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

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21

22 **Abstract**

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

44

45 **Keywords:** motifs; mutualistic networks; pollen analysis; pollen network; species roles; visit  
46 network.

47

48 **Data accessibility:** The data analysed during the current study will be available in Zenodo upon  
49 acceptance or at the reviewers' request.

50

## 51 **1. Introduction**

52 Plant-pollinator interaction networks are critical to the maintenance of ecosystems (Ashworth et al.,  
53 2009; Bronstein et al., 2006; Memmott, 2009; Vázquez et al., 2009). Pollinators indeed provide an  
54 invaluable service, on which much of current agriculture depends (Deguines et al., 2014; Gallai et  
55 al., 2009; Klein et al., 2007), and they maintain genetic diversity in plant populations (Kearns et al.,  
56 1998). Reciprocally, wild plants provide various resources to pollinators, usually food and other  
57 type of nutrients, hence maintaining pollinator populations (Bronstein et al., 2006; Kearns et al.,  
58 1998; Ollerton, 2017). Understanding the structure and functioning of these networks (i.e. how  
59 species interact and how these interactions shape species abundance dynamics) and obtaining more  
60 accurate information on plant-pollinator networks are among the current important goals of  
61 ecology. Thus, it is essential to manage and maintain insect pollination - which constitutes an  
62 ecosystem service of global importance – because disruption of interactions can affect the diversity,  
63 abundance and distribution of both plants and pollinators, with cascading consequences affecting  
64 the whole network (Gill et al., 2016). Most plant-pollinator networks are based on direct  
65 observations of contact between an insect and a flower in the field. However, some links are  
66 biologically (*i.e.* morphologically) or temporally (*i.e.* phenologically) forbidden, while other links  
67 can remain unobserved (Olesen et al., 2011). Thus, such visit-based networks can only represent a  
68 subsample of all possible interactions. Alternative methodologies or more intense sampling can  
69 reduce the probability of missing some existing interactions. One such alternative method is the  
70 identification of pollen found on pollinator bodies.

71 Pollen is a major attractant for many pollinators since it is an important part of their diet (Kearns  
72 and Inouye, 1993). Moreover, it can favour long-term associative learning in wild bees (Muth et al.,  
73 2016), influencing the floral choice of pollinators and their foraging strategy (Somme et al., 2015).  
74 As a result of this “visitation activity”, *i.e.* when pollinators visit a flower, pollen becomes attached  
75 to their bodies. Thus, it becomes a natural marker indicating the recent history of pollinator visits  
76 (Jones, 2012a) since a significant part of the pollen grains generally stay on the pollinator’s body.  
77 The identification of this pollen provides valuable information on the spectrum of pollen resources  
78 and it is an important method to elucidate the foraging behaviour and the floral preferences of wild  
79 pollinators, such as solitary and social bees (Beil et al., 2008; Carvell et al., 2006; Fisogni et al.,  
80 2018; Marchand et al., 2015), hoverflies (Lucas et al., 2018a; 2018b, Rader et al., 2011), butterflies  
81 and other pollinators (Macgregor et al., 2019; Stewart and Dudash, 2016). Pollen is also often used  
82 to assess pollinator effectiveness both at the community level (Ballantyne et al., 2015; King et al.,  
83 2013; Willmer et al., 2017) and at the individual level (Marchand et al., 2015; Tur et al., 2015).  
84 Indeed, not all the visits recorded in the field correspond to actual pollination (King et al., 2013;

85 Popic et al., 2013) and not all the pollinators are equally efficient. For example, not all pollen grains  
86 transported by corbiculated-bees are available for the pollination event, since the moistening (using  
87 nectar) may cause physiological changes in the pollen grain (Parker et al., 2015).

88 The identification of pollen found on insect bodies can be used as an alternative method to study  
89 plant-pollinator interactions (Jones, 2012b). This methodology can provide a more extended history  
90 record of plant-pollinator interactions than the observation of visits. Moreover, observing pollen  
91 grains rather than visits removes some of the sampling biases associated with short sampling  
92 periods and can provide an alternative view to the ‘plant’s perspective’ provided by the observation  
93 of visits (Bosch et al., 2009; Gibson et al., 2011; King et al., 2013). However, few studies have  
94 compared visit-based networks to pollen-based ones (Alarcón, 2010; Bosch et al., 2009; Olesen et  
95 al., 2011; Pornon et al., 2017, 2016), mostly because the identification of pollen grains is time-  
96 consuming and depends on the availability of experts with skills in palynology. The precision of  
97 pollen identification depends on knowledge of the floral community in the study sites (Westrich and  
98 Schmidt, 1990), thus suggesting that the use of a complete pollen atlas of the co-flowering species  
99 of the study site, as we used in the present study, may enhance the precision of identification. An  
100 alternative method to microscopic identification that recently garnered interest is the use of DNA  
101 barcoding (Bell et al., 2019, 2017; Macgregor et al., 2019; Pornon et al., 2017, 2016; Richardson et  
102 al., 2015). It is, however, a recent methodology not widely used in the study of plant-pollinator  
103 networks and it can have some limits (Bell et al., 2017; Macgregor et al., 2019).

