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How wild bees find a way in European cities: pollen metabarcoding unravels multiple feeding strategies and their effects on distribution patterns in four wild bee species

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1

2 **Title:** How bees find a way in European cities: pollen metabarcoding unravels multiple feeding
3 strategies and their effects on distribution patterns in four wild bee species

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42 **ABSTRACT**

43 1. Urban ecosystems can sustain populations of wild bees, partly because of their rich native and
44 exotic floral resources. A better understanding of the urban bee diet, particularly at the larval stage, is
45 necessary to understand biotic interactions and feeding behaviour in urban ecosystems, and to
46 promote bees by improving the management of floral resources in cities.

47 2. We investigated the larval diet and distribution patterns of four solitary wild bee species with
48 different diet specialization (i.e. *Chelostoma florissomne*, *Osmia bicornis*, *Osmia cornuta* and *Hylaeus*
49 *communis*) along urban intensity gradients in five European cities (Antwerp, Paris, Poznan, Tartu and
50 Zurich) using two complementary analyses. Specifically, using trap-nests and pollen metabarcoding
51 techniques, we characterized the species' larval diet, assessed diet consistency across cities, and
52 modelled the distribution of wild bees using species distribution models (SDMs).

53 3. Our results demonstrate that urban wild bees display different successful strategies to exploit
54 existing urban floral resources: not only broad generalism (i.e. *H. communis*), but also intermediate
55 generalism, with some degree of diet conservatism at the plant family or genus level (i.e. *O. cornuta*
56 and *O. bicornis*), or even strict specialization on widely available urban pollen hosts (i.e. *C.*
57 *florissomne*). Furthermore, we detected important diet variation in *H. communis*, with a switch from a
58 herbaceous pollen diet to a tree pollen diet with increasing urban intensity.

59 4. Species distribution modelling indicated that wild bee distribution ranges inside urban ecosystems
60 ultimately depend on their degree of specialization, and that broader diets result in less sensitivity to
61 urban intensity.

62 5. *Policy implications.* Satisfying larval dietary requirements is critical to preserving and enhancing
63 wild bee distributions within urban gradients. For high to intermediate levels of specialization, we
64 found considerable consistency in the preferred plant families or genera across the studied cities,
65 which can be generalized to other cities where these bees occur. Identifying larval floral preferences
66 (e.g. using pollen metabarcoding) could be helpful for identifying key plant taxa and traits for bee
67 survival and for improving strategies to develop bee-friendly cities.

68

69 **KEYWORDS:** cavity-nesting bees, feeding behaviour, remote sensing, trap-nests, urbanization, urban
70 biodiversity, species distribution models

71 Title page= 238; Abstract = 333; Manuscript+Captions+References = 8421

72

73 1. INTRODUCTION

74

75 Wild bees are responsible for major ecosystem functions and make many contributions relevant to
76 people, including pollination and maintenance of ecosystem stability, and they represent social and
77 cultural values (e.g. Potts et al., 2016). Over the last decades, bee populations have dramatically
78 decreased (Zattara & Aizen, 2021). Multiple causes have been identified (Goulson et al., 2015), and
79 the loss of floral resources is among the most important (Goulson et al., 2015). Wild bees critically
80 depend on large amounts of nectar and pollen to survive and reproduce during their life cycle, and
81 most species display some degree of fidelity to specific plant taxa (Goulson, 1999; Vanderplanck et
82 al., 2014). Consequently, diet specialization and diet preference are two key traits determining the
83 sensitivity to land-use changes (e.g. urbanization; Dharmarajan et al., 2021) and the distribution
84 patterns of wild bees (e.g. Fournier et al., 2020).

85

86 Urban ecosystems can harbour large and diverse bee communities, helping to preserve and promote
87 wild bee diversity. Although urbanization has major negative impacts on biodiversity (Theodorou et
88 al., 2020), a significant number of wild bee species can thrive in cities. Documentation of wild bees in
89 urban ecosystems has frequently indicated diverse wild bee communities (Baldock et al., 2015;
90 Casanelles-Abella, Chauvier, et al., 2021), although this ultimately depends on each species' traits
91 and its response to urbanization. Urban ecosystems are warmer (Roth et al. 1989), have higher
92 landscape heterogeneity (Turrine & Knopp, 2015) and are generally less polluted by pesticides
93 (Scheyer et al. 2007) than intensive agricultural areas. Moreover, while intensified agricultural
94 systems have impoverished floral resources, in cities these resources might be maintained, thanks to
95 social investment, high availability of woody species (e.g. street trees) in highly urbanized areas , and
96 the presence of flower-rich habitats (Somme et al., 2016; Tew et al., 2021). In both public and private
97 greenspaces, there are important efforts to establish and maintain flowering plant assemblages, with

98 each phase reflecting the preferences and needs of the specific owners and managers (Harrison &
99 Winfree, 2015). Therefore, there is a major opportunity to promote wild bee fitness and reproduction
100 by increasing and improving wild bee habitats.

101

102 Urban ecosystems can induce dietary changes in species, due to their distinct availability of various
103 food resources. In urban ecosystems, natural food resources are complemented with anthropogenic
104 food resources (Faeth et al., 2005), whose accessibility is modulated by each species' diet
105 specialization. Urban floral resources are especially diverse in cities, due to gardening and
106 horticultural activities, with many native and exotic species planted for different purposes. Some of
107 these species provide additional sources of food for pollinators within their range of foraging
108 preference, phenology and trait matching (Garbuzov & Ratnieks, 2014; Harrison & Winfree, 2015).
109 Therefore, generalist wild bee species with a broad dietary range might be better able to exploit the
110 existing urban resources, access and forage on a greater variety of patches, and consequently be
111 more widely distributed.

112

113 Knowledge on bee diet preferences could reveal which plant species are important for their survival
114 and reproduction and could be translated into important decisions concerning the planning and
115 management of floral resources, e.g. what species to plant. Plant identification with DNA
116 metabarcoding techniques has increased in diet studies and provides new knowledge about the
117 feeding preferences of animals, which can help us to understand their distribution along
118 environmental gradients (Pitteloud et al., 2021). So far, diet preferences have mostly been assessed
119 indirectly through observations of adult bee plant visitation (e.g. Marquardt et al., 2020) or through the
120 morphological identification of pollen grains (Haider et al., 2013; Sedivy et al., 2011). Nonetheless,
121 specific sampling methodologies, such as trap-nests that allow standardized sampling (Staab et al.,
122 2018), combined with metabarcoding techniques promise to be a powerful tool to characterize and
123 study larval bee diets (Bell et al., 2016; Keller et al., 2015). Trap-nests target larval pollen and thus
124 can better describe bee diet preferences than measurements of adult visitation, while pollen
125 metabarcoding techniques can identify a larger number of taxa with a higher taxonomic resolution
126 than pollen morphological identification; this is particularly useful in urban ecosystems with unique

127 and rich plant pools. Metabarcoding techniques reduce the need for taxonomic expertise associated
128 with pollen morphological identification, thus broadening its application across multiple sites.

