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**Title:** Phylogenetic, functional and taxonomic responses of wild bee communities along urbanisation gradients

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## 1 **Introduction**

2 The last century has witnessed a widespread loss of diversity due to the anthropisation of  
3 natural habitats (Barnosky et al., 2011; Ceballos et al., 2015; Dirzo et al., 2014). Terrestrial  
4 insects have suffered a drastic decline (Hallmann et al., 2017; Wagner et al., 2021), and  
5 pollinators are no exception (Biesmeijer, 2006; Duchenne et al., 2020; Ollerton et al., 2014;  
6 Powney et al., 2019; Zattara and Aizen, 2021). Pollinator decline has been closely coupled  
7 with the intensification of agriculture and its management practices as well as with the  
8 increasing urban development, both of which result in an effective loss of natural habitats for  
9 pollinator's forage and nesting sites (Duchenne et al. 2020; Ollerton et al. 2014; Potts et al.,  
10 2010; Vanbergen et al., 2013; Winfree et al. 2009). By 2050, approximately two-thirds of the  
11 world's population will be living in urban areas, and this population increase will inevitably  
12 increase the metropolitan land area (Seto et al., 2012). In Europe, although a modest reduction  
13 over the last decade, land use for urban purposes still amounted to 711 km<sup>2</sup>/year between  
14 2012-2018 (European Environment Agency, 2019).

15 Wild bees are essential for reproducing many wild plants and crops of high economic interest  
16 (Klein et al., 2007; Ollerton et al., 2011). With the loss of natural habitats, a growing concern  
17 in the potential resilience of cities to host a certain degree of pollinator biodiversity has  
18 emerged (reviewed by Buchholz and Egerer, 2020; Wenzel et al., 2020). This interest in urban  
19 ecosystems has the additional advantage of bringing unique opportunities to increase citizen  
20 engagement with nature and biodiversity conservation (Stevenson et al., 2020).

21 The quality of the urban environment for sustaining bees depends on multiple biotic and  
22 abiotic factors. It worsens with the increase in impervious surfaces and the reduction in green  
23 spaces, leading to decreased habitat amount and further landscape fragmentation (reviewed in  
24 Baldock, 2020; Harrison and Winfree, 2015). These effects, combined with less studied  
25 drivers such as the urban heat island (Fisogni et al., 2020; Hamblin et al., 2017), non-native

26 plants (Koyama et al., 2018; Lentola et al., 2017; Staab et al., 2020), pesticides and other  
27 pollutions including chemical, light, noise, fumes and heavy metal residues (reviewed in  
28 Phillips et al., 2020) might have varying effects on bee populations. The response of wild bee  
29 communities to the degree of urbanisation ranges from those species showing a positive or  
30 neutral response (Baldock et al., 2019; Baldock et al., 2015; Hall et al., 2017) to those  
31 showing a deleterious effect (Banaszak-Cibicka and Żmihorski, 2020; Choate et al., 2018;  
32 Fortel et al., 2014; Geslin et al., 2013; Geslin et al., 2016; Pereira et al., 2021). In addition to  
33 impacts on species richness, urbanisation can induce compositional changes filtering out wild  
34 bee communities depending on the species functional traits (Banaszak-Cibicka and  
35 Żmihorski, 2020; Fortel et al., 2014; Geslin et al., 2016). Identifying the mechanisms involved  
36 in differences in species composition can indicate whether urbanisation allows the  
37 establishment of specific urban species (i.e. spatial turnover, which is the replacement of  
38 some species by others from sites to sites) or whether it filters species from a richer regional  
39 pool (i.e. nestedness, a pattern characterized by the poorest sites being a subset of the richest  
40 ones).

41 The environment where a species lives can be favourable to it on the basis of abiotic and  
42 biotic factors (Cohen et al., 2021; Gámez-Virués et al., 2015; Hamblin et al., 2017; Hung et  
43 al., 2019). Since these environmental factors differ between urban and peri-urban  
44 environments, cities will exert a different environmental filter for wild bees than peri-urban or  
45 agricultural environments. These factors will determine the potential ecological niche of a  
46 species, since what is beneficial to some species may be detrimental to others (Bartomeus et  
47 al., 2013; Tucker and Rehan, 2018; Williams et al., 2010). As a result the combination of  
48 functional traits exhibited by each bee species will determine their ability to persist in an  
49 urban or peri-urban environment. (Banaszak-Cibicka and Żmihorski, 2012; Bartomeus et al.,  
50 2013; Buchholz and Egerer, 2020; Geslin et al., 2016; Harrison et al., 2018; Normandin et al.,

51 2017). The growing understanding of the relationships between bee functional traits and urban  
52 attributes is essential for establishing conservation and management practices in urban spaces  
53 (reviewed in Buchholz and Egerer, 2020). The resilience of cities to perturbations and their  
54 ability to provide pollination services could therefore be inferred from the study of the  
55 functional diversity of wild bee communities (Banaszak-Cibicka and Dylewski, 2021;  
56 Buchholz et al., 2020).

57 Since functional traits evolve in response to the environmental context, species with a shared  
58 evolutionary history are expected to have similar functional traits (Losos, 2008; Wiens &  
59 Graham, 2005). Urban-induced species loss is expected to be non-uniform in the phylogeny of  
60 the community and depend on the suitability of certain functional traits to the urban  
61 environment. In this sense, those traits with a phylogenetic signal are expected to be shared  
62 and conserved in urban communities, thus conditioning the presence of some lineages in  
63 response to land-use changes. The extent to which changes in land use impact the  
64 evolutionary history of wild bee communities has been studied in the agricultural context  
65 (Grab et al., 2019). However, few studies have addressed the effect of urbanisation on the  
66 phylogenetic diversity of wild bees (Harrison et al., 2018).

67 The objective of our study was to investigate the effects of urbanisation on the multiple  
68 components of wild bee diversity sampled through a replicated and standardized design of 29  
69 sites located along urbanisation gradients of three medium-sized cities in the Loire valley  
70 (France). A functional and phylogenetic community approach was used to infer the effect of  
71 the urban landscape on wild bees. We benefited from the availability of wild bee published  
72 time-calibrated molecular phylogenies (Cardinal and Danforth, 2013; Peters et al., 2017) and  
73 a DNA barcode library for the studied wild bee community (Villalta et al. 2021). Specifically,  
74 we addressed the following questions : (i) does urbanisation lead to a decline in the abundance  
75 and richness of wild bee communities? (ii) Does urbanisation induce changes in the

76 composition of wild bee communities, and what is the mechanism most involved in site  
77 dissimilarities? (iii) Does urbanisation favour wild bees on the basis of their biological traits?  
78 And (iv) does it lead to a loss of functional and phylogenetic diversity?