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

116 The aim of this study is to understand to what extent networks obtained using pollinator visit  
117 records can introduce biases in the representation of the network when compared to those obtained

118 through pollen identification. For a constant sampling effort, we could expect that richer  
119 communities are more likely to be undersampled than poorer communities. Then, the addition of  
120 pollen information can lead to changes in the network structure and species roles (*i.e.* the set of  
121 positions occupied by a species in the network), since apparent specialised species may be more  
122 generalist than observed and thus separate groups of species may be more connected, revealing a  
123 biased picture of the network. To test these hypotheses, we used simulated networks mimicking the  
124 ones obtained through observation of insect visits but based on the pool of possible interactions  
125 given by the pollen-based network. In a sense, these randomized networks can be considered as  
126 different “virtual observers” sampling from all the possible interactions detected using the pollen on  
127 insect bodies, but with a sampling effort equal to that used in the field. This technique allowed us to  
128 compare two networks of the same size and to check for congruence between networks. Armed with  
129 this methodological framework, we studied the plant-pollinator networks encountered in three  
130 different calcareous grasslands in three different regions in France. We compared the two types of  
131 networks (visit- and pollen-based networks) using a new methodological approach combining  
132 different analyses. First, we compared the network structure using latent block models (LBM) and  
133 motif analyses (Leger et al., 2015; Simmons et al., 2019a). Second, we compared species  
134 specialisation level (Blüthgen et al., 2006) and species roles, based on the frequency of species  
135 positions within motifs (Simmons et al., 2019a).

## 136 **2. Materials and methods**

### 137 *2.1. Study sites and plant inventories*

138 In this study we recorded interaction between wild bees and native herbaceous plant species in three  
139 calcareous grasslands located in three different French regions (Fig. A.1): one in Hauts-de-France  
140 (Regional natural reserve Riez de Noeux les Auxi, noted R, 50°14'51.85"N 2°12'05.56"E), one in  
141 Normandie (Château Gaillard – le Bois Dumont, noted CG, 49°14'7.782"N 1°24'16.445"E) and one  
142 in Occitanie (Fourches, noted F, 43°56'07.00"N 3°30'46.1"E). We chose calcareous grasslands since  
143 they are characterised by highly diverse plant communities with a high proportion of entomophilous  
144 species (Baude et al., 2016; Butaye et al., 2005; WallisDeVries et al., 2002). The three sites are  
145 three protected areas of 1 hectare each, which are included in the European NATURA 2000  
146 network. We sampled wild bees and we recorded their interactions with flowering species during  
147 one-day sessions in the month of July 2016 (see paragraph 2.3 for sampling details). Flowering  
148 plants were identified at the species level in the field and their abundances recorded. All plant  
149 inventories were performed by the same two surveyors to avoid biases.

## 150 2.2. *Pollen atlas*

151 During each field session we sampled plant anthers of all species flowering within the study site.  
152 We put the anthers in individual Eppendorf tubes filled with 70% ethanol to preserve them. From  
153 this collection, we prepared a pollen atlas representative of the pollen diversity present in the three  
154 areas. In the laboratory, we extracted and transferred the pollen released by anthers in the  
155 Eppendorf tube on a microscopic slide mounted with a cube of glycerine jelly (Kaiser's Glycerol  
156 Gelatine for microscopy) to maintain the natural colour of the pollen grains, and we sealed the  
157 cover slip with nail varnish. For each slide we recorded the plant species and the site and date of  
158 collection, and we photographed the pollen grain as reference.

## 159 2.3. *Direct observations of plant-pollinator interactions in the field*

160 For this study, we recorded plant-pollinator interactions for three days (one day per site) in the  
161 month of July 2016, since it was one of the richest months in terms of plant and pollinator diversity.  
162 Surveys of plant-pollinator interactions were performed under suitable weather conditions for  
163 pollinators (following Westphal *et al.* 2008). The surveyors (from 4 to 5 at each session) walked  
164 slowly and randomly within the site (following a variable transect as explained in Westphal *et al.*  
165 2008) and hand-net sampled all wild bees visiting open flowers, recording the observed plant-  
166 pollinator interaction. The sampling period consisted of 4 hours split into 2 hours in the morning  
167 (about 10am-12am) and 2 hours in the afternoon (about 2pm-4pm), to cover the daily variability of  
168 pollinator foraging behaviour (Vaudo *et al.*, 2014) and flower communities. All sampled insects  
169 were immediately put into individual killing vials with ethyl acetate and were later prepared and  
170 pinned in the laboratory for identification at the species level by expert taxonomists. For some  
171 individuals we recorded and attributed several interactions, since they were observed interacting  
172 with more than one plant species before we were able to collect them.

## 173 2.4. *Bee pollen load analysis*

174 We focused on wild bees (superfamily: Apoidea, clade Anthophila) because, with their hairy bodies  
175 and their ability to quickly fly from one flower to another, they are one of the most efficient  
176 pollinator groups worldwide and many herbaceous plants and wild flowers depend on them for their  
177 reproduction (Ballantyne *et al.*, 2017; Michener, 2000; Stavert *et al.*, 2016). Moreover, wild bees  
178 present different specialized structures for pollen collection which allow them to transport large  
179 amount of pollen to feed their larvae (Alarcón, 2010; Michener, 2000). Pollen was collected from  
180 the bodies of female bees and prepared on two different microscope slides as follows: one slide with

181 the pollen passively transported on the body (scattered pollen, PS), and the other slide with pollen  
182 actively collected in specialised structures (*i.e.* corbiculae or scopae, PC). Since we want to  
183 compare the information provided by PS and PC slides, we only used female bees because males  
184 lack adapted structure to carry pollen, such as corbiculae or scopae, (and hence cannot provide PC).  
185 We collected PS from insect bodies using a small cube of glycerine jelly (volume 2 mm<sup>3</sup>) following  
186 Kearns and Inouye (1993). PC was removed by brushing the specialised structures with a small  
187 needle or a small spoon and put in an Eppendorf tube filled with 70% ethanol for conservation.  
188 Only a fraction of PC (10 µl) was used to prepare pollen slides. We prepared a total of 782 pollen  
189 slides, considering both types of pollen and with information on sampling date, hour and site  
190 (Fourches 346 slides, Chateau Gaillard 256 slides and Riez 180 slides). Pollen identification was  
191 performed at the lowest taxonomic level (at species level in 90% of the cases) by an expert (K. Bieri  
192 at the *Biologisches Institut für Pollen analyses*, Kehrsatz, Switzerland) using a combination of  
193 diagnostic keys and comparison with the pollen atlas described above. When it was not possible to  
194 discriminate between two closely related species, we aggregated them at higher categories (family,  
195 genus or morphotype). Microscope slides were observed at 400x magnification by random transects  
196 until we counted 100 pollen grains, then the rest of the slide was searched for undetected pollen  
197 types. However, for the statistical analyses we did not consider plant species for which we detected  
198 ≤ 5 pollen grains per bee individual, which we considered as infrequent or accidentally collected  
199 (Bosch et al., 2009; Fisogni et al., 2018).