129

130 Here, we investigated the larval diet and distribution of four widespread wild bee species of urban
131 ecosystems, representing a gradient of decreasing diet specialization (*Chelostoma florissomne*, *Osmia*
132 *bicornis*, *Osmia cornuta* and *Hylaeus communis*), along urban intensity gradients in five European
133 cities (Antwerp, Paris, Poznan, Tartu and Zurich). In particular, we asked the following questions: (1)
134 What is the taxonomic and trait-based composition of the bee diets in different urban areas? (2) How
135 consistent are the bee diets across urban areas? (3) How does diet specialization influence the bee
136 distribution in urban ecosystems? We expected that specialized bee species (i.e. *C. florissomne*) would
137 have strong preferences for specific plant taxa and thus a highly consistent diet across urban areas
138 and within urban gradients. Conversely, we predicted that more generalist bees (i.e. *O. cornuta*, *O.*
139 *bicornis* and *H. communis*) would have a more flexible diet and be capable of switching to alternative
140 floral resources, including exotic taxa, and thus have a higher turnover in the diet composition (at the
141 plant family, genus and species level) and a less consistent diet. Finally, we hypothesized that bee
142 species with greater diet specialization would have low flexibility in terms of switching their diet to
143 other plant taxa and thus would be more sensitive to urban intensity.

144

1452. MATERIALS AND METHODS

146 2.1 Cities and study sites

147 We investigated wild bee diets in five cities in Europe: Antwerp (Belgium), Greater Paris (France,
148 hereinafter referred as Paris), Poznan (Poland), Tartu (Estonia), and Zurich (Switzerland), covering a
149 large part of the climatic variability in mainland Europe. Site selection followed Casanelles-Abella,
150 Frey et al. (2021). Overall, we selected sites from the urban green areas mapped and defined in the
151 pan-European Urban Atlas (EEA, 2012). We used an orthogonal gradient of patch area and
152 connectivity. In particular, we calculated connectivity using the proximity index (PI), which considers
153 the area and the distance to all nearby patches with a favourable habitat, within a given search radius.
154 We considered as favourable habitat the land cover classes urban green areas, forest and low urban
155 density with < 30% impervious surface. We set the search radius to 5 km from each focal patch, the

156 maximum value possible with the available cartography. This resulted in the final selection of 80 sites:
157 32 in Zurich and 12 in each of the remaining four cities (Fig. S1, Table S1). We maintained a
158 minimum distance of 500 m between selected sites (except for two sites in Zurich selected based on
159 their position in the patch and connectivity gradient, which were separated by 260 m).

160

161 **2.2 Bee sampling**

162 At each site we installed trap-nests in trees, and in three cases (one in Paris, one in Tartu and one in
163 Zurich) in other vertical structures (e.g. lamp post). We constructed trap-nests with reeds and
164 cardboard tubes (Fig. S1). Our sampling trap-nests consisted of a standardized wood box with three
165 plastic pipes 15 cm in diameter and 20 cm long. We assembled the first two pipes using 200–300
166 reeds from *Phragmites australis* (Cav.) Trin. and 5–10 bamboo reeds with diameters of 1–10 mm and
167 a length of 20 cm to cover all requirements of the cavity-nesting bee community. We assembled the
168 third pipe only using cardboard tubes of 7.5 mm diameter specific for *Osmia* spp. (WAB
169 Mauerbienenzucht, Konstanz, Germany). We installed trap-nests at 2.5–3.5 m height with direct
170 sunlight and SE or SW exposition, and kept them in the field from January until October 2018. In
171 October, we collected the trap-nests and stored them at ca. 5°C until February 2019, and then
172 transferred them to a new room at ambient temperature to recreate spring-like conditions. Bees
173 hatched and were identified to the species level from February to June 2019.

174

175 **2.3 Study organisms**

176 We collected pollen from the nests of four solitary bee species, *Chelostoma florissomne* (Linnaeus,
177 1758), *Hylaeus communis* (Nylander, 1852), *Osmia bicornis* (Linnaeus, 1758), and *Osmia cornuta*
178 (Latreille, 1805). These species encompass a gradient of diet specialization (i.e. the number of
179 different plant families exploited as resources, from the oligolectic *C. florissomne* to the highly
180 polylectic *H. communis*), differ in phenology, and are common species in urban areas in Europe. In
181 our study, each species was present in at least three of the studied cities. For more details about the
182 ecology of these four wild bee species, see Text S1.

183

184 **2.4 Pollen identification**

185 We extracted a total of 464 pollen samples (Table S1) from undeveloped cells (i.e. where the larva
186 had died) in nests where at least one adult had emerged and thus taxonomic identification of the bees
187 was possible. Specifically, for *C. florisomne* we used 121 samples distributed in 3 cities and 18 sites
188 (2 in Antwerp, 1 in Tartu and 15 in Zurich), for *O. cornuta* we used 66 samples distributed in 3 cities
189 and 20 sites (6 in Antwerp, 5 in Paris and 9 in Zurich), for *O. bicornis* we used 176 samples
190 distributed in 5 cities and 37 sites (3 in Antwerp, 10 in Paris, 8 in Poznan, 1 in Tartu and 15 in Zurich),
191 and for *H. communis* we used 101 samples distributed in 5 cities and 33 sites (4 in Antwerp, 6 in
192 Paris, 6 in Poznan, 9 in Tartu and 8 in Zurich). DNA metabarcoding (isolation, amplification and
193 sequencing) was performed by AllGenetics laboratories (AllGenetics & Biology SL, A Coruña, Spain).
194 We followed the method described by Sickel et al. (2015) and Vierna et al. (2017) to produce a pooled
195 amplicon library on the ITS2 genomic region for the Illumina platform (Illumina, 2017). See Text S3 for
196 details on the laboratory procedure of pollen metabarcoding. Bioinformatics followed mainly the
197 procedure described in Campos et. al. (2021) with minor modifications: We used VSEARCH v2.14.2
198 (Rognes, Flouri, Nichols, Quince, & Mahé, 2016) to join paired ends of forward and reverse reads. We
199 also used VSEARCH to remove reads shorter than 200 bp, complete quality filtering (EE < 1 Edgar &
200 Flyvbjerg, 2015) and de-novo chimera filtering, and define amplicon sequence variants (ASVs), as
201 previously done successfully for pollens (e.g. Wilson et al., 2021). The ITS2 rDNA reads were first
202 directly mapped with VSEARCH global alignments and an identity cut-off threshold of 97% against a
203 floral ITS2 reference database generated with the BCdatabaser (Keller et al., 2020), which consisted
204 of plants recorded within the study regions. For the remaining unclassified reads, we first used global
205 alignments against a global reference database (Ankenbrand 2015; Keller et al., 2015). For reads that
206 were still unclassified, we used SINTAX (Edgar, 2016) to assign taxonomic levels as deep as possible
207 but a maximum of genus level with the same global reference database. In total, 82% of species
208 recorded at the sites were present in the local database, 83% of species in the global database (direct
209 classification). Further, 92% of genera were covered by the global database for hierarchical
210 classification. Please note that the global database contains 112.115 unique species, 11.321 genera
211 and 710 families in total, with a very high likelihood of coverage for species and genera of any interest
212 for anthropogenic use, including exotic garden species.