79

## 80 **Methods**

### 81 1. Study sites

82 We conducted the insect sampling in three mid-sized cities of the Loire valley (Orleans, Tours  
83 and Blois) located in the administrative region Centre-Val de Loire in France. The city  
84 populations are 45000 in Blois, 115000 in Orleans and 136000 inhabitants in Tours. The  
85 area's climate is temperate, with persistent rainfalls even during the driest months of the year.  
86 The average temperature and precipitation per year in the three cities are similar, around 12  
87 °C and 650 mm, respectively. The Loire riverbanks offer a relatively homogeneous local  
88 habitat to set up the sampling facilities while moving away from each city's centre. We chose  
89 six sampling sites in Blois, 11 sites in Orleans and 12 in Tours. The number of plots was  
90 chosen according to the percentage of urbanisation, which differs between cities. The  
91 sampling sites varied in their urban area's extent (Fig. 1; Table S1).

### 92 2. Bee sampling and identification

93 Bees were collected at each of the 29 monitoring sites using a combination of pan traps and  
94 net sampling (O'Connor et al., 2019). Three triplets of coloured (yellow, blue and white UV-  
95 reflecting) pan traps spaced at 50 m apart were placed at each sampling site for an exposure  
96 time of 96 h, repeated for 5 sessions from April 23rd to July 17th 2018. At the end of each  
97 trapping session, the traps were lifted and filtered. Bees were conserved in 96° ethanol and  
98 stored at -20°C until identification. Pan trapping was completed with five netting sessions of  
99 ten minutes each on a 100m transect at the end of each trapping session.

100 All 2944 wild bee specimens collected were pinned and photographed. Bee identification was  
101 based on a combination of morphology and DNA barcodes of 2419 wild bees published in a  
102 recent study (Villalta et al., 2021). In addition, we also used these published COI barcodes to  
103 calculate bee genetic diversity (see below).

### 104 3. Landscape composition

105 To characterize land uses in a 500m-radius landscape around sampling sites, we used land  
106 cover maps OSO 2017 (Inglada et al., 2017; [https://www.theia-land.fr/ceslist/ces-occupation-](https://www.theia-land.fr/ceslist/ces-occupation-des-sols/)  
107 [des-sols/](https://www.theia-land.fr/ceslist/ces-occupation-des-sols/)) which were generated from Landsat 8 to Sentinel-2 into a 10m resolution land use  
108 database for the whole of metropolitan France. We choose a 500m radius to keep buffer zones  
109 surrounding sites as entities independent of each other while maximizing the landscape area  
110 attributed to each point and encompassing the average flight distance of most wild bees as in  
111 (Geslin et al., 2016). From the 17 land classes provided by OSO 2017 and after photo-  
112 interpretation of each site to validate the assignment of the surfaces, we determined the area  
113 of six simplified land uses of interest towards insects at a 500m radius surrounding sites,  
114 including urban landscape cover (Table S2). Based on their land-use composition, sites were  
115 also classified as “urban” vs “peri-urban” (Fig S1) following Ward's hierarchical clustering  
116 (hclust function) carried out on principal components (prcomp function) from R Statistical  
117 Software (v4.1.2; R Core Team 2020).

### 118 4. Local floral resources

119 We recorded the flowering vegetation present locally on each site in June 2018 by arranging  
120 75 quadrats (50 x 50 cm) so that 25 quadrats at 5m apart were placed around the three  
121 sampling triplets (except when arranging the quadrat was too dangerous such as in riverbanks  
122 or roads). Since the triplets were arranged in open vegetation made up of herbaceous plants  
123 showing little covering, quadrats could be photographed from the top for later identification  
124 of flowering plants and count of floral units. A floral unit was defined as a single flower head

125 or part of multiple heads, from which a medium-sized bee has to fly rather than walk to reach  
126 another floral unit of the same species (Dicks et al., 2002). The sum of floral units of all  
127 species per square meter was used as a proxy of local floral abundance. In addition to the  
128 species encountered in the quadrats, the flowering plants present locally were identified to the  
129 species or morphospecies level. Plant species richness was used as a proxy of local floral  
130 diversity.

## 131 5. Insect metrics

132 Our data set comprises each sampling site unit, including the pooled data from all three pan  
133 traps and net samplings per study site and all sampling periods in 2018.

### 134 5.1 Taxonomic diversity

#### 135 5.1.1 *Abundance and species diversity*

136 For each sampling site, we have recorded the abundance (n) and the number of species (nsp)  
137 for the whole dataset and by family. In addition to the species richness, we estimated bee  
138 diversity through the Simpson diversity index (Simpson, 1949) to account for the species  
139 presence and their relative abundance in sampling sites and the Chao1 diversity index (Chao,  
140 1984) to account for the diversity of undetected species. Both indices were calculated with the  
141 R vegan package v. 2.5 6 (Oksanen et al., 2020).

142

#### 143 5.1.2 *Genetic diversity*

144 We used 2419 barcodes out of 2944 collected specimens obtained from a recently published  
145 study (Villalta et al., 2021) to calculate the total number of haplotypes (nhap) and the number  
146 of haplotypes per family at each sampling site. The number of COI haplotypes per species  
147 corresponds to the number of unique sequences that differ from others at one or more base  
148 pairs. It was calculated with the R Pegas package v.0.14 (Paradis, 2010). They were summed

149 to calculate the total number of haplotypes and the number of haplotypes per family at each  
150 sampling site.

151

## 152 5.2. Community compositional changes

153 We visualized the dissimilarity in the species composition of wild bee communities using  
154 Non-metric Multidimensional Scaling (NMDS; metaMDS function of the R vegan package v.  
155 2.5-6, Oksanen et al., 2020) based on Bray-Curtis distances for the abundance weighted  
156 community matrices. We tested whether dissimilarity was explained by the urban landscape  
157 class through Permutational Multivariate Analysis of Variance with 999 permutations  
158 (PERMANOVA; adonis function of the R vegan package). We checked that geographic  
159 distance between sites did not significantly explain dissimilarity by carrying out a Procrustes  
160 analysis using the protest function of the R vegan package .

