Monteiro, V.M., Nelson, W., Nilsson, L.,
723   Pattemore, D.E., Pereira, N. de O., Pisanty, G., Potts, S.G., Reemer, M., Rundlöf, M.,
724   Sheffield, C.S., Scheper, J., Schüepp, C., Smith, H.G., Stanley, D.A., Stout, J.C.,
725   Szentgyörgyi, H., Taki, H., Vergara, C.H., Viana, B.F., Woyciechowski, M., 2016.
726   Non-bee insects are important contributors to global crop pollination. PNAS 113, 146–
727   151. https://doi.org/10.1073/pnas.1517092112
728   Requier, F., Odoux, J.-F., Tamic, T., Moreau, N., Henry, M., Decourtye, A., Bretagnolle, V.,
729   2015. Honey bee diet in intensive farmland habitats reveals an unexpectedly high
730   flower richness and a major role of weeds. Ecological Applications 25, 881–890.
731   https://doi.org/10.1890/14-1011.1
732   Rimet, F., Chaumeil, P., Keck, F., Kermarrec, L., Vasselon, V., Kahlert, M., Franc, A.,
733   Bouchez, A., 2016. R-Syst::diatom: an open-access and curated barcode database for
734   diatoms and freshwater monitoring. Database (Oxford) 2016.
735   https://doi.org/10.1093/database/baw016
736   Roger, N., Moerman, R., Carvalheiro, L.G., Aguirre-Guitiérrez, J., Jacquemart, A.-L., Kleijn,
737   D., Lognay, G., Moquet, L., Quinet, M., Rasmont, P., Richel, A., Vanderplanck, M.,
738   Michez, D., 2017. Impact of pollen resources drift on common bumblebees in NW
739   Europe. Global Change Biology 23, 68–76. https://doi.org/10.1111/gcb.13373
740   Rollin, O., Pérez-Méndez, N., Bretagnolle, V., Henry, M., 2019. Preserving habitat quality at
741   local and landscape scales increases wild bee diversity in intensive farming systems.
742   Agriculture, Ecosystems & Environment 275, 73–80.
743   https://doi.org/10.1016/j.agee.2019.01.012
744   Sjödin, N.E., 2007. Pollinator behavioural responses to grazing intensity. Biodiversity and
745   Conservation 16, 2103–2121. https://doi.org/10.1007/s10531-006-9103-0

746 Sjödin, N.E., Bengtsson, J., Ekbom, B., 2008. The influence of grazing intensity and
747   landscape composition on the diversity and abundance of flower-visiting insects.
748   Journal of Applied Ecology 45, 763–772. https://doi.org/10.1111/j.1365-
749   2664.2007.01443.x
750 Sponsler, D.B., Grozinger, C.M., Richardson, R.T., Nurse, A., Brough, D., Patch, H.M.,
751   Stoner, K.A., 2020. A screening-level assessment of the pollinator-attractiveness of
752   ornamental nursery stock using a honey bee foraging assay. Scientific Reports 10, 831.
753   https://doi.org/10.1038/s41598-020-57858-2
754 Steffan-Dewenter, I., Tscharntke, T., 1999. Effects of habitat isolation on pollinator
755   communities and seed set. Oecologia 121, 432–440.
756   https://doi.org/10.1007/s004420050949
757 Sutter, L., Jeanneret, P., Bartual, A.M., Bocci, G., Albrecht, M., 2017. Enhancing plant
758   diversity in agricultural landscapes promotes both rare bees and dominant crop-
759   pollinating bees through complementary increase in key floral resources. Journal of
760   Applied Ecology 54, 1856–1864. https://doi.org/10.1111/1365-2664.12907
761 Tiusanen, M., Hebert, P.D.N., Schmidt, N.M., Roslin, T., 2016. One fly to rule them all-
762   muscid flies are the key pollinators in the Arctic. Proc. R. Soc. B-Biol. Sci. 283,
763   20161271. https://doi.org/10.1098/rspb.2016.1271
764 Venjakob, C., Klein, A.-M., Ebeling, A., Tscharntke, T., Scherber, C., 2016. Plant diversity
765   increases spatio-temporal niche complementarity in plant-pollinator interactions.
766   Ecology and Evolution 6, 2249–2261. https://doi.org/10.1002/ece3.2026
767 Villemey, A., van Halder, I., Ouin, A., Barbaro, L., Chenot, J., Tessier, P., Calatayud, F.,
768   Martin, H., Roche, P., Archaux, F., 2015. Mosaic of grasslands and woodlands is more
769   effective than habitat connectivity to conserve butterflies in French farmland. Biol.
770   Conserv. 191, 206–215. https://doi.org/10.1016/j.biocon.2015.06.030
771 Weiner, C.N., Werner, M., Linsenmair, K.E., Blüthgen, N., 2014. Land-use impacts on plant–
772   pollinator networks: interaction strength and specialization predict pollinator declines.
773   Ecology 95, 466–474. https://doi.org/10.1890/13-0436.1
774 Weiner, C.N., Werner, M., Linsenmair, K.E., Blüthgen, N., 2011. Land use intensity in
775   grasslands: Changes in biodiversity, species composition and specialisation in flower
776   visitor networks. Basic and Applied Ecology 12, 292–299.
777   https://doi.org/10.1016/j.baae.2010.08.006
778 Willmer, P.G., Cunnold, H., Ballantyne, G., 2017. Insights from measuring pollen deposition:
779   quantifying the pre-eminence of bees as flower visitors and effective pollinators.
780   Arthropod-Plant Interactions 11, 411–425. https://doi.org/10.1007/s11829-017-9528-2
781 Wood, T.J., Holland, J.M., Goulson, D., 2016. Diet characterisation of solitary bees on
782   farmland: dietary specialisation predicts rarity. Biodivers Conserv 25, 2655–2671.
783   https://doi.org/10.1007/s10531-016-1191-x
784 Woodcock, B.A., Edwards, M., Redhead, J., Meek, W.R., Nuttall, P., Falk, S., Nowakowski,
785   M., Pywell, R.F., 2013. Crop flower visitation by honeybees, bumblebees and solitary
786   bees: Behavioural differences and diversity responses to landscape. Agriculture,
787   Ecosystems & Environment 171, 1–8. https://doi.org/10.1016/j.agee.2013.03.005
788 Woodcock, T.S., Larson, B.M.H., Kevan, P.G., Inouye, D.W., Lunau, K., 2014. Flies and
789   flowers II: floral attractants and rewards. Journal of Pollination Ecology 12, 63–94.
790