213

214 **2.5 Environmental variables**

215 We assembled variables that were potential drivers of bee diets and distributions and that
216 represented different aspects of the urban environmental gradients. Specifically, we focused on
217 proxies of stress (particularly thermal stress), amount of habitat and resource availability at different
218 spatial scales. We inferred resource availability at the local scale by performing floristic inventories on
219 standardized plots, as explained in Casanelles-Abella, Frey et al. (2021) and in Supplementary Text
220 S4. Furthermore, we collected information on two functional plant traits sensitive to bee–plant
221 interactions, i.e. growth form (Tables S2–S3) and blossom type (Tables S2–S3) using information
222 available in Casanelles-Abella, Frey et al. (2021). See Text S4 and Tables S2–S3 for additional
223 information on the definition of the traits.

224

225 We used local and landscape connectivity metrics, local land cover metrics and landscape remote
226 sensing based indices to infer thermal stress and the amount of available habitat, particularly
227 regarding resource availability. As connectivity metrics we used patch size and the proximity index.
228 We obtained the local land cover metrics by mapping grasslands, artificial surfaces, bare land,
229 coniferous trees and deciduous broad-leaved trees and then calculating their proportions at different
230 spatial scales (i.e. 8, 16 and 32 m) from the focal trap-nest (see Text S5 for additional details). Finally,
231 we used remote sensing based indices on land surface temperature, impervious surfaces, soil, water
232 and vegetation at different spatial scales (50, 100, 200, 400, 800, 1600 m). Specifically, we used land
233 surface temperature (LST), the urban index (UI), the colour index (CI), the normalized difference
234 water index (NDWI) and the normalized difference vegetation index (NDVI), which can be used to
235 characterize existing vegetation and urban infrastructure. In addition, we performed a principal
236 component analysis (PCA) on the explanatory variables to define new meaningful underlying
237 variables while reducing the dimensionality of the data set (see section 2.6). See Text S6 for details
238 on the calculation of the indices and Fig. S2 for the distribution of values of each predictor in each
239 city.

240

241 **2.6 Statistical Analysis**

242 We conducted all analyses in R version 4.0.2 (R Core Team, 2019) with RStudio version 1.4.1106
243 (RStudio Team, 2020).

244 *Species diet analysis*

245 We performed taxonomic and trait-based metrics on the bee diets at the city and site level.
246 Specifically, we computed the proportion of different plant taxa at the family, genus and species level
247 (Table S4). Furthermore, we calculated the species, genus and family richness and the Shannon
248 diversity index. Concerning trait-based responses, we calculated the proportion of the different
249 categories of the three studied traits (Table S5). For each of the four studied bees, we performed
250 Pearson correlations to investigate the relationships between the taxonomic and the trait-based diet
251 metrics with the proxies of urban intensity, habitat amount and resource availability. We assessed
252 these relationships: (1) for each single city and (2) for all the cities combined.

253 We calculated the pairwise correlations between cities for each bee species to study diet consistency.
254 Specifically, we first assembled binary trophic interaction matrices between the four bee species and
255 the plant taxa at the family, genus and species level and then calculated the Pearson correlations of
256 the binary trophic interaction matrices between pairs of cities for each bee and plant level. However,
257 the trophic interaction matrix for a given city, and thus the pairwise correlations between cities, is
258 influenced by the available plant pool. To avoid effects of plant composition, we first created a list with
259 the plant pool occurring in each city at the family, genus and species level. We used the plant species
260 sampled within a 100-m buffer by Casanelles-Abella, Frey et al. (2021) and complemented with the
261 plant species recorded in GBIF at each city for the period 2000–2018 (Fig. S3). If a plant family,
262 genus or species was missing in one of the plant pools of a pair of cities, we removed the interaction
263 when performing the correlations. Moreover, we computed a Chi-squared (χ^2) test on the family and
264 trait composition between cities' plant species pools (Text S7, Table S6).

265

266 *Species distribution of urban gradients*

267 We studied bee distribution patterns with species distribution models (SDMs). We assembled
268 occurrence matrices indicating the occurrence of each bee species in the different sites. From the
269 candidate environmental predictors, we evaluated the statistical relevance of each predictor using the
270 predictive power (D^2 ; Table S7) and then manually picked three predictors that had correlations <0.7
271 to avoid collinearity (Fig. S4) for each bee separately. We used an ensemble of two common
272 modelling techniques to account for model uncertainty and specificity (Buisson et al., 2010).
273 Specifically, we used two regression-based models, i.e. generalized linear models (GLMs) and

274 generalized additive models (GAMs), and two tree-based models, i.e. gradient boosting machines
275 (GBMs) and random forests (RFs), that show a higher complexity in their fitting procedures than
276 GLMs and GAMs. We used city as a fixed factor to account for the nested structure of the data with a
277 binomial probability distribution. We parameterized each modelling technique in the following way: we
278 calibrated GLMs with first-order polynomials, GAMs with a spline smoothing term of intermediate
279 complexity ($k=4$), RFs with a node size of 5 ($nodesize = 5$) and 1000 trees, and GBMs with an
280 interaction depth of 1, a shrinkage of 0.001 and 1000 trees. We ran the models using the R packages
281 *mgcv* version 1.8-30, *RandomForest* version 4.6–14 and *gbm* version 2.1.5. We randomly split the
282 species records of the four bees into two sets containing 80% of the data for model calibration and
283 20% of the data for model evaluation. We repeated the procedure five times. We assessed model
284 performance with the True Skill Statistic (TSS; Allouche, Tsoar, & Kadmon, 2006). TSS evaluates
285 model skill in distinguishing absences from presences. The predictive performance of the different
286 models was deemed acceptable when $TSS > 0.4$, following a commonly used minimum threshold
287 (Thuiller et al., 2019). Thus, we discarded models with TSS values lower than 0.4. We used the
288 selected models of each studied bee species to predict the probability of occurrence over the
289 environmental space of the studied cities.