161 We used the “nestedbetasor” function of the R vegan package to evaluate the contribution of  
162 nestedness and turnover to community dissimilarity. Overall community dissimilarity was  
163 evaluated by calculating the Sørensen dissimilarity, split into the turnover component  
164 evaluated by calculating the Simpson dissimilarity and the nestedness-resultant component  
165 (Baselga, 2012).

166 To explore the pattern by which species would be lost across the urban gradient, we also  
167 calculated the abundance weighted NODF (Nestedness metric based on overlap and  
168 decreasing fill) of the bee communities ordered along the urbanisation gradient (nestednodf  
169 function from the bipartite package; Dormann et al., 2008) which was then compared to what  
170 would be expected by chance using null models, as in Grab et al., 2019 (File S1).

171 The species involved in the partition of sites in urban vs peri-urban (Fig. S1) were identified  
172 through the “multippatt” function of the R indicpecies package (De Cáceres et al., 2011).

173 Beta diversity analyses were carried out with the R *vegan* package v 2.5-6 (Oksanen et al.,  
174 2020) for the overall wild bee community data and separately by city (results by each city in  
175 File S2).

176

### 177 5.3 Functional traits

178 Functional traits of bee species (Table S3) were selected according to the possible response to  
179 floral resource availability, landscape composition and bee social behavior. They included  
180 female body size, duration of activity per year (flight period length in months), sociality  
181 (*sensu stricto*) regarding the presence of a reproductive caste (i.e., ‘solitary’, ‘social’,  
182 ‘polymorphic’, ‘social parasite’ or ‘kleptoparasite’), nesting structure (‘ground’, ‘cavities’,  
183 ‘dead stems or ‘carder’), diet (‘opportunistic’ or ‘specialised’) and voltinism (“univoltin”,  
184 “bivoltin” and “partly bivoltin”). All functional traits were retrieved from the literature (Table  
185 S3) and completed in some cases by expert's evaluation.

186 Changes in the functional composition of wild bee communities were measured by computing  
187 at each site the community-level weighted means of trait values (CWM) and functional  
188 dispersion (FDis from the dbFD function, FD package in R, Laliberté and Legendre, 2010) for  
189 each quantitative trait separately and by calculating trait frequencies for nominal or binary  
190 traits.

191

### 192 5.4 Phylogenetic tree

193 Representative COI sequences for each species (153 species out of 156) in the study area  
194 (Table S4; File S3) were aligned with Geneious v. 8.0 (Kearse et al., 2012) using the MAFFT  
195 plugin (Katoh, 2002) with the auto option and other parameters set as default. BEAUTI  
196 v.1.8.1 (Drummond et al., 2012) was used to create input files for BEAST v.1.8.1  
197 (Drummond et al., 2012). Time-calibrated phylogeny construction details can be found in File

198 S1. The maximum clade credibility newick tree file obtained (File S4) was used to calculate  
199 the subsequent metrics related to phylogenetic diversity.

200

### 201 5.5 Functional and phylogenetic diversity

202 Three different metrics of the phylogenetic and functional community structure were  
203 calculated at each sampling site: the Faiths' diversity (PD for phylogenetic diversity and FD  
204 for functional diversity), the mean nearest taxon distance (MNTD) and the mean pairwise  
205 distance (MPD). The Faiths' diversity index is related to species richness (Schweiger et al.,  
206 2008). PD represents the evolutionary relatedness within a community and is calculated by  
207 summing the total lengths of the branches of the phylogenetic tree of the species present in the  
208 community (Faith, 1992). Similarly, FD is the total branch length of the functional  
209 dendrogram (Petchey and Gaston, 2002) computed from functional dissimilarity among  
210 species based on the Gower distance. MNTD represents the mean of the branch lengths  
211 connecting each species to its closest relative in the dendrogram (Webb, 2000). The MPD  
212 index calculates the mean pairwise phylogenetic (or functional) distance among all species in  
213 a community, is equivalent to the average taxonomic distinctiveness and is independent of  
214 species richness.

215 We also used richness-independent standardized effect sizes for PD and FD (hereafter sesPD  
216 and sesFD), MPD (hereafter sesMPD) and MNTD (hereafter sesMNTD) metrics using a null  
217 model for each city that maintains species richness and abundance constant while  
218 randomizing species from the set of species occurring in at least one community of the city  
219 transect (sample.pool option) 999 times through the “ses.pd”, “ses.mpd” and “ses.mntd”  
220 functions of the R Picante package v.1.8.2. (Kembel et al., 2010).

221

222 6. Statistics

223 We tested the effect of landscape land-use variables at 500 m radius surrounding sites and  
224 local floral resources on above insect metrics using generalised linear mixed effects models  
225 (GLMMs) with the R package glmmTMB (Brooks et al., 2017), using the city as a random  
226 factor. Since some land-use variables were correlated (Table S5), we included the proportion  
227 of urban surfaces and uncorrelated variables only, such as the proportion of water, the  
228 proportion of forests and the proportion of sandbanks and dunes at a 500m buffer. Local floral  
229 resources factors included the scaled ‘local floral abundance’ and ‘local floral richness’.  
230 Haplotypic and taxonomical analyses were carried out for (i) the entire dataset and (ii)  
231 separately for each of the six bee families.

232 From the list of models with combinations of fixed effect terms in the global model, we  
233 selected the top best model (dredge and get.models functions of the R MuMIn package,  
234 Bartoń, 2019) on the basis of the second-order Akaike Information Criterion (AICc; Anderson  
235 and Burnham, 2004). Poisson error distribution (or negative binomial when overdispersion  
236 was detected) with a log link function were used for count data (abundance, number of  
237 species and number of haplotypes). Gaussian error distribution with an identity link function  
238 were used for the following variables species diversity indices (Simpson and Chao1),  
239 functional and phylogenetic diversity indices (PD and FD, MTD, sesPD and sesFD, sesMPD  
240 and sesMNTD) and quantitative trait-related indices (CWM and FDis). Gaussian error  
241 distribution with a log link was used for the MNTD metrics. Betabinomial error structures  
242 with a logit link function were used to analyse the shifts in the frequencies of categorical traits  
243 (nesting, sociality, lectism, parasitism, voltinism). To validate the goodness of the fit and the  
244 assumptions of each GLMM, simulated residuals were plotted using the ‘*simulateResiduals*’  
245 function in the ‘DHARMA’ package (Hartig, 2020).