## 200 2.5. Characterisation of plant-pollinator interactions

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

206 We divided plant species in three groups: (a) plant species which were present in the study area  
207 (and included in the botanic inventory) and that were visited by pollinators; (b) plant species which  
208 were present in the study but whose interaction with pollinators was detected only by pollen  
209 analysis; (c) plant species present only in the surroundings of the study sites but not within them  
210 (and whose interaction with pollinators was detected only by pollen analysis). Plant species which  
211 were present in the study area but were never visited by pollinators and whose pollen we did not  
212 find on the insect bodies (*i.e.* plant species with no interactions) were excluded from the analysis



213 altogether, and group (c) was not used for the purpose of comparing networks obtained from direct  
214 observation of visits and pollen identification. Results of this classification were represented using a  
215 heat map (function *quilt.plot* in R, see Supplementary Information Fig. A.2, A.3, A.4).

216 Prior to conducting the rest of the analyses, we tested whether the information provided by the two  
217 types of pollen (PS and PC) was different. To do so, we compared the two interaction-based  
218 rarefaction curves (Fig. A.5) using a Wilcoxon test. We found that there was not significant  
219 difference in the number of observed links between PS and PC, even if the percentage of unique  
220 links was higher for PS than for PC (results not shown). Thus, we decided to merge the information  
221 given by the two pollen types and we further refer to them as “pollen-based network” in the  
222 following analysis.

## 223 2.6. Plant-pollinator network analysis

224 We constructed two weighted (*i.e.* quantitative) bipartite networks including all pairs of interacting  
225 plant and insect species (i) directly observed as visits in the field (“visit-based” network) or (ii)  
226 retrieved from the pollen found on insect bodies (“pollen-based” network). Overall, we built three  
227 visit-based networks and three pollen-based networks (using the two types of pollen – PC and PS –  
228 found on insects), one for each site.

229 Raw networks were weighted networks accounting for the intensity of interactions between species  
230 pairs – in the case of visits, intensity equals the number of recorded visits of the focal pollinator  
231 species on the focal plant species; in the case of pollen identifications, intensity equals the number  
232 of insects of the focal pollinator species found with at least 5 pollen grains from the focal plant  
233 species. For some analyses (connectance, motifs and position analyses) we transformed weighted  
234 networks into binary ones.

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

239 To model compartmental structure within networks, we applied latent block models (LBM) to each  
240 network, visit-based, simulated or pollen-based. We used the *BM\_poisson* method for Poisson  
241 probability distribution implemented in the *blockmodels* package (Leger et al., 2015) to calculate  
242 blocks on the weighted networks. The algorithm finds the best groupings of insects and plants that

243 maximize the Integrated Completed Likelihood (ICL; Biernacki *et al.* 2000; Daudin *et al.* 2008) of  
244 a model that fits the intensity of interactions between each pair of species as a Poisson draw with a  
245 parameter defined by the blocks each species belong to.

246 While the LBM approach reveals consistent species groups in complex networks, it neither informs  
247 on how species interact within each block, nor does it seek regularities in the arrangement of links  
248 within a small group of species. Such information can be obtained by counting the number of motifs  
249 observed within networks (Simmons *et al.*, 2019b, 2019a). Motifs are defined by Simmons *et al.*  
250 (2019a) as “building blocks” of the network, *i.e.* patterns of possible interactions between a small  
251 number of species. If we compare the network to a toy brick house, motifs would be the “building  
252 bricks” with different sizes, shapes and colours that can be used to build the house. Motifs contain  
253 between two (one pollinator and one plant species) and six species. Motifs do not only consider  
254 direct interactions, but they also consider indirect ones, when the impact of one species on another  
255 is mediated by one or more intermediary species (Wootton, 2002, 1994). To calculate how  
256 frequently different motifs occurred in our networks, we used the function *mcount* implemented in  
257 the new package *bmotif* in R (Simmons *et al.*, 2019b) and normalized these values using the  
258 maximum number of times each motif could have occurred given the number of species in the  
259 network (correction “*normalise\_nodesets*”).

## 260 2.7. Insect roles and specialization index

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

266 We also calculated the standardized specialization index  $d'$  (Blüthgen *et al.*, 2006), but we did not  
267 use the  $d'$  values provided by the *dfun* function in the bipartite package (Dormann *et al.*, 2009) as  
268 they sometimes yielded spurious results based on the computation of the minimal  $d$  value (*e.g.*  
269 reporting low  $d'$  for species with only one partner in the network). However, we used the  $d$  and  
270  $d_{max}$  values, obtained from the *dfun* function, and we calculated the  $d'$  index, for each plant and  
271 insect species, as the ratio of the  $d$ -value (Kullback-Leibler divergence between the interactions of  
272 the focal species and the interactions predicted by the weight of potential partner species in the

273 overall network) to its corresponding *d*<sub>max</sub>-value (maximum *d*-value theoretically possible given  
274 the observed number of interactions in the network).