290

291 **3. RESULTS**

292 **3.1 Species diet analysis**

293 A total of 41 plant families, 93 genera and 135 species were identified from the nests of the four bee
294 species (Fig. 1, Tables S8–S9). Over half of the species were native (55%), there were more herbs
295 (42%) than trees (34%), and dish-bowl blossoms were more common (56%) (Figs S6–S8, Table S9).
296 The number of plant species per bee nest was similar among bee species (Table S9). The total
297 number of collected plant taxa varied greatly among bee species, reflecting their differences in diet
298 specialization: 1 family and 4 species in *C. florisomne*, 12 families and 33 species in *O. cornuta*, 18
299 families and 51 species in *O. bicornis*, and 32 families and 81 species in *H. communis*. (Fig. 1, Table
300 S9). At the city level, we found dominance patterns in pollen abundance for some bees (Fig. 1). In *O.*
301 *bicornis*, the most abundant species in pollen were *Quercus robur* (Antwerp, 70%), *Acer*
302 *pseudoplatanus* (Paris, 64 %; Poznan 44 %; and Zurich 33 %). In *H. communis*, *Styphnolobium*

303 *japonicum* was the most abundant species in pollen but only in Paris (52 %) and Poznan (32 %), with
304 the vast majority of species representing 1-14 % of the pollen abundance. Interestingly, in *C.*
305 *florisomne*, the most abundant *Ranunculus* spp. in pollen changed between cities (*R. acris* in
306 Antwerp, *R. repens* in Tartu and *R. bulbosus* in Zurich). Finally, in *O. cornuta*, no species made up
307 more than 37 %, being the most abundant ones *A. pseudoplatanus* (Antwerp, 37%; Paris, 21 %;
308 Zurich, 24 %) and *Prunus lusitanica* (Paris, 33 %). In addition, very few nests were dominated by a
309 single plant species and mostly in *C. florisomne* (Fig. S6).

310 We found different levels of diet conservatism across cities at the plant family and plant genus level,
311 according to the bee specialization degree and taxonomic resolution of the plant taxa (Fig. 2). At the
312 family and genus level, diet consistency was high for *C. florisomne* and declined with broader feeding
313 niches, particularly at the genus level (Fig. 2a, Table S10). Conversely, we found major variation at
314 the plant species level, which was particularly prominent for the broad generalist *H. communis*, which
315 switched from herbaceous pollen to tree pollen with increasing urbanization (Fig. 3b).

316

317 The extent to which bee diet taxonomic and trait-based composition were conserved also varies
318 according to the degree of specialization. *Chelostoma florisomne* had the most conserved diet,
319 composed exclusively of native *Ranunculus* spp. (Figs 1, 2a, Tables S8–S9). *Osmia cornuta* primarily
320 collected the pollen of native tree and shrub species, mainly with dish-bowl or brush type blossoms,
321 from the families Sapindaceae, Salicaceae and Rosaceae (Figs 1, S7–S10, Tables S4, S5, S8–S11).
322 Nevertheless, in Paris and Zurich we also found a considerable proportion of Ranunculaceae (Fig.
323 1b). Both *C. florisomne* and *O. cornuta* taxonomic and trait-based metrics showed no or little variation
324 along urban intensity gradients (Fig. S11, Table S12). In *O. bicornis*, native tree species with dish-
325 bowl or brush blossoms from the families Sapindaceae and Fagaceae represented a large part of the
326 diet (Figs 1, S7–S10, Tables S4, S5, S8–S11), but we found some variation across cities concerning
327 the remaining species in the diet (Figs. 1b, S10).

328 *Hylaeus communis* had the most diverse and variable diet. The Fabaceae family represented 34% in
329 Paris and 42% in Poznan of the species found in the larval diet (Fig. 1b) and a minor part in the
330 remaining cities (Fig. 1b). Furthermore, exotic species were more frequent for *H. communis* than for
331 the other three bee species (Figs 3a, S7, Tables S5, S9). In addition, we found family richness,

332 species richness and pollen diversity to be positively correlated with NDVI for *H. communis* for all
333 cities except Poznan (Fig. S11, Table S12). Finally, in Paris, an important part of the diet was trees
334 with flag type blossoms (Fig. 3b), in part due to the contribution of *Styphnolobium japonicum* (Fig 1a,
335 Table S8), which became more dominant in the diet with increasing urban intensity (e.g. decreasing
336 NDVI and increasing UI and CI at different scales; Fig. 3, Table S12).

337

338 **3.2 Species distributions along urban gradients**

339 The PCA conducted on the explanatory variables returned two main axes that explained 38% and
340 12% of the variation, respectively. The first axis was composed of remotely sensed variables, with
341 larger values of the PC axis indicating less vegetation (i.e. higher UI, CI and LST, and lower NDVI;
342 Fig. S12) independently of the landscape scale considered. The second axis was mostly composed of
343 local land cover variables and metrics representing the available floral resources. Specifically, larger
344 values on the PC axis indicated larger proportions of grasslands and lower proportions of deciduous
345 trees and artificial surfaces, independently of the local scale considered (Fig. S12).

346 We found two distinct distribution patterns of the four bee species along urban intensity gradients. The
347 first type of response was composed of *C. florisomne*, *O. cornuta* and *O. bicornis*. The probability of
348 occurrence of these three bee species decreased rapidly with increasing urban intensity at the
349 landscape scale (Figs 4a–b, S13) and increased with higher proportions of grasslands at the local
350 scale (Fig. 4c–d). Strikingly, the probability of occurrence peaked at low proportions of broad-leaved
351 trees (Figs 4c–d, S13), even though the diets of *O. cornuta* and *O. bicornis* were composed largely of
352 tree pollen. By contrast, the probability of occurrence of *H. communis* remained constant for larger
353 values of urban intensity (Figs 4a–b, S13). Moreover, the probability of occurrence was positively
354 affected by the amount of deciduous broad-leaved trees at local scales (Figs 4c–d, S13).

355

356 **4. DISCUSSION**

357 With biodiversity loss increasing at an unprecedented speed (Leclère et al., 2020) and urbanization
358 expanding worldwide, there is a pressing need to better understand the niche requirements of
359 different taxa in order to satisfy them in urban ecosystems through targeted planning and

360 management (Aronson et al., 2017). Here, using trap-nests and pollen metabarcoding techniques, we
361 studied the larval diet and distribution patterns of four wild bee species in five European cities.

362

363 The larval diet of the studied oligolectic and intermediate polylectic bees was conserved in terms of
364 plant family composition, representing successful strategies in urban ecosystems as an alternative to
365 generalism. As hypothesized, diet consistency decreased with decreasing wild bee degree of
366 specialization and was relatively high at the plant family level and to lower at the plant genus level.
367 Moreover, for *C. florisomne*, *O. cornuta* and *O. bicornis*, bee diet at the plant family level in the
368 studied cities was consistent with prior studies in non-urban ecosystems (e.g. Haider et al., 2014).
369 Diet conservatism regarding plant family composition has also been observed in other polylectic bee
370 species such as *Bombus* spp. (Wood et al., 2021) and *Osmia* spp. (Haider et al., 2014; Vaudo et al.,
371 2020), indicating that diet conservatism might be widespread despite a lack of data. The observed
372 patterns contrast with the low predictability of diet composition based on the available floral resources
373 at all plant levels. Finally, diet consistency at the plant species level, with the exception of highly
374 specialized *C. florisomne*, was very low, possibly because the specific trait values (e.g. pollen
375 properties imposing cognitive or physiological restrictions; Vaudo et al., 2020) driving plant–pollinator
376 interactions in less specialized wild bees might be relatively consistent within plant families or genera.