246

## 247 **Results**

248 1. Wild bee community description

249 Over the year of the survey, a total of 2944 wild bee specimens were collected, 647 from  
250 Blois (85 species), 1673 from Orleans (115 species) and 624 from Tours (82 species). They  
251 were assigned to six families, 25 genera and 156 species. The Halictidae was the most  
252 abundant family and represented 50% to 71% of samples depending on the city (Table S6).

253 Out of 2944 specimens, 382 belonging to 75 species were netted, of them only 16 species  
254 were exclusively caught by netting. Eleven out of these sixteen species were represented by  
255 singletons (10) and doubletons (1). Given that the abundance estimates for pan trap and  
256 netting samples could be very different we assessed whether analysing the data with and  
257 without considering netting samples had any impact on the results obtained before using the  
258 complete database.

259 The species accumulation curve reaches saturation, indicating that we captured most of the  
260 species in our study area (Fig. S2). Notwithstanding, the haplotype accumulation curve did  
261 not reach saturation, thus reflecting an incomplete capture of the genetic diversity in our  
262 sampling with a total number of 666 haplotypes (Andrenidae 122, Apidae 92, Colletidae 28,  
263 Halictidae 375, Megachilidae 41, Melittidae 8).

264

265 2. Does urbanisation lead to a decline in wild bee abundance and richness?

266 Overall wild bee abundance was not explained by the urban landscape cover or any other  
267 landscape factor (Table 1; Fig. 2). A significant reduction of both Melittidae and  
268 Megachilidae bee abundance associated with the urban landscape cover was found (Table 1;  
269 Fig. S3).

270 The overall species richness and the Simpson and Chao1 indices of wild bees responded  
271 strongly to the urban landscape cover. (Table 1; Fig. 2). They showed a significant decrease  
272 with the increase in the proportion of urban landscape cover (p-value <.001), revealing a

273 negative effect of urbanisation on wild bee taxonomic diversity, whether we consider  
274 dominant or account for undetected species. Since the effect of urban landscape cover on wild  
275 bee species richness remains significant even after the inclusion of abundance as a co-variable  
276 (estimate= -0.877191, se=-0.191015, p-value = 4.38e-06), this indicates that this trend is  
277 independent of the sample size. As expected, the proportion of water surrounding sites  
278 negatively influenced the species richness of collected wild bees (but not the Simpson and  
279 Chao1 indices) since water also constitutes an impervious surface. The drop in the overall  
280 species number with the proportion of urban landscape cover is mainly driven by a significant  
281 reduction in Andrenidae and Halictidae species (Table 1; Fig. S3). This overall species  
282 reduction is not concomitant with a significant decrease in the total number of haplotypes  
283 (Table 1). Despite the variation in haplotypes number for each family mirrored the  
284 abundances trends, we did not find a significant relationship between urban landscape cover  
285 and the number of haplotype in any family (Table 1; Fig. S3).

286 Local floral abundance and plant species richness did not significantly influence overall wild  
287 bee abundance or taxonomic richness. Floral abundance was, however, positively associated  
288 with the Chao1 diversity index, with the abundance of Halictidae and Apidae as well as with  
289 the number of haplotypes in Halictidae. Plant species richness was only positively associated  
290 with the number of haplotypes in Apidae (Table 1).

291

292 3. Does urbanisation induce changes in species composition of wild bee communities?

293 The NMDS reveals strong dissimilarity in species composition between urban vs peri-urban  
294 classes (Fig. 3a). Bee communities from the urban sites are taxonomically different from the  
295 peri-urban ones (Permanova,  $F(1,29)=2.31$ , p-value = 0.003). From Procrustes analysis,  
296 geographic and community dissimilarity matrices are not significantly correlated (p-value =

297 0.177, Procrustes correlation= 0.415), indicating that species community dissimilarity is not a  
298 function of the Euclidean distance between communities.

299 The decomposition of overall community dissimilarity in turnover and nestedness  
300 components (0.875 and 0.039, respectively) indicates that species turnover is the main  
301 contributing factor in community dissimilarity overall (Fig. 3b).

302 We found that the ordered matrix's observed value of weighted NODF is significantly lower  
303 than the nestedness values obtained with null models ( $t=66.14$ ,  $df=99$ ,  $p\text{-value}<2.2e-16$ ),  
304 confirming that the ordered matrix does not present a nested structure. Hence, urban sites do  
305 not contain a subset of species from the more complete communities present in peri-urban  
306 landscapes but seem to harbour a set of urban species distinct from the peri-urban ones.

307 Nine species were found to be involved in the partitioning of sites into urban and peri-urban  
308 classes. Species associated with urban sites are *Lasioglossum laticeps*, *L. nitidulum* and  
309 *Andrena pusilla*. In contrast, species associated with peri-urban sites are *L. zonulum*, *Halictus*  
310 *maculatus* *A. haemorrhoea*, *Dasypoda hirtipes*, *L. marginatum*, and *Panurgus calcaratus*. Beta  
311 diversity analyses carried out separately on each city reveal similar results (File S2).

312

313 4. Does urbanisation favour wild bees on the basis of their biological traits?

314 We found a significant negative relationship between the urban landscape cover and the  
315 community weighted mean of body size of wild bees (Table 2, Table S7), suggesting that  
316 smaller species dominate urban wild bee communities compared to periurban communities  
317 although the functional dispersion of body size remains similar. The community weighted  
318 mean of bee size also significantly decreased with the proportion of water and increased with  
319 forests. A significant increase in the community weighted mean of the duration of activity  
320 with urban landscape cover and local floral richness was also found (Table S7), suggesting  
321 that urban wild bee communities have a broader period of activity compared to periurban ones

322 (Table 2). This relationship was however depended on the netted samples, since the  
323 relationship disappeared when netted individuals were excluded from analysis. The frequency  
324 of solitary bees significantly decreased with an increase in urban landscape cover and local  
325 floral abundance (Table 2; Table S7). Contrary to our expectations, the frequency of ground-  
326 nesting bees was not significantly influenced by the urban landscape cover (Table 2). Also,  
327 none of the frequency of specialist and univoltine bees showed a significant response to urban  
328 landscape cover (Table S7).

