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 km2/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-

107   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 indicspecies 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 ('opportunist' 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 contruction 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 aboved 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

11

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-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. haemorrhoa, 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 %)

346 and taxonomical diversity indices with increasing urban landscape cover (Banaszak-Cibicka

347 and Żmihorski, 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 Żmihorski (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 *haemorrhoa, 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 Żmihorski, 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 Żmihorski, 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 Żmihorski,

392 2012; Hinners 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 Żmihorski,

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 ornemental 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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Figure and Table captions

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

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

Figure 3: a) Non-metric multidimensional scaling (NMDS) plot based on Bray-Curtis dissimilarities for the periurban and urban bee communities. Sites were classified as "urban" or "periurban" land cover classes based on ward hierarchical clustering depending on their simplified land use classification proportions (Fig. S1). b) Partition of overall dissimilarity among sites (Sørensen dissimilarity index) between the turnover (Simpson dissimilarly) and the nestedness-resultant components.
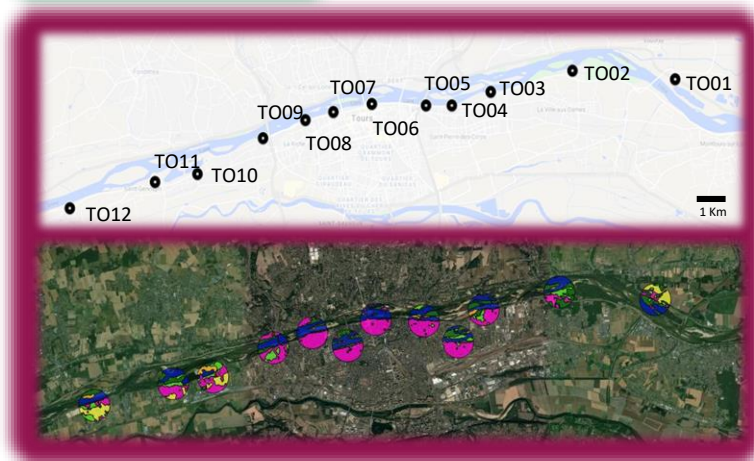
Table 1: Summary of the generalised mixed-effects models (glmmTMB) testing for the effects of urban landscape cover and other land classes at a 500m radius surrounding sites on abundance and diversity metrics of wild bees. Overall abundance, species richness, Simpson index, Chao1 index, and haplotype numbers are presented in (a). Per family abundance, species and haplotype richness are shown in (b).