377 Across different animal taxa, broad feeding niches (i.e. low diet conservatism) have been identified as
378 a key characteristics to pass the urban ecological filtering, and hence to thrive in cities (Fournier et al.,
379 2020), with some species even undergoing rapid phenotypic changes to broaden their diet (e.g.
380 Eggenberger et al., 2019). While widely distributed species inside cities are typically generalists,
381 especially when highly mobile and with specific nesting and sociality modes (i.e. cavity nesting or
382 social), intermediate specialization might be an alternative advantageous strategy in urban
383 ecosystems. Focusing on wild bees, species whose preferred plant families are selected and
384 facilitated by stakeholders (in Central European cities, e.g. Rosaceae, Fagaceae, Salicaceae; Ossola
385 et al., 2020) could have very diversified resources, due to the co-occurrence of spontaneous and
386 cultivated native and exotic plant species. Here, we show that this is the case for *O. cornuta* and *O.*
387 *bicornis*. Consequently, wild bees could still switch pollen hosts at the species level and better exploit
388 existing resources while avoiding the costs associated with broad niches, as some pollen types are
389 indigestible or toxic (Eckhardt et al., 2014; Praz et al., 2008). On the other hand, strict specialization,

390 as in *C. florisomne*, can still be a successful strategy in urban ecosystems when pollen hosts are
391 highly facilitated and widespread in urban ecosystems. Nonetheless, strict specialists are vulnerable
392 to the partial or total loss of pollen hosts, due to e.g. urban sprawl across the habitat of the bee or
393 pollen host species, changes in social investment when gardening or managing greenspaces, or the
394 arrival of pests.

395

396 The degree of specialization in bees is associated with distribution patterns along urban gradients.
397 Our results suggest that increasing specialization leads to a higher sensitivity towards increased
398 urban intensity. Diet specialization determines the possibility of occupying new patches, and greater
399 specialization represents a strong limitation when the nutritional requirements are not met (e.g. with
400 agricultural intensification; Peters, Keller, & Leonhardt, 2021). Rarity in bumblebees has been
401 associated with narrower feeding niches (Goulson, 1999), and this is also the case for other wild bees
402 (e.g. Deguines et al., 2016). Some types of urban greenspaces consistently contain high bee
403 diversity, including several specialists (Baldock, 2020; Salisbury et al., 2015). Still, only a handful of
404 usually broad generalists can colonize various types of urban greenspaces. Hence, these species are
405 widespread within urban ecosystems (Fournier et al., 2020), even though not only the degree of
406 specialization but also other correlated functional traits are involved in the response to urbanization
407 (e.g. stress tolerance, dispersal; Harrison & Winfree, 2015). To promote a larger number of bees in
408 previously unoccupied areas of the city, wild bee habitat must be strategically increased, including
409 enhancing the availability of high floral resource diversity. A first step to achieve this is to map the
410 existing floral resources within the different urban land covers, making use of ongoing inventories
411 (Ossola et al., 2020) or sampling schemes from research projects (Baldock et al., 2019; Casanelles-
412 Abella, Chauvier et al., 2021). These products could be combined and compared with wild bee diet
413 preferences to detect where and what kind of planning actions can be taken. For instance,
414 strategically increasing plant diversity rather than overall quantity with targeted taxonomic and trait
415 groups is a measure that has been successful in other ecosystem types (e.g. agroecosystems; Sutter
416 et al., 2017), although other wild bee requirements (e.g. nesting mode) must also be satisfied to
417 successfully promote them (Requier & Leonhardt, 2020).

418

419 **4.1 Limitations and prospects**

420 Pollen metabarcoding only yields relative abundance data and can be subject to PCR or taxon
421 dependent biases as discussed in several studies. (e.g. Bell et al., 2016; Keller et al., 2015) The
422 semi-quantitative abundances obtained in metabarcoding analyses are however still useful to identify
423 the relative proportions of taxa and differentiate dominant, common and rare contributions to a mixed
424 pollen sample by showing correlations (even though not perfect) between read and grain numbers
425 (Keller et al., 2015) . It is further agreed that pollen metabarcoding can identify to deep taxonomic
426 levels, is able to detect rare taxa and is well comparable between studies (Bell et al., 2016). In
427 addition, the prevalence of certain plant families and species across cities (Fig. 1), and the existing
428 information on *C. florisomne*, *O. cornuta* and *O. bicornis* (Haider et al., 2013, 2014; Sedivy, Praz,
429 Müller, Widmer, & Dorn, 2008) showing patterns similar to those observed here support our findings.
430 Combining molecular and morphological approaches would provide more robust relative abundance
431 estimates as suggested by Sickel et al. (2015). Further, if taking dry weight of the collected pollen
432 samples is an option (e.g. Lihoreau et al., 2012), these relative data could be converted to absolute
433 abundances per sample. Furthermore, we could only use morphological plant traits to investigate diet
434 composition and shifts along urban gradients. However, bee choices when selecting larval food are
435 likely to be influenced by the nutritional properties of the pollen, such as the content of protein, sugar
436 or other essential nutrients (Vanderplanck et al., 2014; Vaudo et al., 2020). Evidence seems to
437 indicate important differences in the nutritional value of the available floral resources between urban
438 land cover types (Tew et al., 2021) and possibly between ornamental plant species (Garbuzov &
439 Ratnieks, 2014). Incorporating nutritional traits in future dietary studies will better elucidate the
440 mechanisms behind diet composition and trophic niche shifts.

441 Finally, our results open future research directions. First, further studies could consider whether there
442 are signals of feeding behavioural changes due to anthropogenic factors (e.g. plant or management
443 preferences by stakeholders), as seen in other taxa (e.g. Cucherousset et al., 2012), and if these are
444 reflected in the variation of other functional traits (e.g. tongue length; Eggenberger et al., 2019). To
445 achieve this, it would be critical to study and compare bee diets in both urban and non-urban
446 ecosystems. Second, assessing the effects of intraspecific diet variability on fitness (e.g. number and
447 sex-ratio of offspring, adult and larval survival) would provide important mechanistic insights for
448 anticipating how plant management choices might affect urban bee communities.