329

330 5. Do favoured traits lead to a loss of functional and phylogenetic diversity?.

331 The functional and phylogenetic Faith diversity (FD and PD) of wild bee communities were  
332 significantly reduced by an increase in urban landscape cover (Table S8, Fig. 2). This  
333 reduction was accompanied by a significant increase in the functional and phylogenetic  
334 MNTD but not in MPD (Table S8), suggesting that urbanisation did not influence functional  
335 nor phylogenetic diversity when decorrelated from species richness. Moreover, we did not  
336 find any significant relationship between the urban landscape cover and functional or  
337 phylogenetic sesPD, sesFD, sesMPD or sesMNTD (Table S8, Fig. 2).

338

## 339 **Discussion**

340 We investigated the effect of urbanisation on the taxonomic, functional and phylogenetic  
341 diversity of wild bees in three medium-sized cities along the Loire river in France. The wild  
342 bee community sampled was relatively diverse, with 156 species representing about 60 % of  
343 the regional bee fauna (Villalta et al., 2021).

344 The total abundance recorded in our study was not related to the urban landscape cover.  
345 However, consistent with previous research we observed a drop in species richness (44 %) and  
346 taxonomical diversity indices with increasing urban landscape cover (Banaszak-Cibicka

347 and Źmihorski, 2020; Bates et al., 2011; Geslin et al., 2013; Geslin et al., 2016; McCune et  
348 al., 2020; Hostetler and McIntyre, 2001).

349 In addition to changes in species diversity, cities as well as and peri-urban sites seem to select  
350 taxonomically and functionally different communities from an overall species pool. While  
351 Banaszak-Cibicka and Źmihorski (2020) found a nested structure in their wild bee  
352 communities, indicating that urban sites have a subset of species from the more complete  
353 communities present in rural and suburban landscapes, here it seems that urban sites harbour a  
354 set of species distinct from the peri-urban ones. In our study, the spatial turnover of species  
355 was the main contributing factor in overall community dissimilarity. *L. laticeps*, *L. nitidulum*  
356 and *A. pusilla* were the species that most represent the urban assemblage, and together with *L.*  
357 *morio* responded positively to the urban landscape cover (Table S9). These “winner” species  
358 are among the most numerous overall. Therefore, an increase in their abundance associated  
359 with urbanisation contributes substantially to reduce the differences in the total abundance  
360 between urban and peri-urban areas. On the contrary, we found *L. zonulum*, *H. maculatus*, *A.*  
361 *haemorrhoea*, *D. hirtipes*, *L. marginatum*, and *P. calcaratus* as the peri-urban related species.  
362 Shifts in bee communities towards smaller, social bees with extended flight periods were  
363 found, resulting in a modification of the functional composition of bee communities with  
364 urbanisation. The flight distance to forage is positively correlated with bee body size  
365 (Greenleaf et al., 2007). In urban areas with a more fragmented landscape, larger species  
366 would potentially benefit from foraging in patches of distant resources, and would therefore  
367 be expected to be selected. Although it is found in some studies (Birdshire et al., 2020), there  
368 is evidence across the literature that urbanisation filters for smaller species (Ahrné et al.,  
369 2009; Banaszak-Cibicka and Źmihorski, 2012; Hamblin et al., 2018) and large body-size are  
370 rare in cities (Banaszak-Cibicka et al., 2018a, 2018b; Glaum et al., 2017). In our case, small  
371 bee species seems to be favoured in urban areas, where small-bodied bees such as

372 *Lasioglossum* display shorter flight ranges. The advantage of being small in an urban  
373 environment has been related to the reduction of resources needed to survive in these areas of  
374 potentially reduced nutrient availability (Fortel et al., 2014). On the other hand, it has also  
375 been related to diverse and abundant floral patches that would ensure the foraging needs of  
376 small species without the need to fly long distances (Buchholz and Egerer, 2020). In addition,  
377 a warmer environment caused by the urban heat island effect promotes ectotherms' increased  
378 metabolic costs that are expected to drive shifts to smaller body sizes (Scheffers et al., 2016)  
379 and select for small ectotherm species with reduced metabolic costs (Merckx et al., 2018).

380 The phenology of bee populations in urban areas is closely linked with the presence of native  
381 or exotic floral resources (Wilson and Jamieson, 2019), the effect of temperature on the  
382 overwintering period of bee species (Schenk et al., 2018) and the phenology of native plants  
383 (Fisogni et al., 2020). Several studies have shown that urban environments favour wild bee  
384 species with later spring emergence dates and extended flight periods (Banaszak-Cibicka and  
385 Źmihorski, 2012; Harrison et al., 2018; Wray and Elle, 2015). In our design, we have found a  
386 predominance of bees with extended and less variable flight periods in urban areas. Unlike  
387 native flora, ornamental flora is selected for longer flowering and renewal several times a  
388 year, contributing to the establishment and selection of bee species with extended foraging  
389 periods (Harrison and Winfree, 2015).

390 Social bees were positively associated with the increase in urban landscape cover as found in  
391 other urban areas studied (Banaszak-Cibicka et al., 2018b; Banaszak-Cibicka and Źmihorski,  
392 2012; Hinnens et al., 2012). Social bees are ecologically dominant for resource foraging  
393 compared to solitary bees, thus facilitating their behavioural and ecological versatility and,  
394 consequently, their survival in urbanised environments (Banaszak-Cibicka and Źmihorski,  
395 2012).

396 We did not find differences in the dietary breadth of wild bee communities according to  
397 urbanisation. Shifts in community composition from specialist to generalist species (Deguines  
398 et al., 2016; Matteson et al., 2008) might be expected once again because of the abundance of  
399 ornamental plants in urban environments. However, there is no clear consensus in the  
400 literature as trait-based selection for specialist or neutral trends have been reported with  
401 urbanisation (Buchholz and Egerer, 2020). Finally, the expected decrease in ground-nesting  
402 bees with increasing urbanisation (Banaszak-Cibicka and Dylewski, 2021; Geslin et al., 2016;  
403 Matteson et al., 2008) was not detected in our study. Locally, the presence of bare soil,  
404 cobblestone paving and sandbanks alongside the Loire might provide nesting resources for  
405 ground-nesting bees, especially *Lassioglossum* species with colonies often found in the  
406 crevices of cracked pavements, well adapted to disturbed environments and with a rapid  
407 response to the appearance/disappearance of nesting niches. The significant increase of 3  
408 *Lasioglossum* species with urbanisation could also dilute the abundance of other soil nesting  
409 bee species in urban sites.

