Title Page

Title: Urban bumblebees diversify their foraging strategy to maintain nutrient intake

Short title: Foraging differences between rural and urban bumblebees

Authors:

Simonetta Selva 1,2

Marco Moretti 1

Fabian A. Ruedenauer 5

Alexander Keller 4

Bertrand Fournier 5

Sara D. Leonhardt 5

Helen Eggenberger 1

Joan Casanelles-Abella 6, 1*

* = corresponding author at joan.casanelles@tum.de

Affiliations:

1. Biodiversity and Conservation Biology, Swiss Federal Research Institute WSL, 8903 Birmensdorf, Switzerland
2. Plant Ecology, Department of Environmental Sciences, ETH Zurich, 8092 Zurich, Switzerland
3. Landscape Ecology, Institute of Environmental Sciences and Geography, University of Potsdam, 14476 Potsdam, Germany
4. Cellular and Organismic Networks, Faculty of Biology, Ludwig-Maximilians University Munich, 82152 Munich, Germany
5. Plant-Insect Interactions, TUM School of Life Sciences, Technical University of Munich, 85354 Freising, Germany

6. Urban Productive Ecosystems, TUM School of Life Sciences, Technical University of Munich, 85354 Freising, Germany

Open science statement:

Morphological trait data can be found in Eggenberger et al., (2019). Plant trait data can be found in Casanelles-Abella et al., (2021), Filipiak et al., (2022) and Tew et al., (2021). Plant composition data can be obtained from GBIF (2022) and from InfoFlora (2022). Raw data on metabarcoding is archived at NCBI, project PRJNA1137320. Processed response and explanatory data used in the analyses are available at EnviDAT under the doi: 10.16904/envidat.532.

Abstract: (150 words; current: 146)

Anthropogenic ecosystems can alter individual functions and ecological processes such as resource use and species interactions. While variability on morphological traits involved in diet and resource use has been observed between urban and non-urban populations of pollinators, the consequences on the dietary and pollen transportation patterns remain poorly understood. Here, we investigate the variability in the diet breadth of rural and urban individuals of two bumblebee species and the consequences for nutrient intake and pollen transportation. We show that urban bumblebees exhibit a broader diet breadth than their rural counterparts, driven by the enhanced floral diversity in cities. However, we found that the nutrient intake remained similar across urban and rural ecosystems. Finally, we found distinct pollen transportation patterns between urban and rural individuals. Our findings highlight the importance of considering complementary facets of species' diet and interactions when assessing the effects of anthropogenic ecosystems.

Keywords: pollination; plant-pollinator interactions; feeding behavior; land-use changes; urban biodiversity; intraspecific trait variability

Introduction

Anthropogenic ecosystems, such as cities and rural areas, have changed the foraging landscape in which animals live, driving phenotypic or genetic divergence (Lowry *et al.* 2013). Land-use changes often reduce and fragment the amount of available habitat by the expansion of impervious or highly managed agricultural surfaces. Food resource availability can lead to shifts in dietary patterns, particularly regarding two dimensions: species' diet breadth and species' nutrient intake (Gámez-Virués et al. 2015; Vaudo et al. 2016), and driving underlying trait syndromes (Hahs et al. 2023). Thus, as food resource availability is modified differently in cities and rural areas, diet-related morphological traits and the foraging strategies between urban and rural populations can also also be distinct (e.g., Cucherousset et al. 2012). Despite the implications of foraging strategies for species health and conservation (Parreño et al. 2022; Di Pasquale et al. 2013), it remains little studied, especially for invertebrates.

Bumblebees are central-place foragers and provide an excellent system for investigating how foraging strategies change between urban and rural areas. This is because the changes on habitat amount, and subsequently, on the availability of resources are expected to strongly influence such strategies (Peters et al. 2022). Links between foraging strategies, feeding specialization and both tongue length and body size have been found in several bumblebee species (Harder 1983; Pyke 1982; Wood et al. 2021). Generally, shorter tongue length is expected to increase accessibility to floral resources and enhance how efficiently floral resources are exploited, through minimizing handling time and maximizing energy return (Harder 1983; Pyke 1982; Sponsler et al. 2022a) (but see also De Keyzer et al. 2016). At the intraspecific level, variability in tongue length/body size might be in part signaling changes in the composition and structure of floral resources (Miller-Struttmann et al. 2015). Eggenberger and colleagues (2019) found reduced tongue length and body size in urban bumblebees compared to their rural counterparts in two generalist bumblebee species, in agreement with the hypothesis that increased temperatures in cities reduce body size (Gérard et al. 2021) but contrary to findings in other studies (Theodorou et al. 2021). The intraspecific differences in foraging-related traits might result in distinct plant visitation and interaction networks, potentially influencing bumblebee diet breadth, nutrient intake as well as pollination services through modified pollen transportation (*sensu* Ellis et al. 2023) in the two main structures, that is, the body and the leg-baskets (i.e., corbicula).

Urban and rural areas have distinct floral diversity patterns including the composition and structure of species and nutrients. On the one hand, rural areas, specifically when management is intensified, often have impoverished plant assemblages with a dominance (Carmona et al. 2020) but also lower proportion of non-native species than urban areas (Tew et al. 2021, 2022). On the other hand, in cities, plant diversity is often significantly enhanced due to both spontaneous and cultivated species including native and non-native species (Kühn et al. 2004), likely resulting in higher variability in floral traits such as corolla length, structural blossom class composition (Tew et al. 2021), and other floral attraction traits (Cabon et al., 2022). Simultaneously, plant distribution in urban areas might be more patchy and dominance patterns be less pronounced (Avolio et al. 2015; Frey & Moretti 2019; Swan et al. 2011) compared to rural areas. Finally, from a nutrition approach, an increased number of plant species might result in larger amounts or altered ratios of macronutrients (Pioltelli et al. 2024; Trinkl *et al.* 2020).

The differences in the structure, composition and distribution of resources in the surrounding landscape of the colony can have different effects on the dietary patterns and pollen transportation networks of urban and rural bumblebees. According to the optimal foraging theory (Fretwell & Lucas 1969), bumblebee individuals are expected to have foraging strategies and behaviors that maximize their colonies' net yield of energy (Goulson 1999). Thus, the foraging distance will depend on the degree to which the resources are accessible (or isolated) and how evenly distributed they are (Pioltelli et al. 2024). Such predictions have been confirmed in social bees, including honeybees (Bartholdi et al. 1993) and bumblebees (Dreisig 1995), with bumblebee flight duration and flight distance negatively related with the coverage of green areas around colonies in several species (Redhead et al. 2016).

In rural areas, bumblebees have been found to have a consistent diet breadth (Timberlake et al. 2019). Nevertheless, in urban areas bumblebees may have larger diet breadths resulting from the visitation of a larger and likely more distinct plant community (Hülsmann et al. 2015) than their rural counterparts. This is supported by the diet diversification hypothesis (Jha & Kremen 2013; Kaluza et al. 2017), but restricted to a certain extent by phylogenetic preferences (Wood et al. 2021). While a broader diet breadth may translate into an improved nutrient intake (Trinkl et al. 2020), this is not always the case (Moerman et al. 2017; Peter et al., 2022). Plants exhibit species-specific variations in the nutrient content of their pollen such as amino acids, fatty acids and carbohydrates (Di Pasquale *et al.* 2013; Ruedenauer *et al.* 2019; Vanderplanck *et al.* 2014), and in the production of secondary compounds and toxicity (Palmer-Young *et al.* 2019; Rivest & Forrest 2020). These differences have consequences for the nutritional landscapes they provide (Tew et al. 2021), for example by changing the composition and distribution of specific macronutrients at the landscape scale or by exposing bees to larger amounts of toxic compounds (Parreño et al., 2020). This implies that pollen diversity may not necessarily correlate with good nutrition; rather, it is the nutrient content of the pollen of the specific diet composed that matters most when making foraging choices (Moerman et al. 2017). In that regard, several bumblebee species have been found to have strict nutritional requirements for instance, focusing on low fatty acid content and high amino acid content (Ruedenauer et al. 2020; Vaudo et al. 2016) as well as maintaining an adequate ratio between fatty acids and amino acids (protein ‘P’ : lipids ‘L’ ratio, (Ruedenauer et al. 2020; Vaudo et al. 2016), adapting their foraging strategies to satisfy their nutritional demands to maximize fitness (Ruedenauer et al. 2020). Consequently, bumblebees may thrive while feeding on a reduced number of plant species if their nutrient requirements are met by the resources provided by the available plants, such as in rural areas. The strict food requirements are likely widespread across bumblebee species, but are still to be tested.