449 4.2 Policy implications

450 The uncovered taxonomic and functional diet preferences can support the planning and management
451 of urban greenspaces to promote wild bees, particularly in wild bee species where the diet patterns
452 are consistent across cities. Typical common urban weeds (e.g. *Taraxacum officinale*, *Bellis perennis*,
453 *Trifolium pratensis*, *T. repens*) that are important floral resources in urban areas (Baldock et al., 2019;
454 Kanduth et al., 2021; Larson et al., 2014) contributed little or nothing to the larval diet of the studied
455 bees. Conversely, both native and exotic woody species proved to be a widely used floral resource.
456 For example, *O. cornuta* and *O. bicornis* collected large amounts of tree pollen from different plant
457 families, which might enable them to exploit secondary pollen hosts by mixing high quality pollens
458 with less digestible ones (e.g. *Ranunculus* spp., Asteraceae; Eckhardt et al., 2014; Praz et al., 2008).
459 Thus, the maintenance of different vegetation and urban habitat types (e.g. meadows, street trees,
460 shrublands) is of major importance for preserving bees. In addition, our results indicate that the
461 occurrence of specific plant taxa (e.g. at the family or genus level) or trait values is more important
462 than the origin of the plants (Harrison & Winfree, 2015). For example, non-native plants have been
463 observed to retain the blooming time of their original region (Godoy et al., 2009), and thus plant
464 species from the Northern Hemisphere (e.g. planted urban trees or shrubs such *Acer* spp., *Salix* spp.,
465 *Crataegus* spp and, *Quercus* spp.) may bloom in synchrony with natives species, providing additional
466 resources for wild bees. Furthermore, urban trees have been shown to be an important resource for
467 several wild bee species (Somme et al., 2016). For instance, in our study, *H. communis* increasingly
468 collected more pollen from ornamental trees (mainly *Styphnolobium japonicum*) with decreasing
469 amounts of greenspace. Because the tree distribution in cities is mostly driven by anthropogenic
470 factors, it represents an important point of action for greening strategies, specifically in densely
471 urbanized city areas with limited herbaceous vegetation. Overall, floral preferences obtained from
472 pollen metabarcoding, particularly when combined with existing information on available floral
473 resources, could help to improve current strategies for developing bee-friendly cities (e.g. in the EU,
474 see Wilk et al., 2020). In particular, characterizing bee diets could inform planning, management and
475 decision making (e.g. what species, genera or families to plant), involving stakeholders, policymakers,
476 nurseries and plant centres, for urban greenspaces to preserve and further promote wild bees in
477 urban ecosystems.

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490 **AUTHORS' CONTRIBUTIONS**

491 JCA, MM and LP conceived the ideas. MM, FC, LL, RS, PP and PT developed the site selection
492 methodology. JCA collected the bee data. AK performed the bioinformatic analyses on the pollen.
493 JCA, SM, ND, TH and MAO collected the plant data. MAO, TH and LL provided the local land cover
494 metrics. CA and PP provided the remote sensing data. JCA analysed the data. JCA led the
495 manuscript writing, with editing and supervision by MM and LP. All the authors reviewed and revised
496 the manuscript and gave final approval for publication.

497 **DATA AVAILABILITY STATEMENT**

498 The raw data on diet composition supporting this paper can be find in the repository EnviDat v.0.1.
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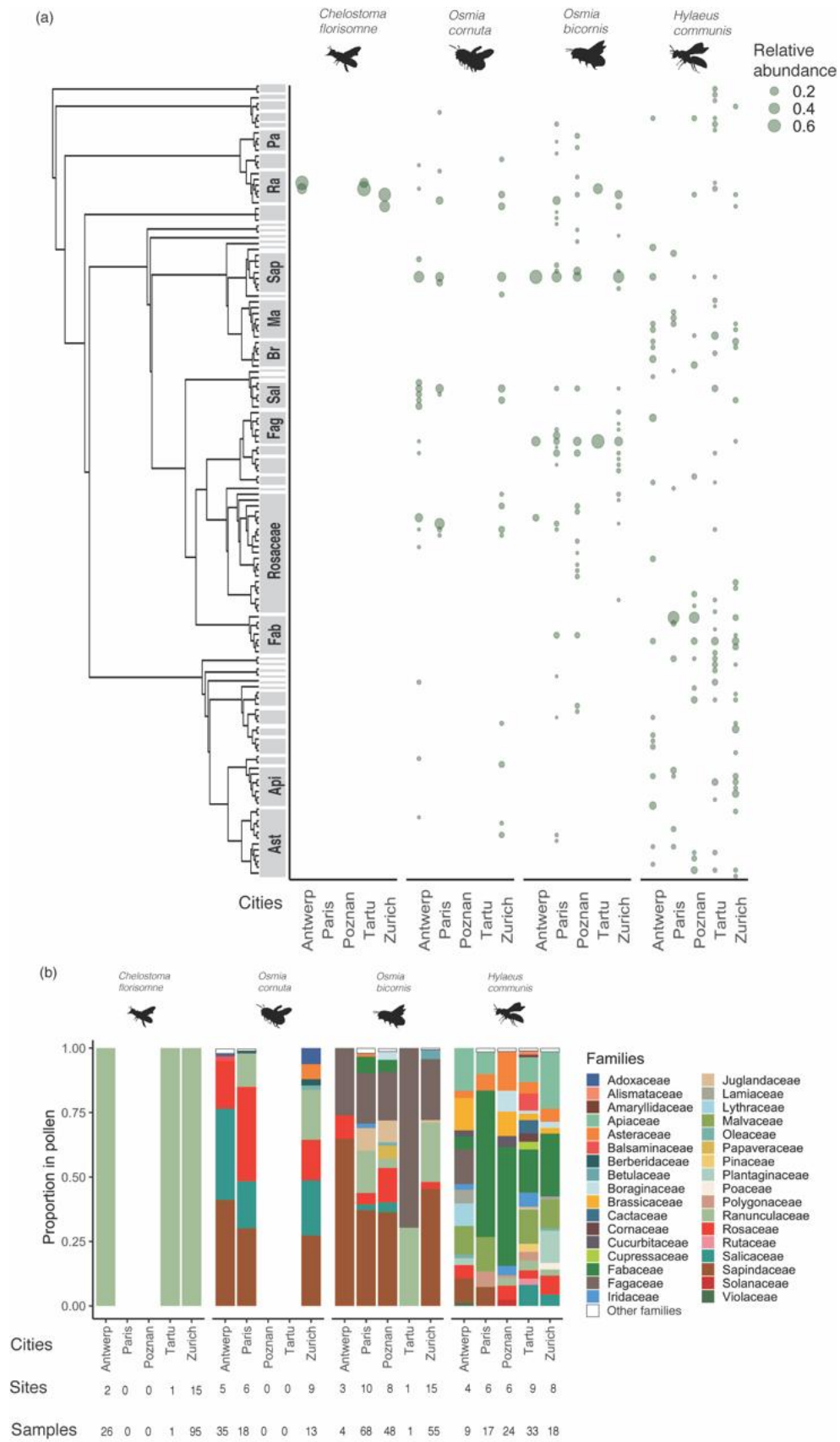
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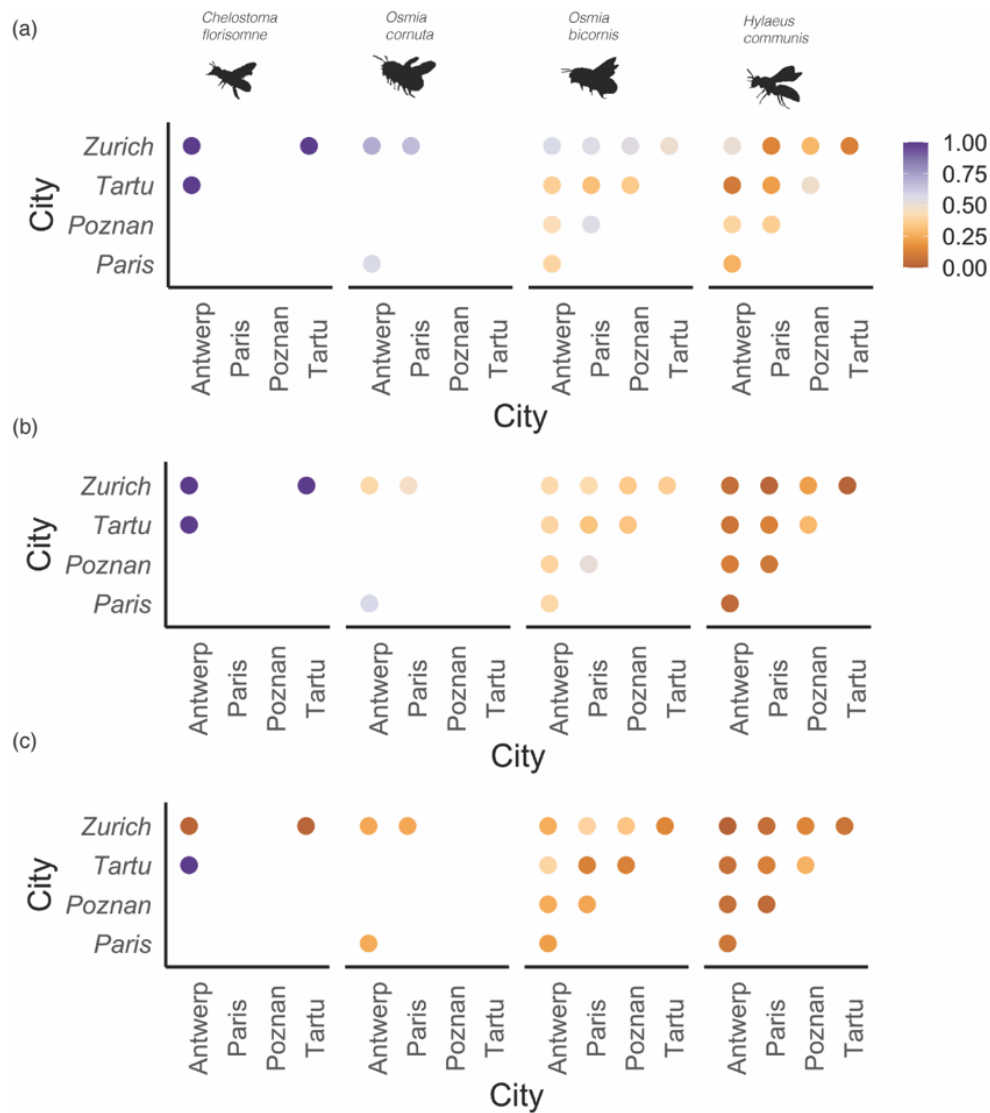
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691 **FIGURE 1.** Bee larval diet composition in the studied cities. (a) For each bee species, the collected
692 plant species in each city where the bee species was recorded (three cities for *Chelostoma florisomne*
693 and *Osmia cornuta*, five cities for *Osmia bicornis* and *Hylaeus communis*) are shown. The size of the
694 circle represents the mean relative abundance of plant species contributing to pollen samples per city
695 and bee species. (b) For each bee species, the proportion in the pollen of the different collected plant
696 families in the studied cities is shown (mean relative abundance of plant species contributing to pollen
697 samples per city and bee species). Only families with a proportion in pollen ≥ 0.01 are plotted,
698 whereas the remaining ones are represented in the category “Other families”. Note that the proportion
699 in pollen for *O. bicornis* in Antwerp and Tartu was obtained using only four and one samples,
700 respectively. For each bee and city, we provide the number of sites where pollen samples were taken,
701 and the total number of samples. Information on the computation of the phylogenetic tree can be
702 found in Text S2 and Fig. S5. Data supporting (a) can be found in Table S8. Ast, Asteraceae; Api,
703 Apiaceae; Fab, Fabaceae; Fag, Fagaceae; Sal, Salicaceae; Br, Brassicaceae; Ma, Malvaceae; Sap,
704 Sapindaceae; Ra, Ranunculaceae; Pa, Papaveraceae.