410 A few studies considered the functional diversity of wild bees in urban environments  
411 (reviewed in Buchholz and Egerer 2020). While functional dispersion has been found to  
412 decrease with urbanisation (Hung et al., 2019; Banaszak-Cibicka and Dylewski 2021), other  
413 studies reported functional diversity to be greater in urban gardens compared to semi-natural  
414 habitats (Martins et al., 2017), and no effects of urbanisation were detected in the studies  
415 conducted by Buchholz et al., 2020, and Zaninotto et al., 2021. In spite of several responses to  
416 urbanisation of individual traits in mean and variance in our study, they seem to be not  
417 sufficient to contribute to significant response of overall multi-trait functional metrics.

418 Along with the reduction in the number of species associated with the increase in the urban  
419 landscape area, we found a decrease in Faith's diversity (FD and PD) and an increase in  
420 MNTD while no effect on MPD, both for functional and phylogenetic dendrograms.

421 However, these effects appear to be purely due to the reduction of specific richness with  
422 increasing urbanisation. The lack of significant differences in the phylogenetic metrics  
423 (sesPD, sesMPD and sesMNTD) with urban landscape cover for observed assemblages and  
424 those expected from simulated communities indicates that the effect of urbanisation on  
425 functional and phylogenetic diversity between sites do not differ from that resulted from a  
426 random loss of species. In our study, despite the decline in species richness, species turnover  
427 contributes to maintaining taxonomically and functionally distinct communities between  
428 urban and peri-urban sites, thereby compensating to some degree for the negative effects of  
429 urbanisation on taxonomic diversity and contributing to the maintenance of functional and  
430 phylogenetic diversity.

431 The availability of floral resources has been shown to increase the diversity and abundance of  
432 bees in urban environments (Baldock et al., 2015; Bennett and Lovell, 2019; Hülsmann et al.,  
433 2015; Matteson and Langellotto, 2010; Threlfall et al., 2015) and has the potential to help  
434 compensate for the negative effects of urbanisation (Wilson and Jamieson, 2019). Despite  
435 having no significant effect on total wild bee abundance or the number of species, floristic  
436 abundance was positively associated with the Chao1 richness index. This pattern suggests an  
437 essential role of floral abundance in conserving rare bee species, either because they benefit  
438 from an increase in net floral resources or a decrease in competition. These results underline  
439 the importance of maintaining floral resources in cities to alleviate other adverse effects of  
440 urbanisation.

441

## 442 **Conclusion**

443 The analysis of bee communities in a replicated design along three urbanisation gradients has  
444 allowed us to show that urbanisation leads to a strong decline in species richness while  
445 maintaining an overall abundance. This might be explained by family-dependent responses to

446 urbanisation, with few dominant species benefiting from the urban area. The urban  
447 environment favours wild bees based on their body size, flight phenology and social structure.  
448 The trait-based approach of wild bee communities has allowed us to highlight some urban  
449 drivers potentially involved in trait filtering, including the urban heat island effect and  
450 abundance of ornamental plants. However, this trait-based filtering does not lead to a non-  
451 random loss of evolutionary history in the urban environment. The species turnover between  
452 urban and periurban areas contributes to maintaining a relatively stable phylogenetic and  
453 functional diversity when considered independently from the reduction in species number  
454 along the urbanisation gradient.

455 Our data show that the city provides a markedly different environment from peri-urban areas,  
456 which exert a contrasting mode of selection. Consequently, cities and peri-urban areas appear  
457 to filter bee communities that are taxonomically and functionally different. These results  
458 underline the key role of wild bee functional traits in assisting conservation policies and  
459 habitat management in anthropised landscapes.

460

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## 791 **Figure and Table captions**

792 Figure 1: Location of the 29 sampling sites across 3 cities in France (Orleans in yellow, Blois  
 793 in green, and Tours in red)

794

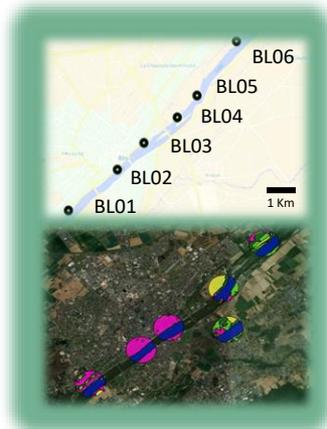
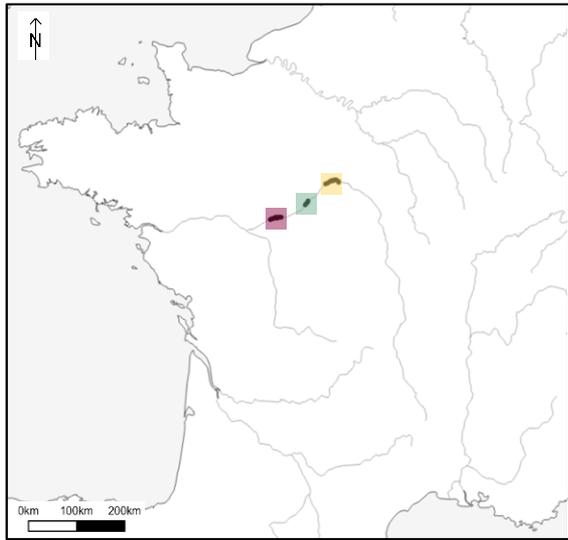
795 Figure 2: Wild bee variation in abundance (a), richness (b) functional and phylogenetic  
 796 diversity observed metrics FDobs (c) or PDobs (d) and standardized effect sizes metrics  
 797 sesFD (e) or sesPD (f) computed from both functional and phylogenetic dendrograms in the  
 798 three cities as a function of the urban landscape cover (ULC). The black line represents the  
 799 overall predicted values from the generalized linear mixed model, and the grey ribbon  
 800 represents the calculated standard errors of the predictions. The coloured dashed lines  
 801 represent trends in the three cities. Signif. codes: "\*\*\*\*" 0.001; "\*\*\*" 0.01 ; "\*" 0.05.  
 802