Moreover, the altered structure, composition, distribution and accessibility of the floral resources might further shape bumblebee foraging decisions (Pioltelli *et al.* 2024). When preferred floral resources are more scarce (e.g., because they are isolated, less abundant and/or more exploited by others), bumblebee might select suboptimal floral resources that require less energy investment to find and access to reduce searching and handling costs, or that are less preferred by other pollinating individuals to avoid competition (Rosenberger et al. 2022). When this results in shortages of certain nutrients, bees may be forced to increase the number of plant species they visit for compensation (e.g., through pollen mixing: Somme et al. 2015), resulting in more plant species visited per individual and a lower degree of niche overlap (Sponsler et al. 2022a, b).

Finally, novel floral landscapes, such as the ones in cities, might represent an additional challenge for bumblebees with consequences on foraging strategies and behaviors, and ultimately, on the colony fitness. Social bees might be well adapted to navigate novel floral landscapes by task division amongst workers and enhanced learning skills by observation of conspecifics (Burns & Dyer 2008; Dukas & Real 1993). However, social bees still rely on specific flower cues to guide their foraging choices (Ornai & Keasar 2020). Hence, these novel floral landscapes can impose complex decisions and trade-offs when foraging (Dukas & Real 1993). Specifically, it might require more training effort to learn how to efficiently use floral resources, for example, identifying and manipulating nutritionally suitable plant species and avoiding unsuitable ones, potentially shaping dietary patterns and pollen transportation (Evans Lisa *et al.* 2017; Laverty & Plowright 1988).

Here, we build on the work by Eggenberger et al. (2019) and compare the foraging strategies of two common intermediate, generalist bumblebee species (Wood *et al.* 2021), *Bombus lapidarius* (Linnaeus, 1761) and *Bombus pascuorum* (Scopoli, 1763), in two distinct anthropogenically-modified ecosystems, that is, urban and rural areas. We do so by analyzing a combination of pollen metabarcoding, pollen chemistry and interaction networks. Specifically, we have four main goals. First, compare the diet breadth (i.e., the multifacet diversity of collected pollen, that is, taxonomic, functional, and phylogenetic diversity) between urban and rural bumblebee populations. Second, test the influence of resource availability in the study sites and the variability in morphological traits (i.e., mouthparts and body lengths) on shaping diet breadth between urban and rural populations. Third, compare the nutrient intake patterns of two key macronutrient groups (i.e., the content and ratios of amino acids and fatty acids) between urban and rural populations. Four, compare the pollen transportation patterns (ie., the similarity in the pollen composition transported in the leg baskets and the body) and pollen transportation networks (*sensu* Ellis *et al* 2022, assessed as the plant-individual bumblebee interaction networks) in urban and rural populations.

We expect (first goal) that our species have, at least to some degree, strict requirements regarding their nutrition, particularly concerning fatty acids and amino acids as seen in other bumblebee species (Ruedenauer *et al.* 2020; Vaudo *et al.* 2016, 2020), and certain conservatism for specific plant families as seen in natural and semi-natural ecosystems (Wood *et al.* 2021). Simultaneously (second goal), according to the optimal foraging theory, we expect bumblebees to adopt foraging strategies that maximize the energy return while reducing the associated costs (i.e., searching, learning and handling effort), determined by the resource availability and the variability in morphological traits. Thus (third goal), we expect diet breadth to increase in urban bumblebees due to the larger numbers of plant species occurring and (fourth goal) the observed bumblebee intraspecific trait variability (Eggenberger *et al.* 2019) also shaping the pollen transportation networks (Biella *et al.* 2022; Vaudo *et al.* 2024) with larger number of plants visited in urban bumblebees and lower niche overlap (Figure 1). Based on this, we consider three scenarios of nutritional intake (Figure 1):

1. *Nutrient intake maintenance:* nutrient intake, specifically key indicators such as fatty acid and amino acid content and ratios, is similar in urban and rural areas, indicating strong overarching preferences for certain nutrients and ratios driving pollen selection (Ruedenauer *et al.* 2020; Vaudo *et al.* 2016). In cities, this could signal a pressure to forage on more resources to fulfill diet requirements.
2. *Urban advantage due to diversification:* nutrient intake, specifically key indicators such as fatty acid and amino acid ratios, is different between urban and rural areas, indicating a distinct foraging. When paired with expanded diet breadth, expected to happen in urban areas, this might lead to diet diversification (Jha & Kremen 2013; Kaluza *et al.* 2017), when foraging in more diverse plant assemblages, resulting in improved nutrition (Trinkl et al.).
3. *Urban disadvantage:* alternatively to scenario (2), the distinct nutrient intake between urban and rural areas might decrease nutrition in cities. This scenario may indicate that the phylogenetic constraints shown in Wood *et al.* (2021) limit the possibilities for diet diversification in cities, resulting in impoverished nutrition, which might contribute to reduced foraging-related traits.

Material and methods

Study sites and bumblebee sampling

We sampled bumblebees in urban and rural areas in three Swiss regions (hereinafter regions), specifically in the Cantons of Basel, Bern, and Zurich (Text S1, Figure S1, Table S1). For each region we selected three sampling sites in both urban (i.e., the cities of Basel, Bern and Zurich) and rural areas (except for rural Bern where only one sampling site could be selected (total = 16 sites)), following Eggenberger et al. (2019). Briefly, urban areas had at least 60% impervious surfaces, and were located in the core of the cities, and 1 km away from suburban areas and urban forests. For rural areas, we standardized sampling sites based on the following criteria: low settlement areas, meadow and pasture lands to standardize management regimes, similar in altitude to urban sites (400–600 m), proximity to water and little to no forest covers (Figure S1). Rural sites were randomly allocated within a specified area defined by a 4 km radius, which represents the maximum radius of the chosen cities.

We studied the common carder bee *Bombus pascuorum* (Scopoli, 1763) and the red-tailed bumblebee *Bombus lapidarius* (Linnaeus, 1761). These two bumblebees are common in the Swiss lowlands and present in both urban and rural areas. They are both generalist, although *B. pascuorum* has a longer tongue than *B. lapidarius*.

Bumblebees were collected by hand-netting following targeted sampling, in the highest activity months of the season for both species, that is, July to mid-August, in 2016. Within each 800-m radius, we collected 30–40 individuals per species, except for one urban site in Bern where only three individuals of *B. lapidarius* were found. Sampling efforts were standardized across all sites and conducted during peak bumblebee activity hours (09:00–17:00) and under optimal weather conditions. We walked the entire 800-m radius searching for the targeted bumblebee species, but limited the collection to a maximum of ten individuals at a given location within the circle. Only active foragers were collected. Species identity of all collected individuals was verified in the laboratory, and specimens that could not be clearly identified were removed. For more detailed information on the study design and sampling of the bees see Eggenberger et al. (2019) and Text S1.

Pollen collection and metabarcoding

We extracted pollen from the corbicula and the body of the bumblebees separately (see Text S2). In total, from the collected individuals in Eggenberger et al., (2019), we used 152 individuals of *B. pascuorum* and 238 individuals of *B. lapidarius* across all sampling sites from which we found pollen in both the corbicula and the body (Table S2-S3). We extracted two samples per individual, one of the pollen of the corbicula (leg pollen) and one from the pollen from the body, resulting in 390 samples of corbicula pollen and 390 samples of body pollen in total. On average, sampling sites had 9.4 individuals of *B. lapidarius* (minimum-maximum: 1-25, Table S2) and 14.4 individuals of *B. pascuorum* (minimum-maximum: 6-27 individuals, Table S2).

DNA metabarcoding (isolation, amplification, and sequencing) of pollen samples was performed by AllGenetics laboratories (AllGenetics & Biology SL; A Coruña, Spain). In summary, the ITS2 region was amplified according to existing protocols (Campos *et al.* 2021; Sickel *et al.* 2015). The libraries were then purified, pooled, and sequenced on an Illumina NovaSeq platform. Taxonomy was assigned to amplicon sequence variants (ASVs) using a pre-trained classifier, and filtering steps were applied to remove singletons and correct for mistagging issues. For more details on the workflow see Text S3.