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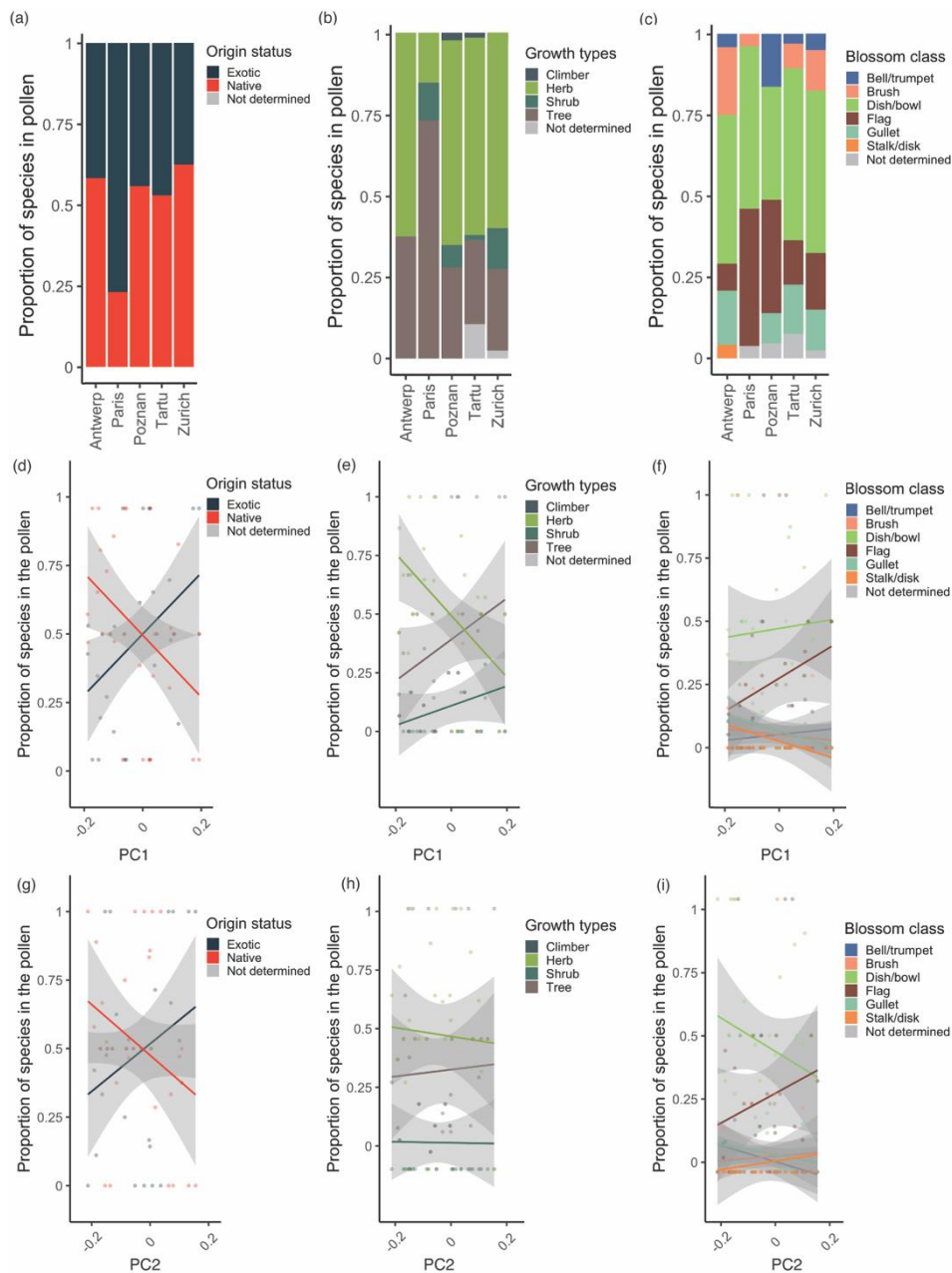
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708 **FIGURE 2.** Pairwise correlations of the larval diet composition among cities. For each of the four
 709 studied bee species, the city pairwise correlations of the collected plant taxa are shown at the family
 710 (a), genus (b), and species (c) level. The colour of the dots indicates the value of the correlation, with
 711 lower and higher values in orange and blue, respectively. Note that the correlation values are
 712 expressed as absolute values. Note also that the pollen for *O. bicornis* in Antwerp and Tartu was
 713 obtained using only four and one samples, respectively. Data supporting Fig. 2 can be found in Table
 714 S10.