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.

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	S	D	d'	Mirecourt	S	D	d'	Marcenat	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 |

806

807 **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

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
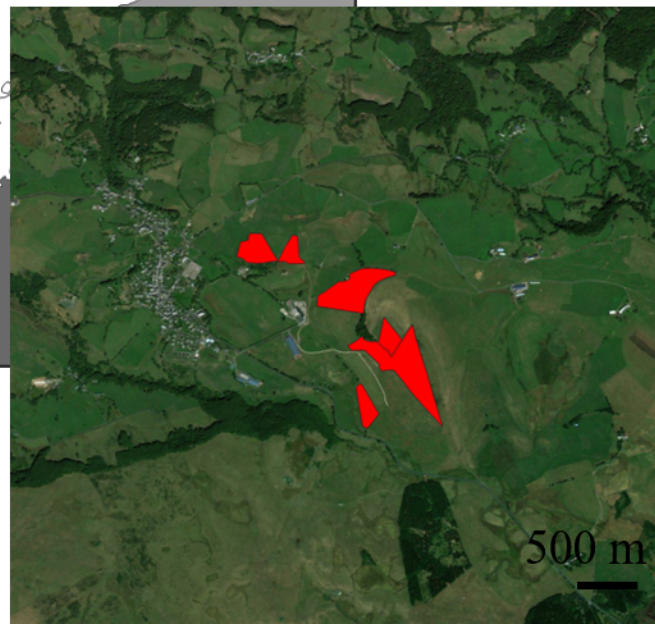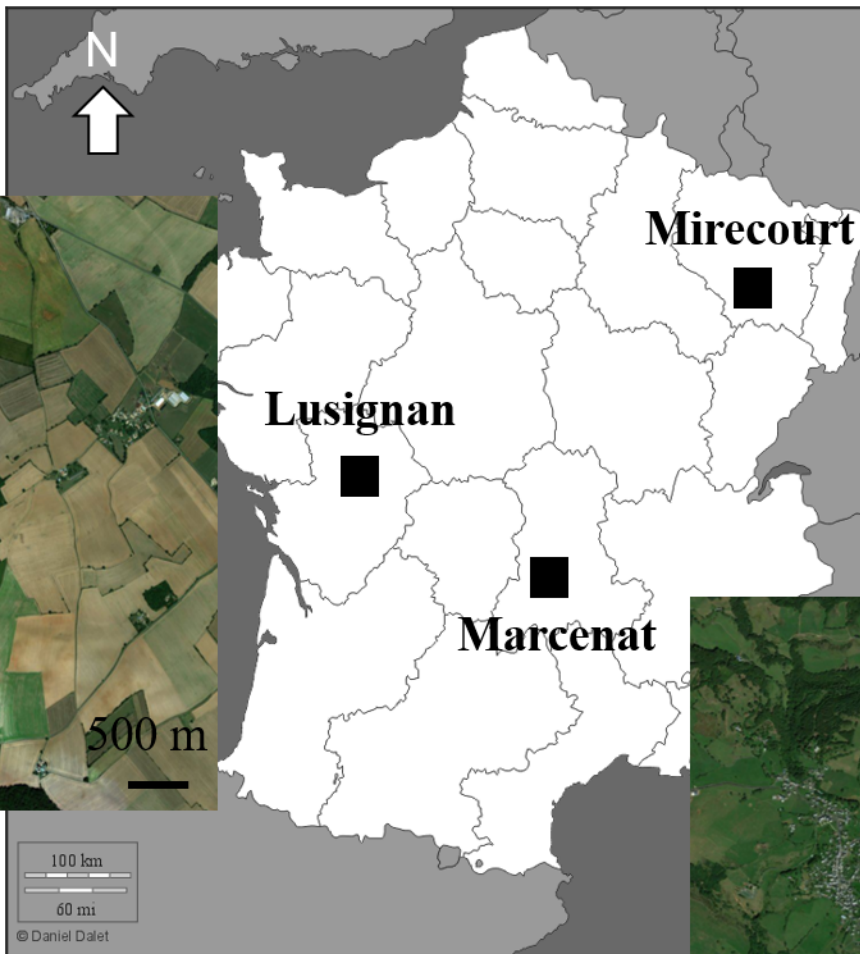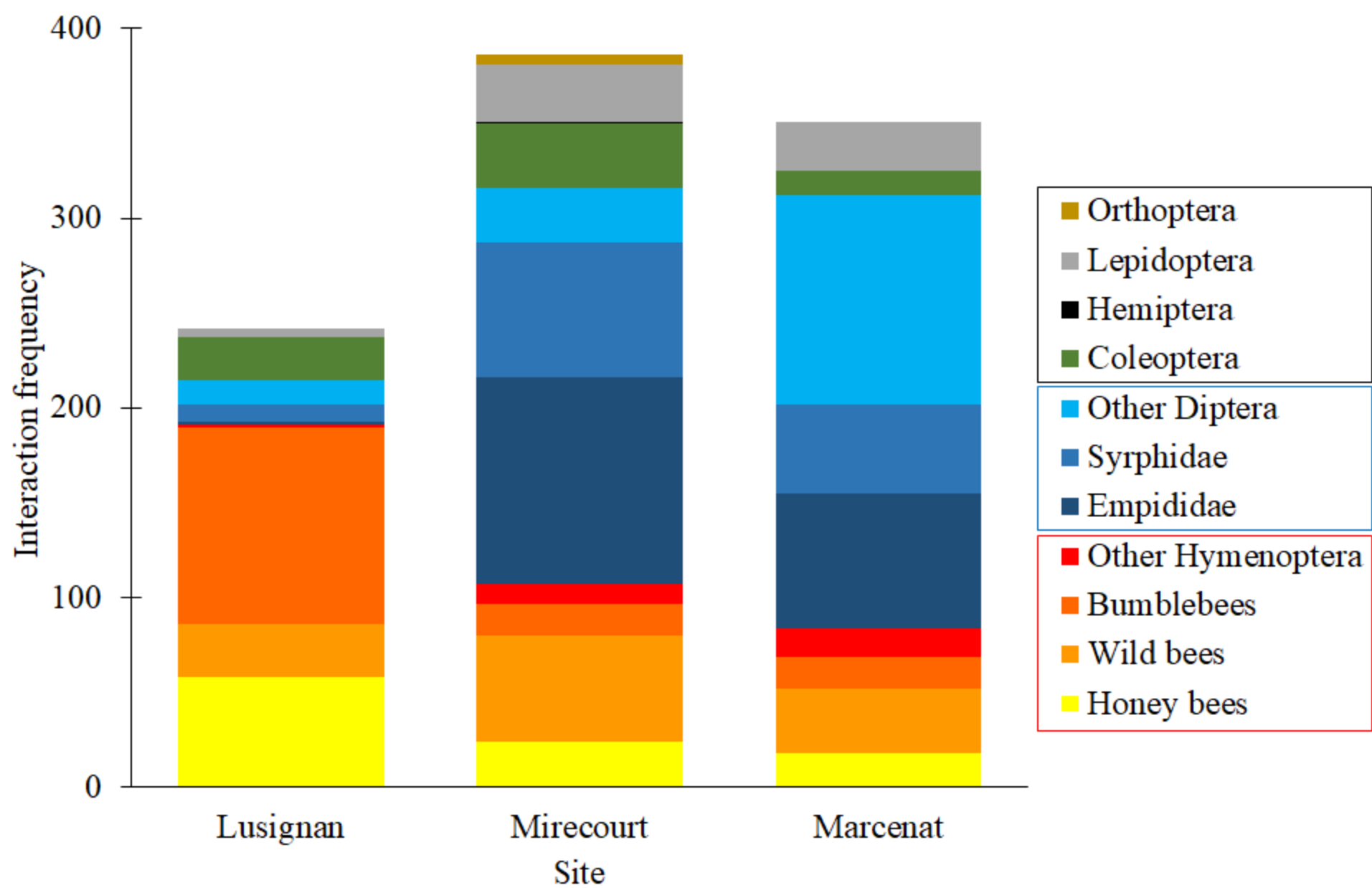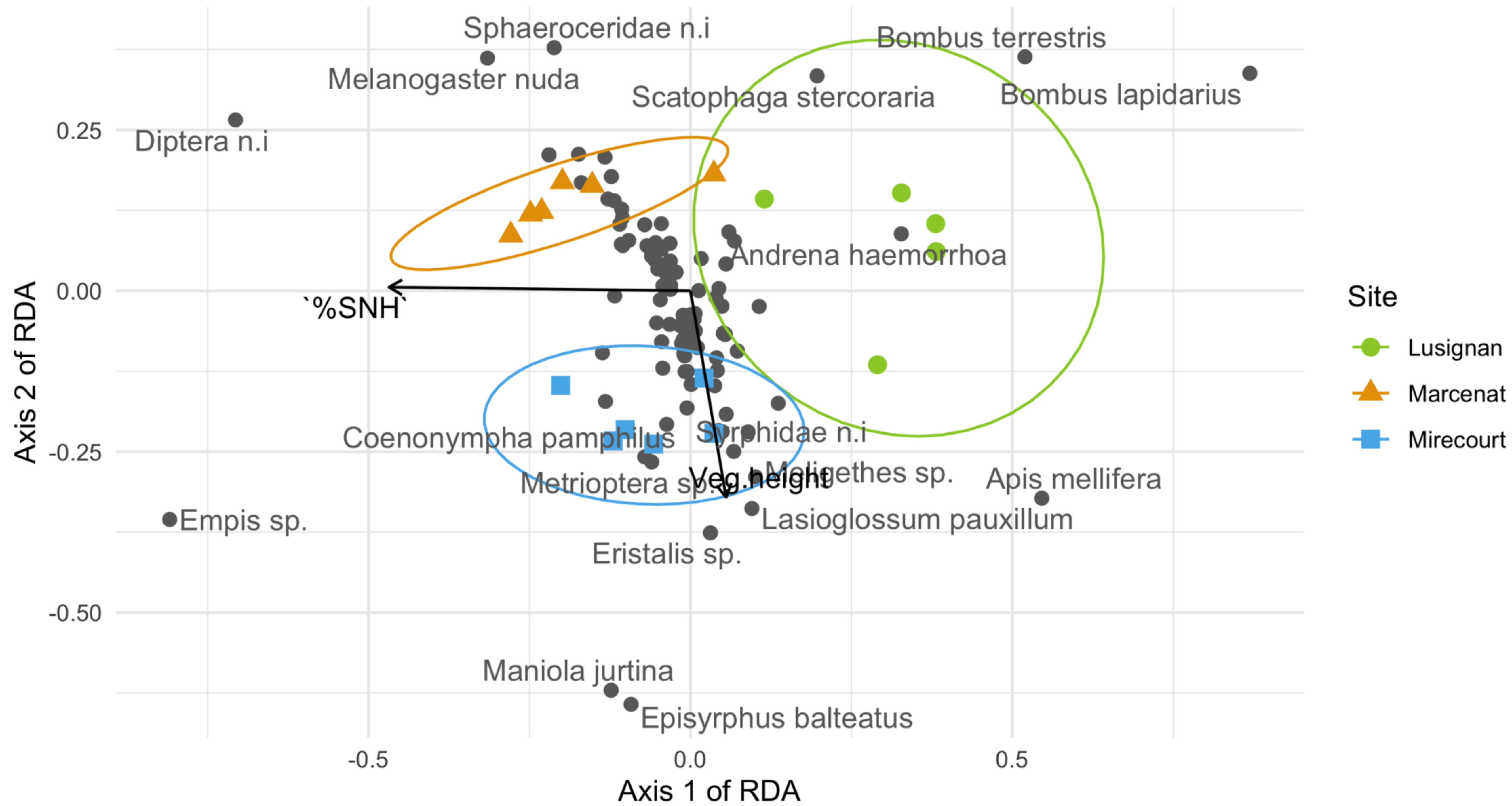
Cichorium intybus