Regarding bioinformatics, we followed the pipeline described at https://github.com/chiras/metabarcoding_pipeline (Leonhardt *et al.* 2022). The pipeline was applied with VSEARCH v2.14.2 (Rognes *et al.* 2016)for merging, quality truncation and filtering (maxEE < 1; 150 bp < sequence length < 300 bp). Cleaned reads were denoised to amplicon sequence variants (ASVs) and Chimera filtered with VSEARCH (Rognes *et al.* 2016). ASVs were first directly mapped with global alignments using VSEARCH against a floral ITS2 reference database for the study region and an identity cut-off threshold of 97%. This database was created with the BCdatabaser (Keller *et al.* 2020) and with a list of potential plants that could be present in the study region. For still unclassified reads, we used SINTAX (Edgar 2016) to assign taxonomic levels as deep as possible with a global reference database (Quaresma *et al.* 2024).

Nutritional analyses

We focused on two critical macronutrients for bumblebee health and fitness: amino acids and fatty acids (Roulston & Cane 2000). In order to have a sufficient pollen mass to perform the nutritional analyses, we pooled pollen (from the leg-baskets) from different bumblebee individuals collected by Eggenberger et al. (2019) within study sites and for each species separately. In total, we gathered 93 samples (*B. lapidarius:* 42, *B. pascuorum:* 51, Table S3) for fatty acid analyses and 85 samples (*B. lapidarius:* 34, *B. pascuorum:* 51, Table S3).

Amino acid analysis

We used ion exchange chromatography (IEC: Biochrom 20 plus amino acid analyzer) to analyze protein-bound amino acids (AAs) in pollen, following the protocol outlined by Kriessell et al. (2017).

Initially, 5-10 mg of the collected pollen was extracted in an ultrasonic bath using 100 µl of deionized water for 30 minutes. The extract was then refrigerated for 60 minutes, followed by centrifugation and membrane filtration for 10 minutes. The residue, kept for protein-bound amino acid analysis, was mixed with 200 µl of 6 N HCl, boiled at 100°C for 4 hours, cooled to room temperature, and centrifuged for 10 minutes. The supernatant had its water and HCl evaporated at 100°C, and the sample was re-dissolved and boiled at 100°C in 200 ml of fresh water until complete dryness, a process repeated twice, followed by another centrifugation. 100 µl of the supernatant was then mixed with 20 µl of 12.5% sulfosalicylic acid, frozen overnight, and extracted in the refrigerator for 30 minutes the next day. The sample was briefly mixed, centrifuged again for 10 minutes, and 100 µl of the supernatant was combined with 100 µl of sample rarefaction buffer (lithium buffer). This mixture was membrane-filtered in the centrifuge for 5 minutes, and 20 µl of the filtrate was diluted in 80 µl of sample rarefaction buffer for IEC analysis. To quantify amino acids, we used an external standard (physiological calibration standard, Laborservice Onken GmbH, Gründau, Germany) containing all proteinogenic amino acids except glutamine and asparagine, which were manually added before running standards and samples. Tryptophan could not be analyzed as it is destroyed in HCl. The total protein content was calculated as the sum of all amino acids. Therefore, in this study, the AA content always refers to the total content of protein-bound AAs. Moreover, we also calculated the total content of essential AAs, which cannot be synthesized by animals and have to be obtained exclusively from the diet, and non-essential AAs, which can be synthesized by animals (Table S4). Additionally, we also calculated the ratio between essential and non-essential AAs (Table S4).

Fatty acid analysis

The analysis of fatty acids (FAs) followed the protocol outlined by Villagómez et al. (2023). Specifically, 0.5 mg of each pollen sample and 7 µl of a 200 ng/µl solution of nonadecanoic acid in chloroform (used as an internal standard) were homogenized in 0.1 ml of a 2:1 mixture of chloroform and methanol (both from Sigma-Aldrich, Taufkirchen, Germany). To achieve further homogenization, an additional 0.4 ml of the chloroform-methanol mixture was added, and the mixture was then transferred to a new, larger vial along with an additional 2.5 ml of the chloroform-methanol mixture, resulting in a total volume of 3 ml. The samples were shaken at 250 rpm for 24 hours and evaporated to dryness. Subsequently, 10 µl of trimethylsulfohydroxide (TMSH) in 150 µl of dichloromethane (both from Sigma-Aldrich) was added, and the samples were analyzed using a gas chromatograph (GC, Agilent Series 8890) coupled via a splitter to both a mass spectrometer (MS, Agilent 5977C) and a flame-ionization detector (FID). Helium was used as the carrier gas. We injected 1 µl of the sample in splitless mode at 300°C. The initial oven temperature was set at 60°C, increased to 150°C at a rate of 15°C/min, held for 10 minutes, then increased to 320°C at a rate of 10°C/min, and held for another 10 minutes. Fatty acids were identified by comparing the mass spectra and retention times of peaks in the resulting chromatograms (MS) to standards (e.g. FAME C8-C24 and single fatty acid standards, Sigma-Aldrich), while the chromatograms obtained from the FID were used to quantify fatty acids via the internal standard. In this method, di- and triglycerides are broken down into fatty acid methyl esters. Therefore, similar to amino acids, the FA content in this study always refers to the total content of free and glyceride-bound FAs. Additionally, we also calculated the content and ratios of specific types of FAs relevant for bee nutrition, survival and health (Mannig, 2015). Particularly, the content of saturated, non-saturated, omega 3, omega 6, and omega 9 FAs, and the ratios between saturated vs. unsaturated FA, and between Omega 3 vs. Omega 6 (Table S4). These different metrics on FAs can be related to nutrition and health.

Floral resource availability in the landscape

Floral resource availability in the landscape was inferred using plant species richness as done in other studies (Kithara et al., 2008), since better measures, such as abundance and biomass were not available. Particularly, floral resource availability was assessed by complying a list of plants occurring at each site within a 1500-m buffer following bumblebee foraging ranges using data from two sources: the Global Biodiversity Information Facility (GBIF, 2022) and the National Data and Information Center on the Swiss Flora (InfoFlora, 2022). InfoFlora is the central organization for monitoring plant diversity in Switzerland, which conducts exhaustive plant surveys throughout the country, from which we retrieved plant data from 1759 species. However, InfoFlora is focused on native and invasive species, which might underestimate the number of plant species in cities avoiding the non-native and ornamental ones that are non-invasive. Therefore, we complemented the plant list with plant occurrences from GBIF (2022), adding 443 species.

Plant traits

We used plant floral traits to understand the mechanisms behind the flower choice of bumblebees (Ornai & Keasar 2020). In particular, we recorded data on seven functional traits, including flowering onset, flowering duration, growth form, plant height, blossom class, symmetry of the flower, and nectar sugar concentration (see details on measurements in Filipiak *et al.* 2022) and one descriptive trait, namely the origin status (native/non-native) (Table S5). Plant traits were collected from multiple published, open-source data sets (Casanelles-Abella *et al.* 2021; Filipiak *et al.* 2022; Tew *et al.* 2023). As the availability of sugar concentration data was limited, we retrieved sugar concentration data for the most abundant plant species among others (Figure S2), which together represented 79 % of the relative abundance of the plant species visited by bumblebees.

Bumblebee morphological traits

We used the morphological traits measured by Eggenberger et al. (2019), which are directly or indirectly linked to foraging behavior (Table S6). Specifically, we used the intertegular distance, proboscis length, forewing length, and corbicula length. Due to the existing allometric relationships between body parts, for forewing, proboscis and corbicula length, we calculated the ratio between the traits and intertegular distance (i.e., proboscis ratio, corbicula ratio, forewing length ratio).

Statistical analysis

We conducted all analyses in R version 4.2.1 (R Core Team, 2023) with RStudio version 2022.7.2.576 (RStudio Team, 2023).