Table 2: Landscape and floral traits effect on functional bee community traits. Significant relationships in community weighted means (CWM) and functional dispersion (FDis) of quantitative traits, and in frequencies of categorical traits are denoted by arrows denoting the direction of change.
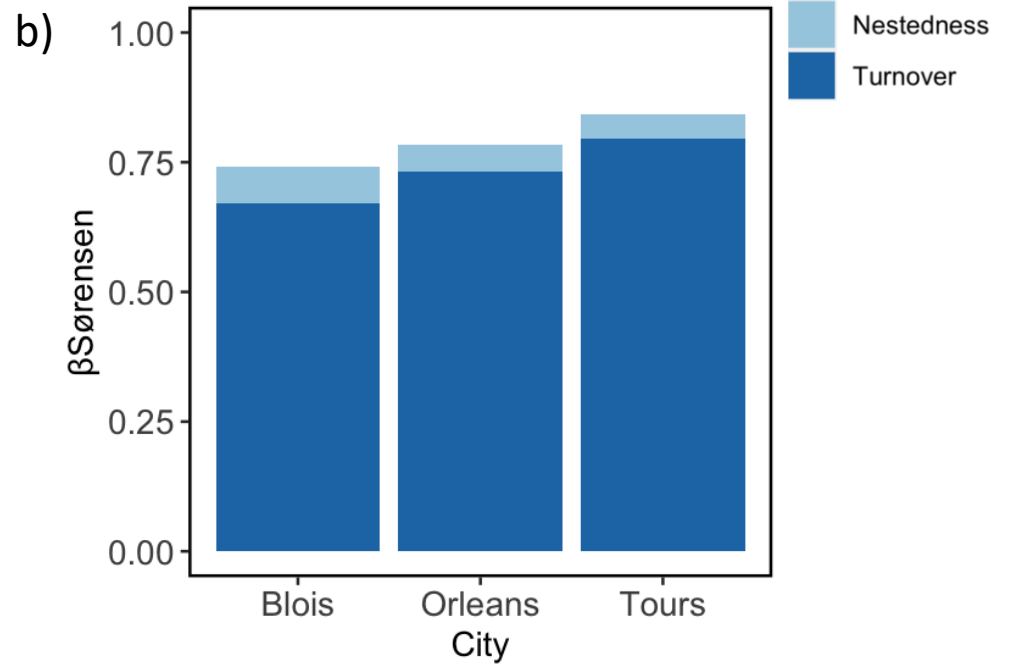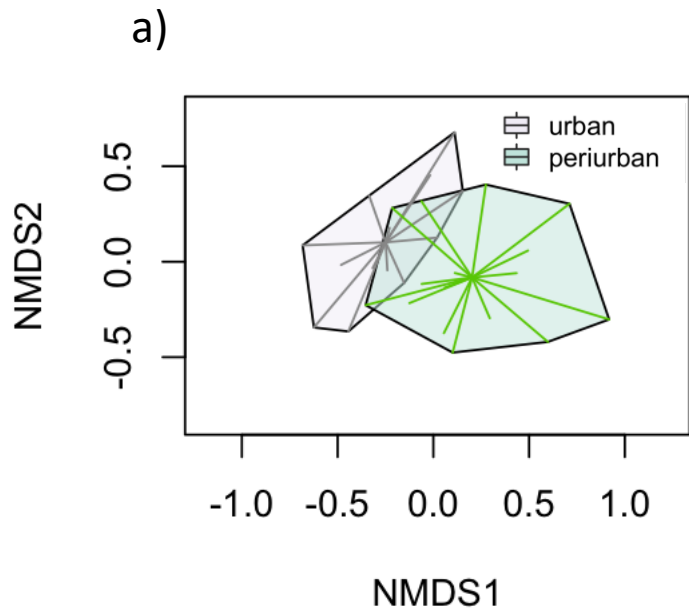
26

Figure 1

Figure 2

a)

b)

Figure 3

Urban landscape cover (ULC) effect on wild bees

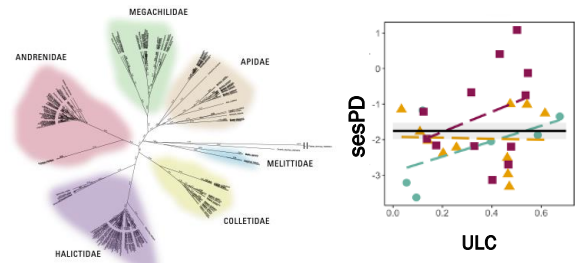**water**
agricultural
forest
semi-opened vegetation
sand banks and dunes
urban

Community composition

-High species turnover
-Functional structure response

social   solitary
small   big
Long flight period   Short flight period

Urban   Peri-urban
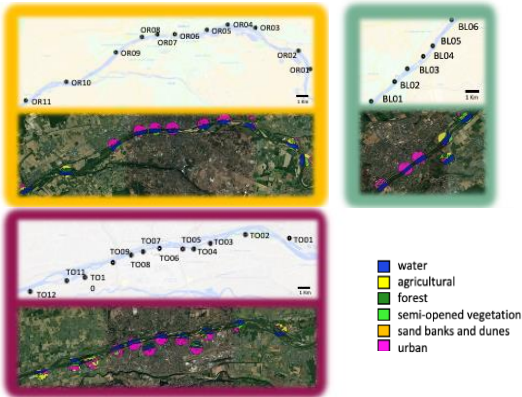
Species abundance and diversity
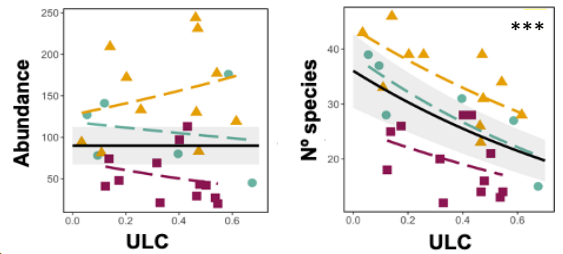
↓ Null models

Phylogenetic diversity

↕

Functional diversity