## 275 2.8. Comparing network structure and species roles using a null model

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

289 To gauge if the network structure changed between the two networks, we compared the results of  
290 LBM and motif analyses between visit-based networks and simulated ones. Then, to detect whether  
291 species roles changed between networks, we compared results on specialization and node positions.  
292 Including connectance, H2, *d*’, NMI, motifs and positions, we performed 197 tests in the site of F,  
293 117 tests in the site of CG and 107 tests in the site of R and we adjusted all p-values in each site  
294 using the function *p.adjust* (package *stat*) and the false discovery rate correction method of  
295 Benjamini-Hochberg (“BH” or “*fdr*”, Benjamini and Hochberg, 1995). The numbers of tests are  
296 different because we had different numbers of species in the three sites.

297 *Latent Block Model* – We performed LBM on weighted versions of the visit-based, pollen-based  
298 and simulated networks (10,000 simulations). To show the species rearrangement among groups  
299 between the visit- and pollen-based networks, we used alluvial diagrams (package *alluvial* in R). In  
300 order to assess whether changes in block memberships of species between pollen- and visit-based  
301 networks was expected due to changes in sampling intensity, we computed the congruence between  
302 the classifications given by node memberships of, first, the visit- and pollen-based networks and,  
303 then, the pollen-based network and each of the simulated networks, using the normalized mutual

304 information index (NMI), implemented as method “*nmi*” of the function *compare* in the R package  
305 *igraph* (Danon *et al.* 2005; Astegiano *et al.* 2017). NMI values range between 0 (no congruence  
306 between classifications) and 1 (perfect congruence). The distribution of NMIs obtained when  
307 comparing the blocks of the pollen-based networks and those of the simulated networks allowed us  
308 to compute the probability (p-value) that the NMI between the visit-based and pollen-based blocks  
309 was significantly less than expected from the null model. Corrected p-values less than 5% were  
310 deemed significantly inferior to the null model expectation.

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

317 *Positions* –To explore if insect and plant species had different roles in the networks based on visits  
318 vs. pollen, we calculated the frequency with which species occurred at different positions within all  
319 possible motifs of 2 to 6 species. This vector of position frequencies represented the species’ “role”  
320 in the network. We then calculated the distance of each species’ role to the centroid of all the  
321 simulated roles for the same species, and compared this distance to the distribution of distances  
322 between simulated roles and their centroid, with observed distances greater than 95% of the  
323 simulated distances deemed as significantly different from the null expectation. To account for  
324 heterogeneous variances and correlations between position frequencies (*i.e.* coordinates in species’  
325 role vectors), we used Mahalanobis distance on modified coordinates obtained by first running a  
326 principal component analysis (PCA) on the set of all roles of all species in all simulated networks.  
327 The covariance matrix used in the Mahalanobis distance was simply the diagonal matrix of singular  
328 values associated with the principal components of the PCA. The modified coordinates of the  
329 centroid and the observed role of a given species were obtained by projecting their position  
330 frequencies into the PCA space.

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

### 332 3. Results

#### 333 3.1. Characterisation of plant-pollinator interactions

334 In the month of July, we recorded a total of 96 flowering species, 63 in the site of Fourches (F), 33  
335 in the site of Château Gaillard (CG) and 32 in the site of Riez (R). However, these species were not  
336 all visited by pollinators, and those with neither visit nor pollen found on insects (12 species in the  
337 site of F, 14 in the site of CG and 16 in the site of R) were not considered in the analysis.

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

341 Visit- and pollen-based networks in the same site have comparable number of species (*i.e.* the  
342 number of insect species is fixed, but the number of plant species can vary depending on the  
343 sampling, *i.e.* visit or pollen), except in the site of Fourches: for the site of Fourches 50 insect  
344 species x 44 potential plant species (29 plant species in the visit-based network vs. 40 species in the  
345 pollen-based one); for the site of Château Gaillard 22 insect species x 18 plant species (13 in the  
346 visit- and 18 in the pollen-based networks) and for the site of Riez 19 insect species x 16 potential  
347 plant species (12 in the visit- and 15 in the pollen-based networks). For three insect species (2  
348 species in the site of CG, *Lasioglossum laticeps* and *Lasioglossum politum*, and 1 species in the site  
349 of R, *Halictus rubicundus*) which were sampled once in the visit-based network, we did not record  
350 any interaction in the pollen-based network due to the low number of pollen grains (< 5) or to  
351 interactions with plant species not included in the botanic inventory (Fig. A.3, A.4). These species  
352 were thus excluded from the analyses in the problematic sites. In the site of Fourches we recorded  
353 179 visit-based interactions and 340 pollen-based interactions; in the site of Château Gaillard we  
354 recorded 130 visit-based interactions and 228 pollen-based interactions and in the site of Riez we  
355 recorded 93 and 173 interactions in the visit- and the pollen-based networks, respectively. Overall,  
356 with the pollen information we doubled the number of interactions in all sites.

#### 357 3.2. Plant-pollinator networks analysis

358 When we compared network connectances, *i.e.* the proportion of realized links over all possible  
359 ones, we found that the pollen-based network connectance was always higher than the visit-based  
360 network connectance in the three sites (Table 1). We observed the opposite pattern for the network  
361 specialization index, since the  $H_2$  values were higher in the visit-based network than in the pollen-  
362 based one in all sites (Table 1). However, when we compared the visit-based network and the

363 simulated networks, we did not find any significant difference both for the connectance and the H<sub>2</sub>  
 364 index (Table 1).