Diversity metrics for the dietary breath

Diet breadth has been defined as the total number of resources in the diet (Kaplan & Hill 2017). In bees, diet breadth has been assessed using taxonomic diversity (Wood *et al.* 2019; Wood & Roberts 2017). However, other diversity facets might provide complementary interpretation of diet breadth. Thus, here we used taxonomic, functional and phylogenetic metrics to infer bumblebee diet breadth. We used plant species richness as the metric for plant taxonomic diversity (Wood & Roberts 2017). We then calculated multidimensional functional diversity metrics, specifically focusing on three main dimensions, that is, functional richness, functional evenness, and functional divergence, using the package "FD" version 1.0-12.1 by Laliberté & Legendre (2010). We used the plant traits flowering duration, structural blossom class, and sugar concentration of the nectar to compute the different functional diversity indices, as the other traits (i.e., symmetry, flowering start, and plant height) had large (>0.7) correlations (see Figure S3). In addition, to compute a functional index the number of plant species in the pollen must be larger than the number of traits. Because our bumblebee individuals often do not carry more than four plant species, we limited the number of traits to three to avoid filtering too many bumblebee individuals, and thus, excluded growth form. Finally, we calculated multidimensional phylogenetic metrics, specifically, phylogenetic variability, phylogenetic richness, phylogenetic evenness, and phylogenetic clustering, using the package "picante" version 1.8.2 by Kembel *et al.* (2010). We used the phylogeny in Jin and Qian (2019). For functional metrics, bumblebee individuals with less than four plant species in the collected pollen were excluded as the convex hull could not be computed. For phylogenetic metrics, bumblebee individuals with less than three species were removed, leading to a total number of 154 bumblebees included for the functional diversity (*B. lapidarius:* urban = 40, rural = 29; *B. pascuorum:* urban = 64, rural = 21; Table S3), and 264 individuals included for the phylogenetic diversity (*B. lapidarius:* urban = 61, rural = 52; *B. pascuorum:* urban = 98, rural = 53; Table S3).

Comparing diet breath and nutrient intake in urban and rural areas

We compared the dietary patterns (i.e., diet breadth and nutrient intake) of urban and rural bumblebees. For diet breadth, we used the computed taxonomic, functional, and phylogenetic metrics at the individual level. For nutrient intake, we considered the different content and ratios of AAs and FAs (see section "Nutrient analyses"). We used linear mixed effects models with landscape (urban and rural) as a fixed factor and region as a random effect. Contrasts were done separately for the two bumblebee species. To correct for multiple comparisons, we used the Holm correction (Holm 1979). Furthermore, we compared the composition of the selected traits between urban and rural populations for each bumblebee species separately using Chi^2 tests.

We also assessed the diet consistency for both bumblebee species separately, following Casanelles-Abella et al. (2022). Specifically, we performed the pairwise correlation between bumblebee diets across study sites. This was done by calculating Pearson correlations between binary trophic interaction matrices at different landscape and region levels (i.e., urban and rural for the three studied regions) for each bee species at the family, genus, and species levels of the plant species. To control for differences in available plant species, we created a list of plants occurring at each site using data from GBIF (2022) and InfoFlora (2022) within a 1500 m buffer following Osborne et al. (2008). Interactions were excluded from the correlation calculation if a plant family, genus, or species was missing from one of the plant species pools of the two groups being compared.

Influence of landscape type, floral resources and bee morphological traits on diet breadth and nutrient intake

We assessed direct and indirect effects of landscape type, floral resources and bee morphological traits on the plant species richness in the collected pollen in the corbicula for each landscape type (urban and rural), available resources at the landscape scale within 1500 m (plant species richness) and two uncorrelated bee morphological traits related to foraging, that is, intertegular distance and proboscis ratio. We used multilevel structural equation modeling, implemented in the *piecewiseSEM* package version 2.3 (Lefcheck 2016), following Shipley (2016), Tresch *et al.* (2019) and Casanelles-Abella *et al.* (2023). We used generalised linear mixed effects models (GLMMs) as composite SEMs. We performed basis set constructions, goodness-of-fit tests, and parameter estimations according to the corrected Akaike information criterion (AIC_c), the Bayesian information criterion (BIC), and Fisher's C statistic ($p < 0.05$; Shipley 2016). We computed pairwise correlation coefficients among predictors before the analyses, and excluded predictors with correlation coefficients larger than 0.7 (Figure S3). All variables were centered and scaled before the analyses. Furthermore, missing paths in the SEM were checked with Shipley's d-separation test (Shipley 2013).

The final SEM model included four components. The main model (component 1) for plant species richness in the collected pollen included the available resources at the landscape scale (within 1500 m radius), the intertegular distance and the proboscis ratio as predictors. In addition, we also assessed the influence of the landscape type on the available floral resources at the landscape scale (component 2). Finally, we also assessed the influence of landscape type (urban and rural) and available resources at the landscape scale on the intertegular distance (component 3) and the proboscis ratio (component 4). In all models, we used site as random factor. Finally, we checked model assumptions, as well as potential spatial autocorrelation patterns in the response variables and the model residuals, by means of Moran's I autocorrelation.

Additionally, we conducted GLMMs on the additional metrics used to assess diet breadth, that is, functional and phylogenetic diversity metrics. We analysed functional and phylogenetic diversity metrics separately from plant species richness as the number of individuals was lower, as functional and phylogenetic metrics could not be computed in bee individuals carrying an insufficient number of plant species in their pollen loads. First, we removed highly correlated metrics (functional richness, phylogenetic clustering, phylogenetic richness). Then, modelled the remaining functional and phylogenetic metrics (i.e., functional evenness, functional dispersion, phylogenetic variance) using as predictors landscape type, available floral resources at the landscape scale, intertegular distance and the proboscis ratio with site as a random factor.

To compare total, essential, and non-essential AA content, as well as total, saturated, unsaturated, omega 3, omega 6, and omega 9 FA content, and the ratio between AAs and FAs (P:L ratio) between urban and rural sites, we used GLMMs with site nested within the region as random factors.

Pollen transportation

We studied pollen transportation patterns classifying the collected plants according to the pollen transportations structure they were found in, that is, in the leg (i.e., corbicula), in the body, or in both structures. We calculated the proportion of plants in these three categories separately per bumblebee species and landscape type (e.g., for urban populations of *B. lapidarius*).

Additionally, to further explore both pollen transportation and dietary patterns, we built bipartite pollen transportation networks and calculated different network metrics using the packages bipartite (Dortman, 2008) and igraph (Csardi & Neputz, 2006). We assembled the networks separately for each species, landscape type (i.e., urban and rural), and two pollen transportation structures (i.e., body and corbicula). We assembled networks considering the individual bumblebees as individual nodes, and using the relative abundances as a measure of strength of interaction. For each network, we calculated the mean number of links per bumblebee individual and per plant species, the niche overlap between bumblebee individuals, the modularity, the generality and vulnerability. These metrics, especially when compared, might further indicate how individuals use the resources and elucidate specific mechanisms (Biella et al. 2022; Ellis et al. 2023; Vaudo et al. 2024).

Results

We found a total of 231 plant species belonging to 47 families across all study sites visited by the two bumblebee species. Although having a longer tongue, *B. pascuorum* foraged on more plant species than *B. lapidarius* (*B. pascuorum* = 176 species, *B. lapidarius* = 157 species). The two bumblebee species predominantly foraged on plants from the family Fabaceae (Figure S4-S5, *B. pascuorum* = 84 %, *B. lapidarius* = 81%), with the species *Trifolium pratense* (*B. pascuroum* = 67%, *B. lapidarius* = 32%) and *T. repens* (*B. pascuroum* = 9%, *B. lapidarius* = 22%) and *Lotus corniculatus* (*B. pascuroum* = 4%, *B. lapidarius* = 23%) representing a substantial part of the collected pollen. However, they differed in the other preferred plant families (see Figure S4-S5). Specifically, *B. lapidarius* also collected a larger amounts of Asteraceae pollen (*B. pascuroum* = 0.1%, *B. lapidarius* = 4%, Figure S4-S5), while *B. pascuorum* preferred Boraginaceae (*B. pascuroum* = 3%, *B. lapidarius* = 0.3%, Figure S4-S5).