803 Figure 3: a) Non-metric multidimensional scaling (NMDS) plot based on Bray-  
 804 Curtis dissimilarities for the periurban and urban bee communities. Sites were classified as  
 805 "urban" or "periurban" land cover classes based on ward hierarchical clustering depending on  
 806 their simplified land use classification proportions (Fig. S1). b) Partition of overall  
 807 dissimilarity among sites (Sørensen dissimilarity index) between the turnover (Simpson  
 808 dissimilarly) and the nestedness-resultant components.  
 809

810

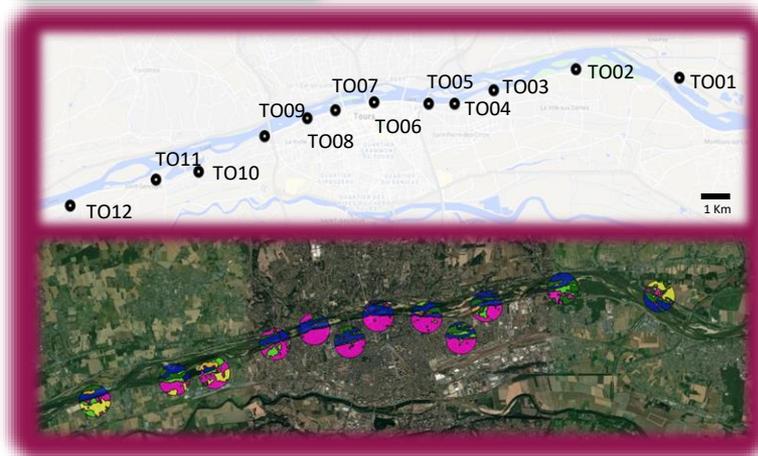
811 Table 1: Summary of the generalised mixed-effects models (glmmTMB) testing for the  
 812 effects of urban landscape cover and other land classes at a 500m radius surrounding sites on  
 813 abundance and diversity metrics of wild bees. Overall abundance, species richness, Simpson  
 814 index, Chao1 index, and haplotype numbers are presented in (a). Per family abundance,  
 815 species and haplotype richness are shown in (b).  
 816

817 Table 2: Landscape and floral traits effect on functional bee community traits. Significant  
 818 relationships in community weighted means (CWM) and functional dispersion (FDIs) of  
 819 quantitative traits, and in frequencies of categorical traits are denoted by arrows denoting the  
 820 direction of change.



- water
- agricultural
- forest
- semi-opened vegetation
- sand banks and dunes
- urban