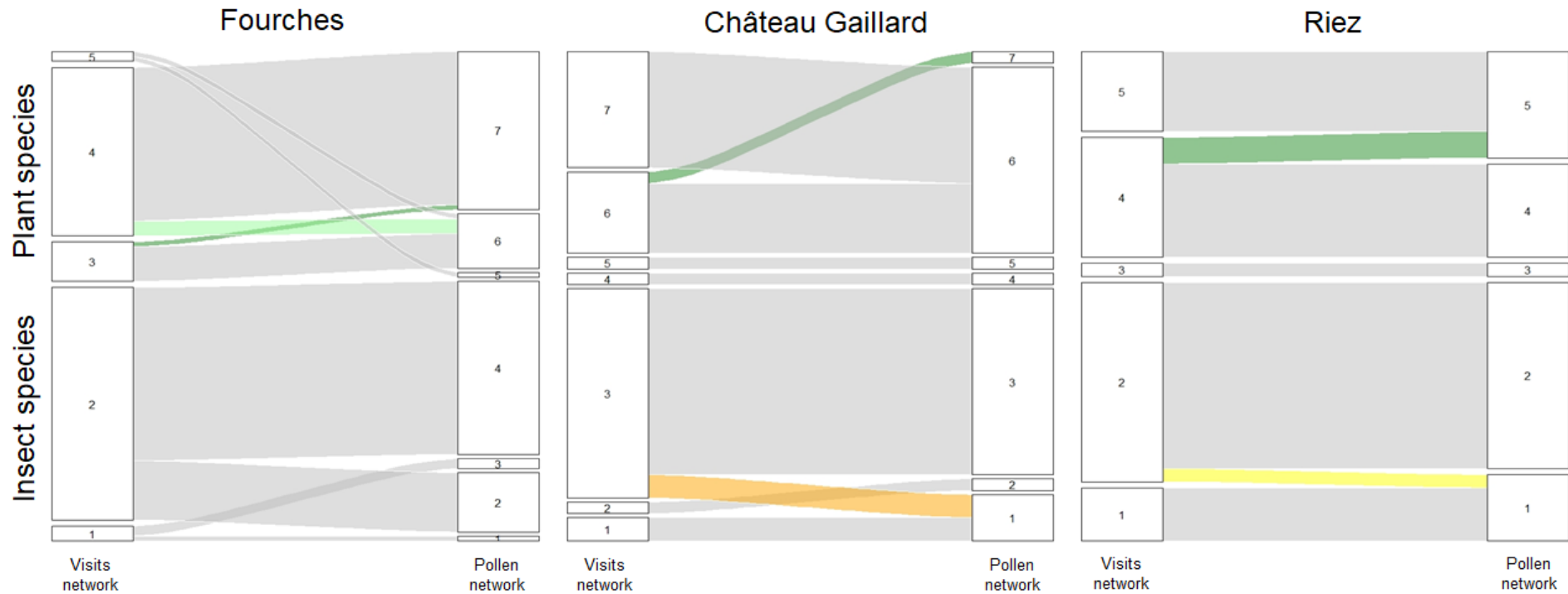
Analysis	Region	Site	Visit-based network	Pollen-based network	Simulated network	adjusted p-value	
<b>Connectance</b>	Occitanie	Fourches (F)	0.07	0.09	0.07	1	n.s
	Normandie	Château Gaillard (CG)	0.17	0.20	0.14	1	n.s
	Hauts-de-France	Riez (R)	0.18	0.22	0.16	1	n.s
<b>H2</b>	Occitanie	Fourches (F)	0.52	0.33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

365

366 **Table 1.** Results for the analyses of networks connectance and H<sub>2</sub> (network specialisation index) in the visit-  
 367 based and pollen-based networks and for the simulated networks in the three sites. The adjusted p-values  
 368 refer to the comparison of statistics between the visit-based network and the simulated networks.

369 In order to compare network structures between visit- and pollen-based networks, we performed  
 370 LBMs (Fig. 1) and compared the classification induced by latent blocks using NMI (Table 2). In the  
 371 site of Fourches, we found a total of 5 blocks (2 insect blocks and 3 plant blocks) in the visit-based  
 372 network and a total of 7 blocks (4 blocks for insects and 3 for plants) in the pollen-based network.  
 373 In the site of Château Gaillard, we found 7 blocks (4 for insects and 3 for plants) in both networks,  
 374 and a similar pattern for the site of Riez, but with 5 blocks (2 for insects and 3 for plants) in both  
 375 networks. Block clustering largely followed species degrees, *i.e.* the number of partners (high,  
 376 medium and low degree, Fig. A.6). We observed plant species rearrangements in all sites (green  
 377 lines in the alluvial diagrams), but insect block rearrangements only in the sites of CG (in two insect  
 378 species, *Andrena flavipes* and *Seladonia tumulorum*) and R (for one insect species, *Lasioglossum*  
 379 *pauillum*). Block rearrangements are mainly due to the higher number of links in the pollen-based  
 380 network but not to substantial changes in the network structure. The higher number of blocks found  
 381 in the pollen-based network in the site of Fourches (Fig. 1) is due to the occurrence of two new  
 382 blocks in the group of insects: the first block in the visit-based network (constituted by three species  
 383 with the highest degrees, Fig. 1 and Fig. A.6 Fourches visits) split in two blocks in the pollen-based  
 384 network (blocks 1 and 3, Fig. 1 and Fig. A.6 Fourches pollen); and the fourth block in the visit-  
 385 based network (Fig. 1 and Fig. A.6 Fourches visits) also split in two other blocks of species  
 386 (respectively with species with medium and low degree in the pollen network, Fig. 1 and Fig. A.6  
 387 Fourches pollen). Even if we found species rearrangements among groups between the visit- and  
 388 pollen-based networks in all three sites (Fig. 1), the network structures were not intrinsically  
 389 different. When we compared the congruence between the memberships of species in the visit- and

390 pollen-based networks using the NMI, we obtained NMI values close to 1 (perfect congruence) in  
391 all sites (Table 2). Moreover, we did not find any significant difference when we compared these  
392 NMIs with those obtained from comparisons of the pollen-based network and each of the simulated  
393 networks.