Differences in the diet breadth

Urban bumblebees had a wider diet breadth than their rural counterparts (Figure 1-2, S7-S9). First, urban bumblebees collected a larger number of plant species in their pollen load than their rural counterparts (*B. pascuroum:* urban = 69, rural = 34; *B. lapidarius:* urban = 55, rural = 21; Figure 1-2, Table S7). Urban bumblebees also visited a wider range of plant families, while rural bumblebees tended to forage from a more limited number of families (*B. pascuroum:* urban = 22, rural = 11; *B. lapidarius:* urban = 20, rural = 9; Table S7-S8, Figure S4-S5). Similarly, we found diet consistency at the plant family level to be higher in rural than urban areas in both species (Figure S6). At the plant genus and species level, the consistency was much reduced in both urban and rural areas (Figure S6). Second, we found differences in the plant trait composition in the pollen collected between urban and rural areas. Specifically, urban bumblebees visited a greater diversity of structural blossom classes (i.e., floral shapes) in urban areas (*B. pascuroum:* χ^2 = 102.66, *p*-value = <0.001; , *B. lapidarius:* χ^2 = 128.62, *p*-value = <0.001; Table S9, Figure S7) and a slightly higher percentage of woody plants in cities (Figure S7), particularly for *B. pascuroum*. Regarding the origin status of the plants (i.e., native vs. non-native), for *B. pascuorum* we did not find a significant increase in the number of visited non-native plants in urban areas compared to rural areas (Table S7, Figure S7). Conversely, for *B. lapidarius* we found a significant increase in the non-native species in cities than in rural areas (χ^2 = 18.74, *p*-value = <0.001; Table S8, Figure S7). The larger diet breadth of urban bumblebees as compared to rural ones was not only visible in terms of taxonomy but also in functional and phylogenetic dimensions of plants visited (Table S8, Figure 2). This indicates that rural bumblebees foraged on a reduced number of plants that in addition, were functionally similar and phylogenetically closely related.

Influence of landscape type, floral resource availability and bee morphological traits on diet breadth

We performed multilevel structural equation models to study the direct and indirect effects of landscape type, resource availability, and morphological traits on the dietary patterns of the two bumblebee species. First, our results revealed a main role of resource availability at the landscape scale (inferred as the plant species richness per site) in shaping diet breadth of both bumblebee species (Figure 3). In both cases, resource availability at the landscape scale positively increased the species richness collected (*B. lapidarius:* 0.376 ± 0.115, p-value = 0.001; *B. pascuorum:* 0.449 ± 0.139, p-value = 0.001; Table S10). Furthermore, resource availability at the landscape scale was much larger in urban areas (mean, min-max: 1457, 947-1884 plant species) than in rural areas (433, 322-530 species) (Figure 3, Figure S8, Table S10). Conversely, plant diet breadth patterns were not affected by bumblebee morphological traits, that is, intertegular distance and proboscis ratio (Figure 3). Furthermore, intertegular distance decreased in urban landscapes and was positively correlated with plant species richness at the landscape scale in both species (Figure 3, Figure S9, Table S10). Proboscis ratio in *B. pascuorum* also decreased in urban landscapes. Finally, the GLMMs on the plant functional and phylogenetic metrics (Table S11) did not indicate any significant effect of plant resources and bee morphological traits in shaping the diversity metrics (Table S11).

Differences in the nutrient intake

Nutritional intake did not significantly differ between urban and rural bumblebee populations for neither of the two species (Figures 4-5, Table S12). Nonetheless, we observed a decrease in the concentrations of total AA, total essential AA, and total concentrations of non-essential AA in urban compared to rural populations particularly for *B. lapidarius* (Figure 4, Table S12), which were significant before applying post-hoc correction. Specifically, we observed a 32% decrease in the total AA content, a 32% decrease of essential AA. and a 27 % decrease in non-essential AA (Figure 4). Regarding FAs, there were no clear differences between urban and rural populations (Figure 5, Table S12). Interestingly, we found more variation in the AA and FA metrics in rural populations than in urban for both bumblebee species, which were stronger in *B. lapidarius.* Finally, we also did not observe differences in the ratio of AAs and FAs between urban and rural areas, which were similar across areas (Figure 6, Table S12).

Pollen transportation

We examined the difference in the pollen transportation patterns in relation to transportations structure (i.e., leg-baskets and body), considering separately the two bumblebee species and the landscape type where they were collected (i.e., urban and rural). We found contrasting differences in the pollen transportation between bumblebee species, and within species, between urban and rural populations (Figure 7, Figure S10-S12). For *B. lapidarius*, we found a similar distribution in the proportion of species that were present both in their body and leg basket pollen load (rural: 52%, urban: 57%; Figure 7). Thus, these shared plant species in the leg-baskets and the body are potentially available for both pollination (pollen in the body) and as food resource for their larvae (pollen in the legs). In addition, the proportion of plant species with pollen transported exclusively on the bumblebee body was also similar between urban and rural populations (rural: 28%, urban: 31%; Figure 7). Moreover, in urban populations, the proportion of plant species whose pollen was only transported in leg baskets, and thus, likely less available for pollination, was lower than in rural populations (rural: 20 %, urban: 12 %, Figure 7). On the other hand, for *B. pascuorum*, we found the proportion of plants with pollen transported in both their body and leg to be much larger in rural than in urban populations (rural: 74 %, urban: 52 %; Figure 7), with the proportion of plant species with pollen transported only on the body being much larger in urban individuals (26%) than in rural (5%). Contrary, in *B. lapidarius*, the proportion of plant species with pollen only present in the leg baskets was similar between urban and rural populations (rural: 21 %, urban: 22%; Figure 7).

Regarding the pollen transportation networks, we found differences in the network metrics between urban and rural areas for both bumblebee species and pollen transportation structures (Table S13). Particularly, we found urban populations to have increased numbers of links per bumblebee individual (*B. pascuorum*$_{leg}$: 33.97%, *B. lapidarius*$_{leg}$: 30.51%, Table S13), and thus, increased generality (*B. pascuorum*$_{leg}$: 14.81%, *B. lapidarius*$_{leg}$: 42.44%, Table S13). Furthermore, we found reduced niche overlap (*B. pascuorum*$_{leg}$: -40.32%, *B. lapidarius*$_{leg}$: -33.33%, Table S13). Finally, we found modularity to increase in urban populations of *B. lapidarius* ca. 57% (Table S13), but to decrease ca. 14% (Table S13) in populations of urban *B. pascuorum*.

Discussion

Species dietary patterns (i.e., diet breadth and nutrient intake) and pollen transportation patterns are still little investigated but critical to understand how species cope with anthropogenic pressures and interact with resources in urban and rural areas. Our integrative approach combining pollen metabarcoding and nutritional analyses on the pollen loads of urban and rural individuals of two bumblebee species, *B. pascuorum* and *B. lapidarius*, provide evidence for our first scenario of diet maintenance, in which an increase in diet breadth, explained by larger floral resources, did not translate into a better nutrient intake. In that regard, bumblebees appear to maintain the acquisition of macronutrients. However, consistency in nutrient intake was less pronounced for AA, and more marked for FA and the ratios between AA and FA. This agrees with previous findings indicating that bumblebees primarily focus on FA rather than AA intake (Ruedenauer *et al.* 2020).

Our results support the diet diversification hypothesis (Jha & Kremen 2013; Kaluza *et al.* 2017), that is, that more diverse foraging landscapes diversify dietary patterns with regard to diet breadth. Urban bumblebees had broader diet breadths than their rural counterparts. This is likely a consequence of two key changes in urban plant communities: (1) an increase in the number of species, and (2) a more even distribution of plant species within the communities (Faeth et al. 2011). Enhanced food resources have been documented to expand diet breadth in urban vertebrates for both herbivores and predators (Anders *et al.* 2022; Gámez-Virués *et al.* 2015), and based on our results, it seems to be the case also for generalist insect florivores. Interestingly, while our two species have a degree of diet conservatism, with preferences for Fabaceae species (specifically, *T. pratense* and *T. repens,* Wood *et al.* 2021, Timberlake *et al.* 2024), these preferences were more marked in rural populations (as seen in other ecosystems, Wood *et al.* 2021) than in urban ones, showing that diet conservatism can be adjusted (Ruedenauer *et al.* 2016). Finally, the diet diversification hypothesis was not supported for nutrient intake, with bumblebees apparently maintaining nutrient intake.