Cirsium palustre

Crepis sp.

Malva sylvestris

Medicago sativa

Phleum pratense

Ranunculus sp.

Taraxacum sp.

Trifolium repens
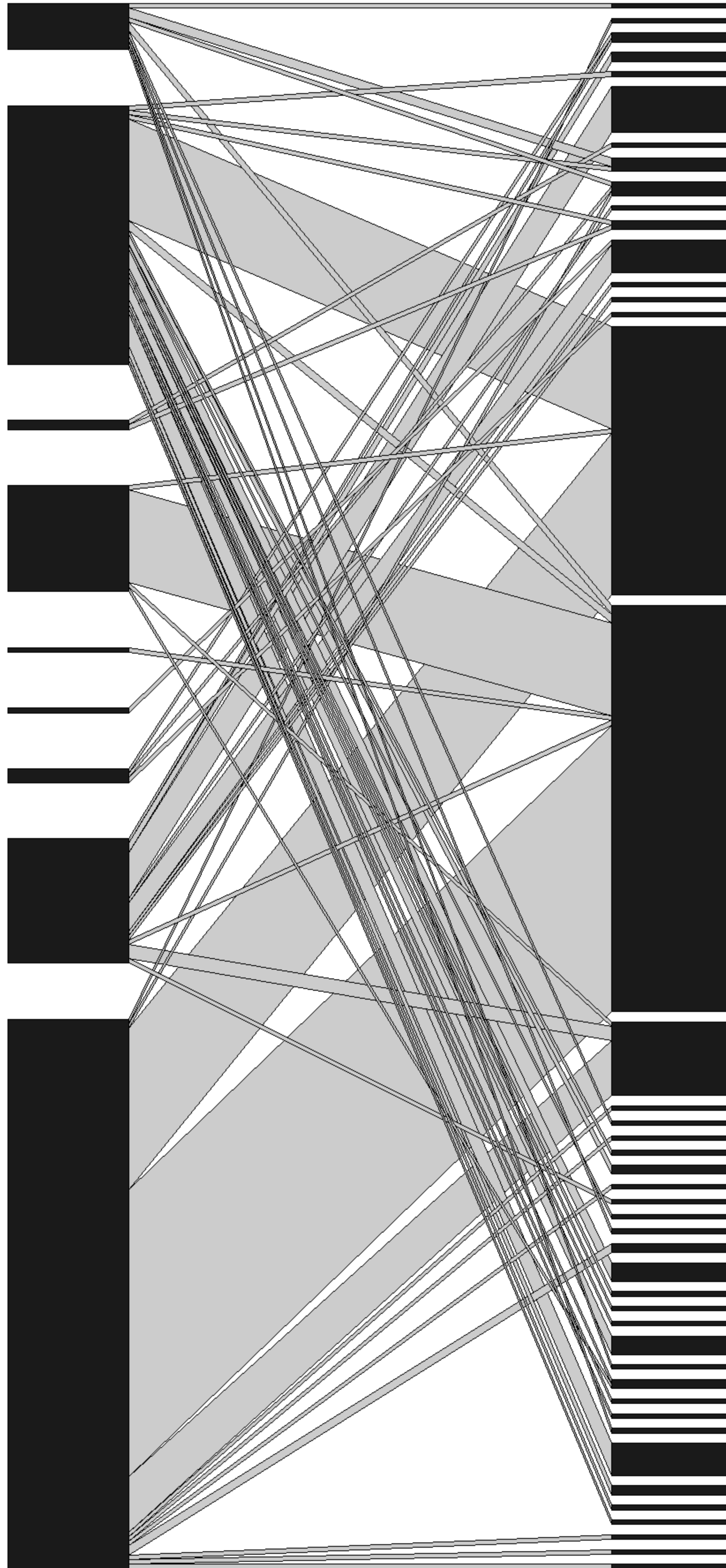
Cheilosia sp.
Chrysotoxum sp.
Diptera n.i
Empis sp.
Eristalis sp.

Scatophaga stercoraria

Sicus ferrugineus
Sphaerophoria sp.

Syrphidae n.i
Andrena cineraria
Andrena flavipes

Andrena 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
Nymphalidae n.i
Thymelicus lineolus
Tyta luctuosa
Metrioptera sp.
Miridae n.i