394

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



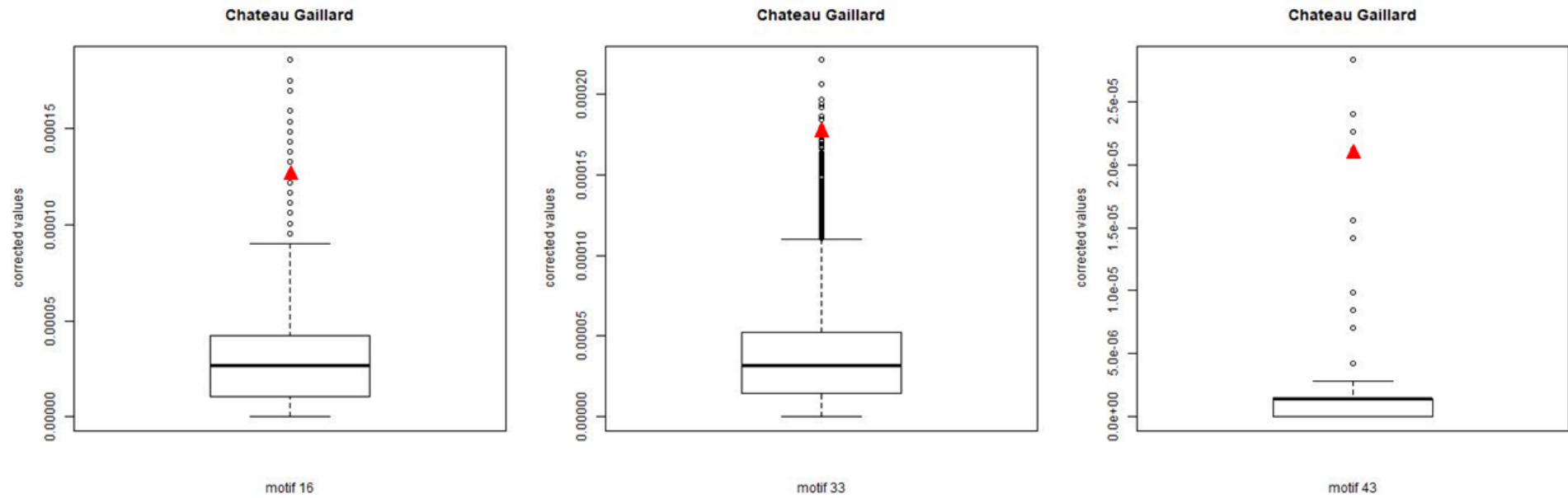
401

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

402

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

408 In general, when we compared the network structure using the motifs, we did not find important  
 409 differences between the visit-based network and the simulated networks. We did not find any  
 410 significant difference when we compared the frequency of each motif in the visit-based network to  
 411 the corresponding frequencies in the simulated networks in the site of Fourches and Riez. However,  
 412 we found significant differences for three motifs (motifs 16, 33 and 43; see Fig. 3 in Simmons *et al.*  
 413 2019a) in the site of Château Gaillard. All three motifs were less represented in the simulated  
 414 networks than in the visit-based network (Fig. 2). Motif 16 is constituted by 5 nodes (*i.e.* species)  
 415 and 6 links, with two species of pollinators (on the top level) and three species of plants (bottom  
 416 level), while motifs 33 and 43 are constituted by 6 nodes and 7 links, with three pollinators and  
 417 three plants species in the motif 33 and two pollinators and four plant species in the motif 43. In  
 418 motifs 16 and 43, all species of one group interact with all species in the other group, thus all the  
 419 possible interactions between the two groups of species are realised. In motif 33 there are two  
 420 pollinators out of three that are generalist species, *i.e.* they interact with all the plant species, while  
 421 the third pollinator is a “specialist” species which interacts with only one “generalist” species in the  
 422 plant group. All the plant species are “generalist” species, but only one plant species interacts with  
 423 all the partners in the pollinator group, while the other two species only interact with two  
 424 pollinators.