Our findings show that bumblebees are able to secure an adequate nutrient intake across variable landscapes. This supports prior findings stressing the importance of an optimal nutrient intake and the foraging strategies evolved to achieve it (Peters *et al.* 2022; Ruedenauer *et al.* 2015). There are several strategies expected to optimize nutrient intake in bumblebees, regarding their cognition and learning skills (Hemingway *et al.* 2024; Rands *et al.* 2023). Bumblebees are able to inform their choices through rapid evaluation of floral rewards (Ruedenauer *et al.* 2015;

Zhou *et al.* 2024), and have been found to consistently discriminate and select high quality pollen (Ruedenauer *et al.* 2016, 2020; Vaudo *et al.* 2016). Bumblebees are able to do so by integrating multiple information cues from flowers (Rands *et al.* 2023), including not only olfactory and visual but also humidity (Harrap *et al.* 2020), and temperature stimuli (Harrap *et al.* 2020), as well as electric fields (Hunting *et al.* 2022). This multimodal integration can be further enhanced by the social learning skills among conspecifics (Avarguès-Weber *et al.* 2018; Bridges *et al.* 2024). Altogether, this might allow generalist bumblebee species to couple with the variations in plant communities such as the ones observed between urban and rural areas.

The nutrient intake maintenance scenario can be explained by mechanisms revolving around the structure of floral resources (Sponsler *et al.* 2023) and the fact that bumblebees are restricted in their nutrient requirements, which forces them to regulate their intake (Ruedenauer *et al.* 2020). Such mechanisms can then be viewed through the lenses of the optimal foraging theory and landscape ecology. While our study does not enable us to disentangle whether neutral or enforced mechanisms drive our results due to the lack of data on the structure of floral resources, bumblebee flight distances in relation to the forage landscape and on bumblebee health and fitness (Parreño *et al.* 2022), it represents a first step towards understanding the factors shaping animal foraging in anthropogenically-modified ecosystems.

Urban and rural areas might have equivalent optimal foraging landscapes and then, diet breadth expansion might emerge from neutral processes. Particularly, this can be expected if (1) the reduction in plant dominance is not affecting preferred plant species (optimal resources), or, if so, if this is compensated by the increase in plant diversity with functionally redundant species, and (2) if resources remain accessible and sufficiently abundant in the cityscape surrounding bumblebee colonies. If so, the expansion of the diet breadth might even be reinforced by having additional benefits for the stability and resilience of bumblebee colonies, such as reducing, to a certain degree, the dependence on specific plant taxa and diluting toxic pollen through mixing different pollen types (Eckhardt *et al.* 2014). In fact, urban areas may have overall similar nutrient landscapes compared to rural areas provided by different plant species (Tew *et al.* 2021).

Alternatively, our results might indicate that urban areas, rural areas or both have suboptimal conditions, enforcing diet diversification. Suboptimal conditions are reflected in the structure of the floral resources, making key floral resources scarcer, less accessible and/or of lower quality, incurring higher energetic costs (searching and handling resources, competition) with potential consequences on individual and colony fitness (Theodorou *et al.* 2022), as seen also in solitary bees (Peters *et al.* 2022). Suboptimal rural foraging landscapes can emerge from impoverished plant communities due to land-use changes and intensity, and a lower degree of compensation from human-investment (e.g, cultivation, rewilding, restoration; Klaus 2013; Swan *et al.* 2011). However, we found pollen transportation networks to have a large degree of niche overlap in rural populations. While this might indicate potential higher competitive pressure, it might also suggest lower competitive pressure. Particularly, if rural areas have sufficiently large amounts of *T. repens* and *T. campestre* preferred resources of our bee species, to satisfy the demand of the bumblebee populations. Importantly, without temporal data it is not possible to disentangle what is driving niche overlap.

Suboptimal urban foraging landscapes might be a consequence of changes in diversity distribution and community structure. Particularly, when preferred optimal resources are reduced and are not compensated by the addition of other plant species. While cities are associated with increasing plant diversity, resulting from multiple habitat types and levels of human facilitation (Swan *et al.* 2011), the diversification effect might be counteracted by the addition of plant species of little or no value for bumblebees. In fact, part of the urban plant communities arises from cultivation, including non-native species, horticultural hybrids and varieties, which are not necessarily nectar and pollen hosts for pollinators (Garbuzov *et al.* 2017; Garbuzov & Ratnieks 2014). The generalist diet of bumblebees might enable them to better use such resources than other pollinating insects, but restrictions can still be expected when certain degrees of diet conservatism exist (Wood *et al.* 2021). Notably, we found low contributions of non-native species in both bumblebee species, indicating potential constraints in the use of novel plant resources. Finally, suboptimal foraging landscapes are expected to intensify competition for optimal resources, further forcing individuals to switch their diet to suboptimal resources to avoid the negative costs of competition and approximate to optimal foraging. In that regard, our pollen transportation networks indicate more dissimilar interaction networks in urban bumblebees, with individuals interacting with a larger, more variable number of plants. Increasing diet breadth has been identified as a mechanism triggered with higher local abundances of bumblebees, potentially as a mechanism to avoid competition (Fontaine *et al.* 2008; Glenny *et al.* 2024).

On a different perspective, the structure of urban floral resources might represent a learning challenge for bumblebees. On the one hand, bumblebees can learn how to use and access new resources, and several generalist species have been found to have a certain degree of flexibility in their diet both experimentally (Zhou *et al.* 2024) and in the field (Jha & Kremen 2013; Sponsler *et al.* 2022a). However, bumblebee learning capabilities have some constraints due to the nature of the associative learning between floral cues, rewards and foraging decisions (Hemingway *et al.* 2024). Enhanced floral types might increase the variability of floral rewards, hampering associative learning, and hence, decreasing the ability to discriminate between suitable and non-suitable plants (Dukas & Real 1993; Hemingway *et al.* 2024). In cities, such conditions might be caused by the addition of many plant species that might not be preferred by the bumblebees, for example, because of their existing trade-offs for foraging efficiency (Pattrick *et al.* 2023) or low nutritional value (Ruedenauer *et al.* 2015). As more complex floral landscapes exist in cities, bumblebees might get exposed to flowers of non-targeted plants in the process of learning and maximizing their foraging strategies and the energy gain. For example, we found *B. pascuorum* to have larger proportions of pollen transported only in the body in cities, perhaps suggesting more learning trials and contact with non-targeted plant species. Although bumblebee individuals tend to specialize between pollen and nectar foragers (Russell et al. 2017), all individuals must still consume nectar to fuel their flights (Combes et al. 2020). Learning challenges in cities, and hence, the derived costs on fitness to maintain an optimal foraging, deserve more attention to better understand what are the challenges and opportunities of novel floral landscapes.

Our study had some limitations that limited assessing the importance of intraspecific trait variability in explaining the variability in the diet breadth between urban and rural bumblebees. Variation in body size and tongue length can be expected to influence foraging of bumblebees as they influence flying distance, amount of pollen that can be carried, and how efficiently flowers can be handled (Chole et al. 2019). There are inconsistent results concerning if short-tongued or long-tongued individuals might better exploit existing resources (Spaethe & Weidenmüller, 2002, Williams & Osborne 2009), with experimental studies supporting (Spaethe & Weidenmüller, 2002) or not (Reverté et al., 2023) the influence of intraspecific variation on foraging . The lack of findings in our study seems to indicate that intraspecific trait variation may be a consequence of physiological factors rather than to diet ones.

There are additional reasons why the effects of intraspecific (morphological) trait variability might have a limited effect on the diet breadth. First, the pollen carried by the sampled individuals represents a snapshot of the current foraging trip. Whether individuals were sampled at the beginning or end of their foraging trips, which can last for some hours (Lihoreau et al. 2012), is unknown. Moreover, bumblebee foraging decisions might be dynamic over time, as the amount of available floral rewards such as nectar changes during the time of the day according to the refill rates of each plant species (Gurevich & Hadany 2021; Torné-Noguera et al. 2016). Furthermore, bumblebee foragers of the same colony can exhibit different fidelity on their foraging sites at the individual level (Heinrich 1976; Lihoreau et al. 2010, 2012): some individuals may prefer visiting one or few patches and thus collect pollen from the same plants, whereas other ones may be constantly changing their foraging sites and possibly their pollen hosts too (Lihoreau et al. 2012).