Figure 1



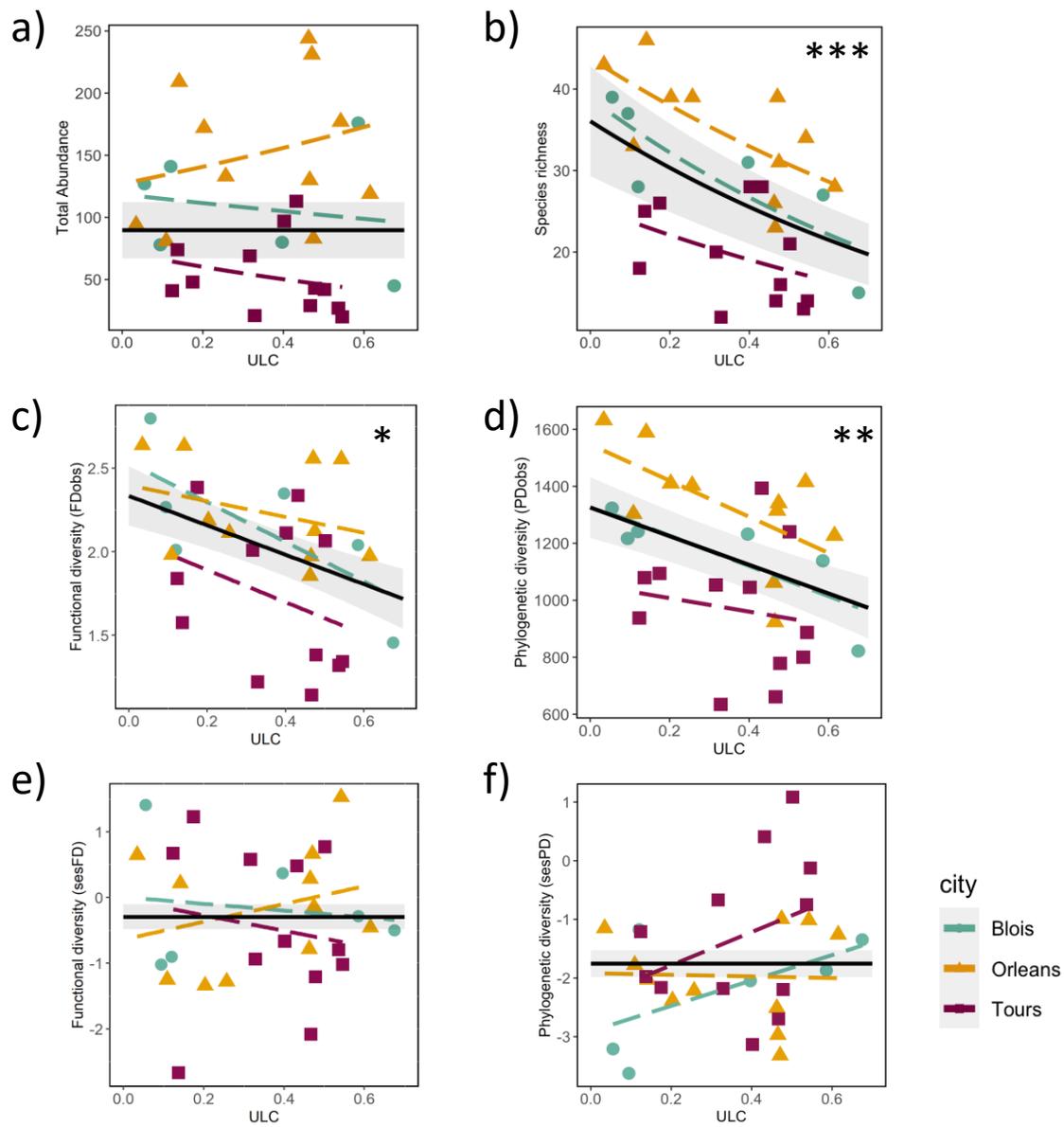


Figure 2

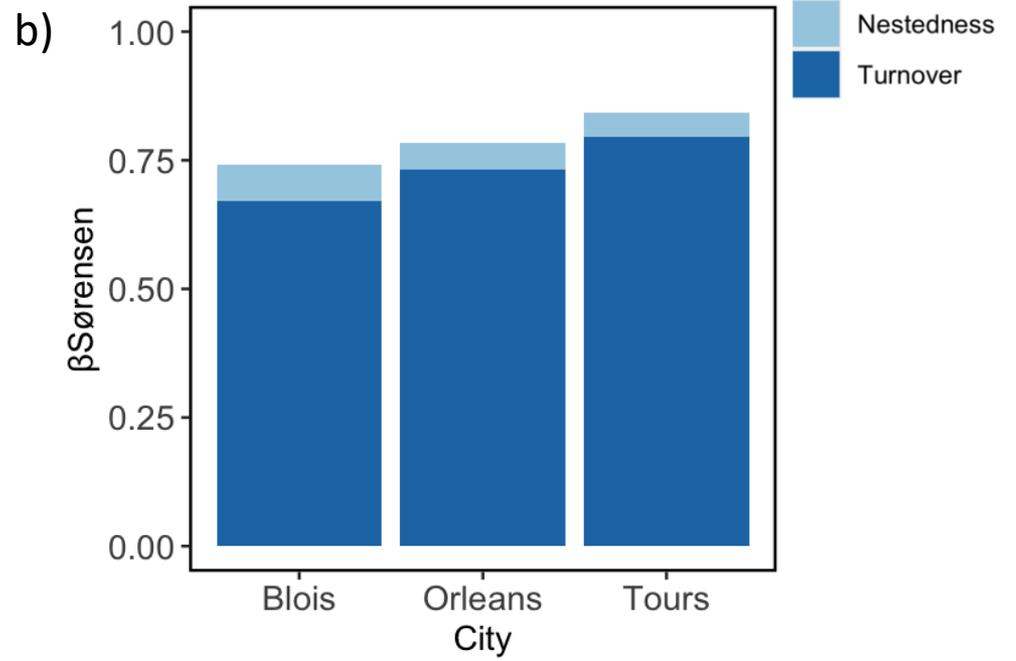
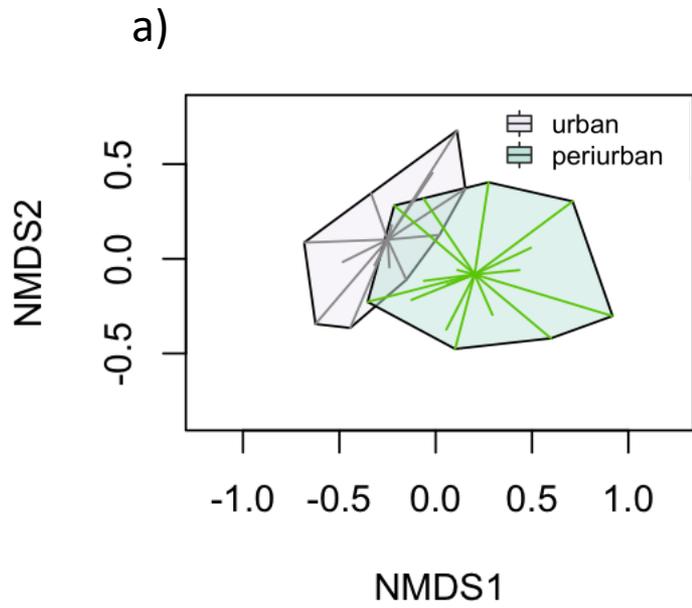
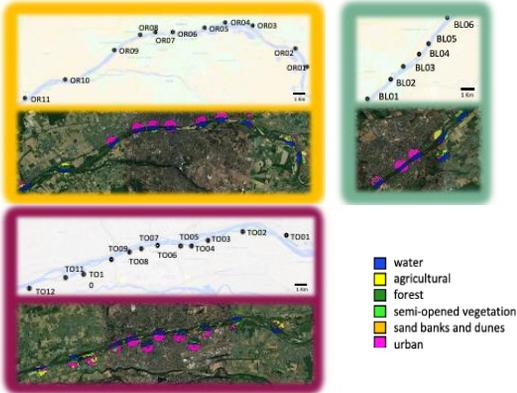
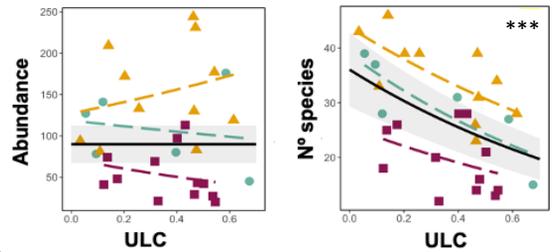


Figure 3

# Urban landscape cover (ULC) effect on wild bees

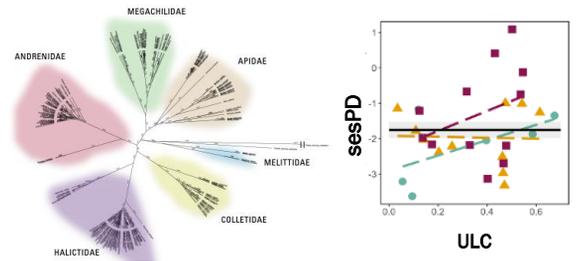


## Species abundance and diversity



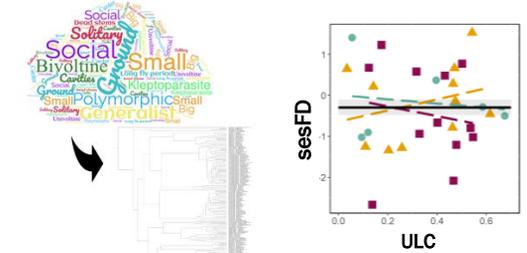
↓ Null models

## Phylogenetic diversity



↓ ↑

## Functional diversity



## Community composition

- High species turnover
- Functional structure response