425

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

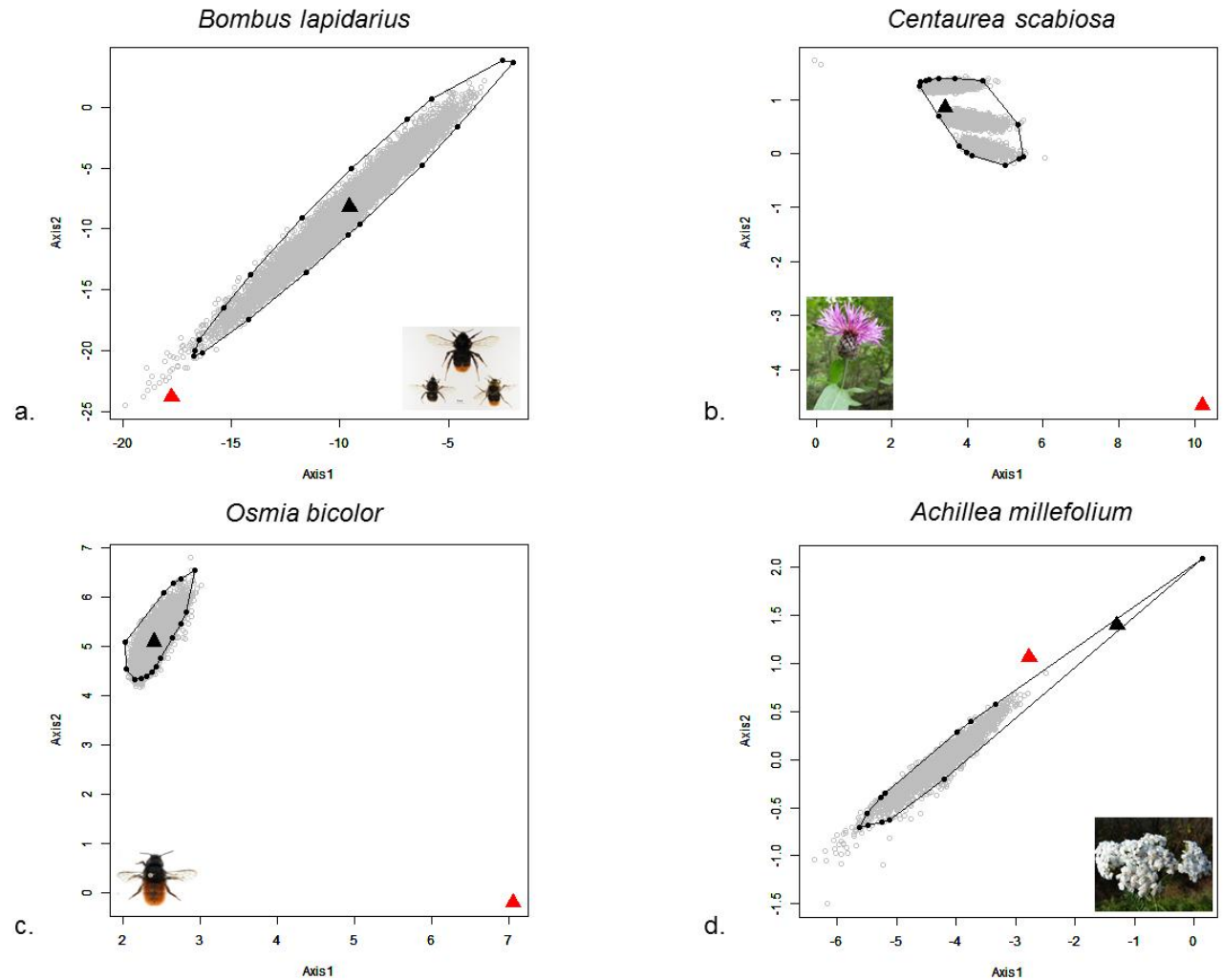
428 3.3. Insect roles and specialization index

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

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

441 We also compared for each species the specialization index  $d'$  calculated in the visit-based network  
442 to the average  $d'$  of the simulated networks. We found that the specialization of most species was  
443 not significantly different in the two networks in all sites (Tables A.1, A.2, and A.3). We recorded  
444 significant differences in the specialization level for 8 species in the site of F (five insect and three  
445 plant species, with two insect and two plant species more specialized in the simulated networks), for  
446 two species in the site of CG (one insect and one plant species, both appearing more specialized in  
447 the simulated networks) and for five species in the site of R (three insects species out of four more  
448 specialized in the simulated networks and one plant species).

449 Overall, nearly half of the species for which we found significant differences in their node positions  
450 or/and in the specialization level were singletons (13 species out of 27) in the visit-based or pollen-  
451 based network, *i.e.* species that had only one observed interaction (Tables A.1, A.2 and A.3). Only  
452 four species out of the 27 (one insect and one plant species both in the sites of CG and R, Tables  
453 A.2 and A.3) showed significant differences in both their role and specialization level.



454

455 **Figure 3.** The PCA plot shows the significant distance (adjusted p-value < 0.05) along principal axes 1 and 2, between the visit-based position (red triangle), the  
 456 simulated centroid (black triangle) and the convex hull (black lines and dots) obtained on the 95% of the simulated positions which were close to the centroid  
 457 (grey dots) in the randomized network, in four examples of species among the 13 species that showed significant differences:(a) *Bombus lapidarius* and (b)  
 458 *Centaurea scabiosa* in the site of Château Gaillard (Normandie) and in (c) *Osmia ruforhirta* and (d) *Achillea millefolium* in the site of Riez (Hauts-de-France).  
 459 The visit-based distance was greater than 95% of all the simulated distances. Photo credits: Atlas Hymenoptera and Acta plantarum.

#### 460 **4. Discussion**

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

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

476 As expected (Bosch et al., 2009), our results show that pollen identification increases the number of  
477 observed links and always yields richer and more connected networks (Table 1), independently  
478 from the site richness and diversity, since in all the sites we doubled the number of links when using  
479 the pollen information. Nevertheless, the pollen-based links often confirm the links observed in the  
480 field (Alarcón, 2010; Popic et al., 2013). We did not find any significant change in any of the study  
481 sites when we compared network structures between visit-based and simulated networks. This  
482 finding, which holds for all three sites, partially invalidates the hypothesis that richer communities  
483 (here, the site of Fourches) would lead to more pronounced differences between networks obtained  
484 by the two methods. However, we found changes in the species roles for some insect and plant  
485 species.

486 Although we observed that the use of pollen data increased the number of interactions (we doubled  
487 the number of interactions in all sites), pollen information mostly increased the number of links for  
488 abundant and already highly connected species (number of links  $> 5$  in the visit-based networks,  
489 Tables A.1, A.2 and A.3), while for rare (singletons) and not abundant species we recorded few  
490 interactions even in the pollen-based network. Since visit-based network construction is essentially  
491 pollinator-based (and not plant-based), the information given by pollen found on insects is  
492 especially useful to add links to plant species that were not observed in the visit-based network

493 (plant species with no links in the visit-based network, Tables A.1, A.2 and A.3). Indeed, block  
494 rearrangements are observed more often in plants than in insects (Fig. 1 and A.6). However, block  
495 changes in the LBM representation did not correspond to changes in species position.