Finally, our results on pollen transportation patterns suggest different plant visitation in urban and rural areas with potential effects on pollination. The larger number of plants visited in urban bumblebee individuals, reflected in the expanded diet breadth, seem to indicate that more floral resources are visited per foraging trip. This in turn might reduce the time spent per plant and could limit the probabilities of successful pollination as seen in other studies (Kendall *et al.* 2022).

Conclusion

Uncovering how animal populations inhabiting different human-modified ecosystems cope with modified environmental conditions (e.g., climatic, biotic) is essential to understand not only the biology and ecology of species and their ecological functions (e.g., pollination), but also to improve species preservation efforts. Our results provide new insights into the various strategies that different bumblebee species apply in response to anthropogenic land-use change, prioritizing the maintenance of their required nutritional intake but varying their diet breadth and plant interactions. In that regard, our results reinforce the importance in nutrient demands, rather than solely morphological trait matching, in driving foraging of pollinators. Thus, accounting for plant nutritional traits should become an additional criteria, besides aesthetics and other traits, when managing and creating urban green spaces. Finding out to what degree species can handle novel ecosystems is a necessary step towards improved conservation, particularly in a momentum, after the Post2020 CBD, where part of the efforts are aimed at reducing the adverse effects of human-modified ecosystems on biodiversity and its contributions to people.

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Authors contributions

SS, MM, JCA, BF, and SDL conceived the study. HE selected the sites, collected the bumblebees and measured the bumblebee morphological traits. SS, JCA and MM processed the pollen samples. AK conducted the bioinformatic analyses. SS, SDL and FAR performed the chemical analyses. SS, JCA, FAR and AK analyzed the data. SS and JCA wrote the first draft. All authors corrected the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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Figures

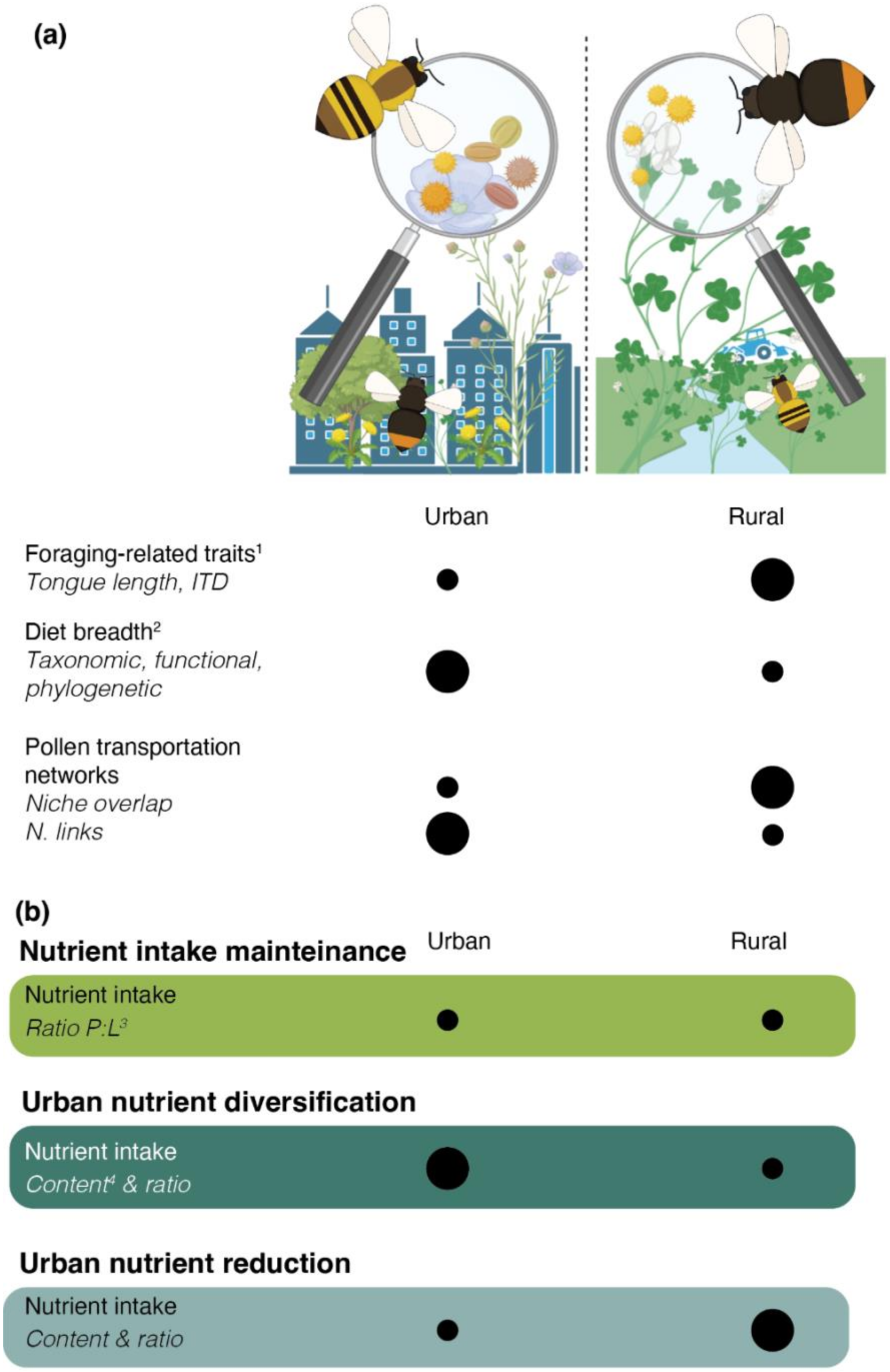


Figure 1. Illustration of the hypotheses regarding bumblebee dietary patterns and pollen transportation in urban and rural areas. (a) General hypothesis regarding differences between urban and rural bumblebees. Overall, urban bumblebees are smaller than their rural counterparts (Eggenberger *et al.* 2019) and are expected to have broader diet breadths due to the enhanced resource availability in cities. Moreover, urban bumblebee pollen transportation networks are also hypothesized to be dissimilar from their rural counterparts, with urban bumblebee individuals

having a larger number of interactions per individual (N. links) and a reduced niche overlap. (b) The three scenarios regarding foraging strategies according to the nutrient intake based on prior studies: in diet maintenance, nutrient intake is similar between urban and rural bumblebees regardless of the expanded diet breadth, showing strong regulation when obtaining macronutrients (Ruedenauer *et al*. 2020). This scenario can indicate four non-mutually excluding mechanisms: increased redundancy of pollen and nectar hosts, loss in the dominance of preferred plant taxa, increased learning challenges, and increased competition. In urban diversification and urban reduction, nutrient intake is dissimilar between urban and rural bumblebees but with different implications on bumblebee fitness and health. In urban diversification, urban bumblebees diversify their nutrient intake due to enhanced plant diversity in urban areas, thus assuming a positive impact on health and fitness (based on Kaluza *et al*. 2017 and Trinkl *et al.* 2020). In urban reduction, urban bumblebees have a worse nutrient intake than their rural counterparts, thus assuming a negative impact on health and fitness. 1= Eggenberger *et al.* 2019; 2 = Kaluza *et al*. 2017; 3 = Ruedenauer *et al*. 2020. Schematic figure created with BioRender.com.