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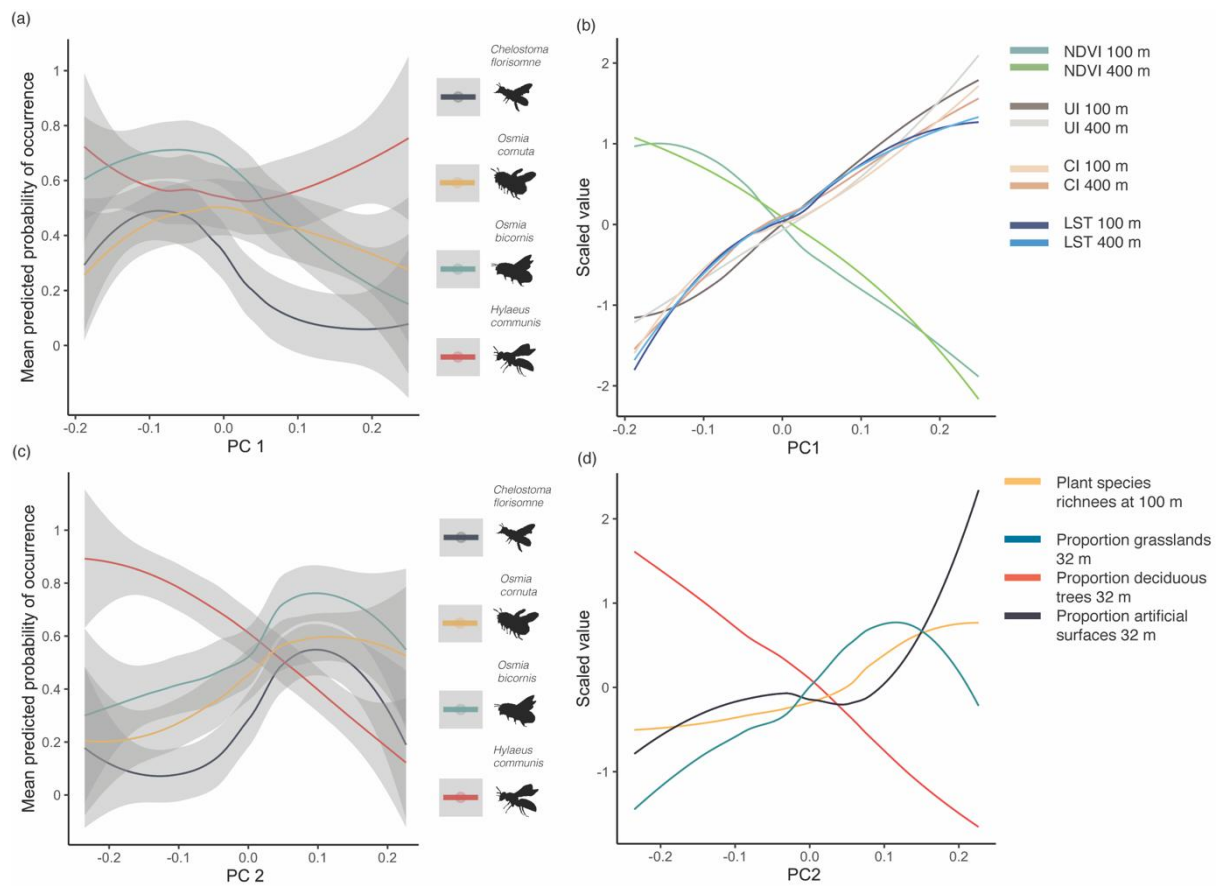
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719 **FIGURE 3.** Trait-based larval diet composition in *Hylaeus communis*. (a–c) Composition of the diet
 720 according to the origin status (a), growth form (b) and blossom class (c) of the plant species in the
 721 larval pollen. (d–g) first-order GLMs of the proportion of the different plant trait levels in relation to the
 722 first (d–f) and second (g–i) PC axes for the origin status (d and f), growth form (e and g), and (f and i)
 723 blossom class. Grey shaded bands indicate 95% confidence intervals. Higher PC1 values indicate
 724 less vegetation (lower normalized difference vegetation index) and more artificial surfaces (urban
 725 index, land surface temperature, colour index). Higher PC2 values indicate a larger proportion of
 726 grasslands and lower proportion of deciduous trees at local scales. PC1 explained 38% of the

727 variation and PC2 explained 12% of the variation. See Figs S7–S9 for the trait-based composition and
728 change along urban gradients of the other three bee species.
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732 **FIGURE 4.** Bee distribution along urban gradients. (a and c) Loess smoothing of the mean predicted

733 probability of occurrence of the four bee species in relation to the (a) first PCA axis (PC1) and (c)

734 second PCA axis, performed on the explanatory variables, representing 38% and 12% of the

735 variation, respectively. The mean predicted probability of occurrence results from the predicted

736 probabilities of occurrence of the models with TSS > 0.4. Bands represent 95% confidence intervals.

737 (b and d) Variation in the explanatory variables contributing the most to PC1 (b) and PC2 (d). (b)

738 Larger values of PC1 correspond to higher values of impervious surfaces (urban index, UI), bare land

739 (colour index, CI) and land surface temperature (LST) and lower vegetation cover (normalized

740 difference vegetation index, NDVI) at different landscape scales (i.e. 100 and 400 m). (d) Lower

741 values of PC2 correspond to higher proportions of deciduous trees and lower proportions of

742 grasslands and floral resources (plant species richness) at local scales. Other scales and variables

743 have been omitted here for simplicity (see Fig. S13). See also Fig. S12 for more details on the PCA.

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