496 Block rearrangements are influenced by the number of links and the species degree, *i.e.* the number  
497 of partners with which a species interacts, but it neither informs on species role nor specialization.  
498 For example, the singleton species *Prunella vulgaris* in the site of Riez clustered in the same block  
499 (block 4) in both visit- and pollen-based networks (Fig. 1). Nevertheless, it was the only plant  
500 species for which we observed a significant change in its role and specialization degree in this site.  
501 In the visit-based network, this species was found in interaction with only one insect species (Fig.  
502 A.6, Riez visits), *Ceratina cyanea*, in a one-to-one interaction (“direct interaction”), and thus only  
503 in position 1 (in motif 1). Conversely, in the pollen-based network, even if *P. vulgaris* always  
504 interacted only with *C. cyanea* (Fig. A.6 Riez pollen), *C. cyanea* interacted with two new plant  
505 species (*Trifolium repens* and *Centaureum erythraea*). Thus, the specialization for *C. cyanea*  
506 changed significantly. Moreover, the specialization level and the role of *P. vulgaris* also changed  
507 significantly since in the pollen-based network its interaction with *C. cyanea* was affected indirectly  
508 by two other plant species, which may be potential competitors. Moreover, all the new positions of  
509 *P. vulgaris* in the pollen-based network, were “unique” in more complex motifs, *i.e.* *P. vulgaris*  
510 interacted with one generalist insect species that had other interactions with other plants (Simmons  
511 et al., 2019a). Similarly to *P. vulgaris*, *Achillea millefolium*, in the same site, was a singleton in the  
512 visit-based network while it gains one link in the pollen-based network. This new interaction was  
513 observed with *Lasioglossum pauxillum* which was a “super-generalist” species (visiting 11 plant  
514 species). Therefore, the role of *A. millefolium* changed significantly (Fig. 3d) through possible  
515 indirect interactions with new potential competitors. These examples show that indirect interactions,  
516 *i.e.* the impact of one species on another mediated by other intermediary species, are important to  
517 give a more complete picture of the species’ role when comparing networks, especially when  
518 accounting for singleton species in the visit-based network.

519 We also found changes in species roles for 6 species which were more connected in the visit-based  
520 network, *i.e.* with more than 5 observed interactions, such as *Bombus lapidarius* and *Centaurea*  
521 *scabiosa* in the site of Château Gaillard (with 20 and 8 interactions, respectively; Fig. 3a, b, Table  
522 A.2). In “complex” motifs where all species are generalists in both groups and all interact together,  
523 changes in species roles through indirect interactions are expected to be stronger than in “simple”  
524 motifs which are composed of specialist species that affect each other indirectly via their effect on  
525 one generalist species (Simmons et al., 2019a).

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

548 Since the focus of our study was to compare representations of networks borne out of two different  
549 methods (observation of pollen vs. observation of visits), but taken at equal sampling intensity, our  
550 results do not comprise the obvious differences seen in raw pollen vs. visit comparisons, *i.e.* that  
551 more detailed approaches (such as pollen-based network building). We did not find any significant  
552 changes in network structures once the intensity of sampling was taken into account, but we  
553 observed important changes at the species level in all the three sites. Indeed, we found differences  
554 both in species role and specialization in a few species, as also evidenced by other studies  
555 (Ballantyne et al., 2015; Lucas et al., 2018b). We showed that non-significant change in network  
556 structure can mask more subtle changes of species roles and specialisation level. However, these  
557 changes are in part observed in singleton species such as *P. vulgaris* in the site of Riez, which  
558 showed a low number of links both in the visit- and in the pollen-based networks. Singleton species  
559 are expected even in well-sampled communities, since they are often considered as rare species

560 accounting for rare interactions (Bascompte and Jordano, 2013; Novotný and Basset, 2000).  
561 Moreover, in our study we focused on wild bee species, but pollination networks are also composed  
562 of other pollinator species (Bosch et al., 2009; Lucas et al., 2018b; Pornon et al., 2016). Hoverflies,  
563 beetles, butterflies and moths, and ants can carry less important amount of pollen grains than bees  
564 (Alarcón, 2010), due to their low hairiness, but can nonetheless influence the network structure and  
565 the comparison between visit-based and pollen-based networks.

566 To conclude, our results suggest that more detailed sampling, obtained from pollen found on insect  
567 bodies, does not lead to the construction of an intrinsically different network, independently from  
568 the site richness and diversity. Almost all of the significant differences are found at the species  
569 level, not at the network structure level, with singleton species accounting for half of these species-  
570 level differences. Overall, this suggests that recording plant-pollinator interactions from pollinator  
571 visit observation is enough to provide a satisfactory representation of the network structure.  
572 However, the use of pollen can provide a more exhaustive image at the species level, highlighting  
573 important changes in species role and specialization, especially for studies investigating pollinator  
574 effectiveness and/or dealing with scarce pollinators. Since pollen identification is a time-consuming  
575 endeavour, new methods such as DNA-barcoding might simplify and accelerate pollen  
576 identification in the future if improved with new specific (regional or local) botanic databases.

577

578 **Author contributions:** NDM and FM conceived the project. NDM, NH, YP, BS and MZ  
579 conducted the fieldwork and provided the data. MZ and NMD prepared the insect slides and ES  
580 prepared the Pollen Atlas. PM provided part of the pollen identification and conducted the  
581 preliminary analyses. NDM conducted the analysis and prepared the manuscript. CM helped with  
582 analyses. FM supervised the analysis and edited the manuscript. NH, YP and BS contributed to all  
583 later versions.

584

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596

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