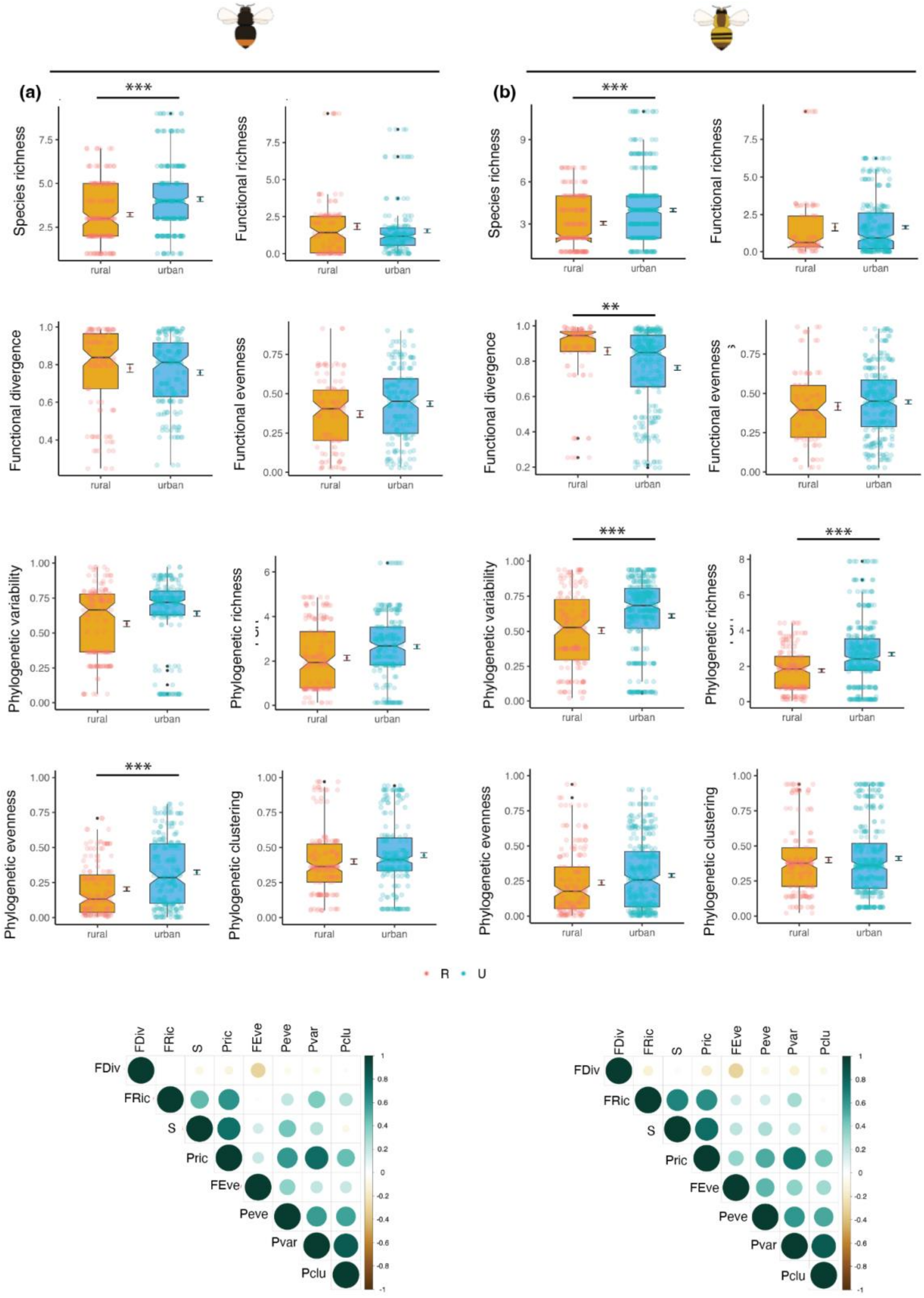


Figure 2. Differences in the dietary patterns in urban and rural bumblebee populations. Boxplots depicting the differences between the pollen taxonomic (species richness), functional (functional richness, FRich; functional evenness, FEve; functional dispersion, FDis), and phylogenetic (phylogenetic richness, Pric, phylogenetic variability, Pvar, phylogenetic evenness, Peve,

phylogenetic clustering, Pclu) diversity metrics between urban and rural populations of *B. lapidarius* (a, left panels) and *B. pascuorum* (b, right panels). Notches indicate the 95% confidence interval of the median. Additionally, on the right side of each boxplot, the mean +- the standard error is also presented. Differences between the means were tested using Generalised Linear Mixed Effects Models with Holm correction for multiple testing. Correlation plots show the correlations between the taxonomic, functional, and phylogenetic metrics. Significance levels: *: 0.05 > *p*-value > 0.01, **: 0.01 > *p*-value > 0.001, ***: *p*-value < 0.001.

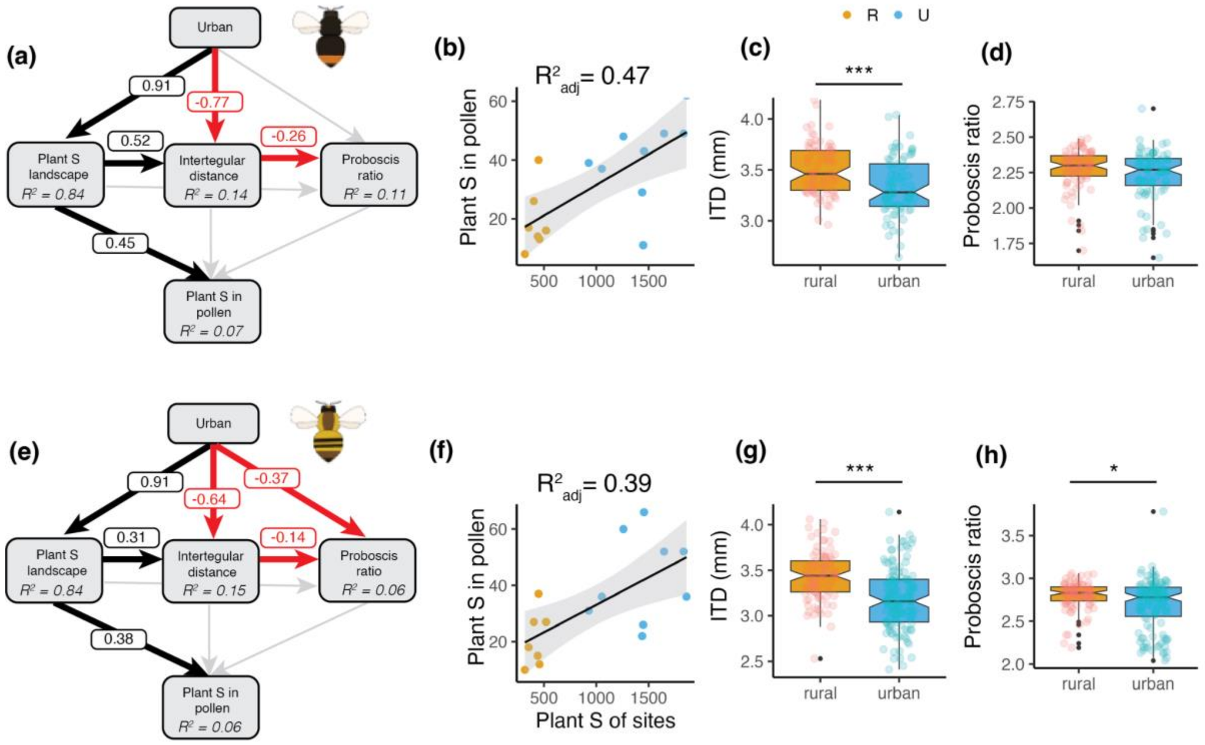


Figure 3. Drivers of bumblebee dietary patterns. (a, e) Piecewise Structural Equation Modeling (pSEM) depicting the direct and indirect effects of landscape type (i.e., urban and rural), floral resources within a 1500 m buffer (i.e., Plant S of sites), and morphological traits related to foraging (i.e., Intertegular distance ITD, and proboscis ratio that results from dividing the proboscis length and the intertegular distance) on the plant species richness of the pollen (Plant S in pollen) collected in the corbicula of individuals of *B. lapidarius* (a) and *B. pascuorum* (e). The pSEM also includes three models explaining the influence of the landscape type on the floral resources in the landscape and the influence of the landscape type and the floral resources within a 1500 m buffer on the intertegular distance and the proboscis ratio. Numbers show standardized path coefficients for significant pathways. Positive paths are depicted in black, negative in red, and nonsignificant ($p > 0.05$) in gray. For each response variable, the R^2 is provided inside the box. *B. lapidarius*: Fisher's C = 0.879, *p*-value = 0.644. *B. pascuorum:* Fisher's C= 0.628, *p*-value=0.731. Additional pSEMs with the functional and phylogenetic diversity metrics are shown in Table S8. (b,f) Linear models depicting the relationship between total species richness in the pollen collected and plant species richness in the landscape at different radii. Shaded bands depict the 95% confidence interval. Points represent the study sites. Species richness in the pollen is calculated by pooling all the bumblebee individuals per study site. (c, d, g, h) Boxplots depicting the differences in the intertegular distance (c, g) and proboscis ratio (d, h) between rural and urban bumblebee individuals. Notches indicate the 95% confidence interval of the median. S = species richness.

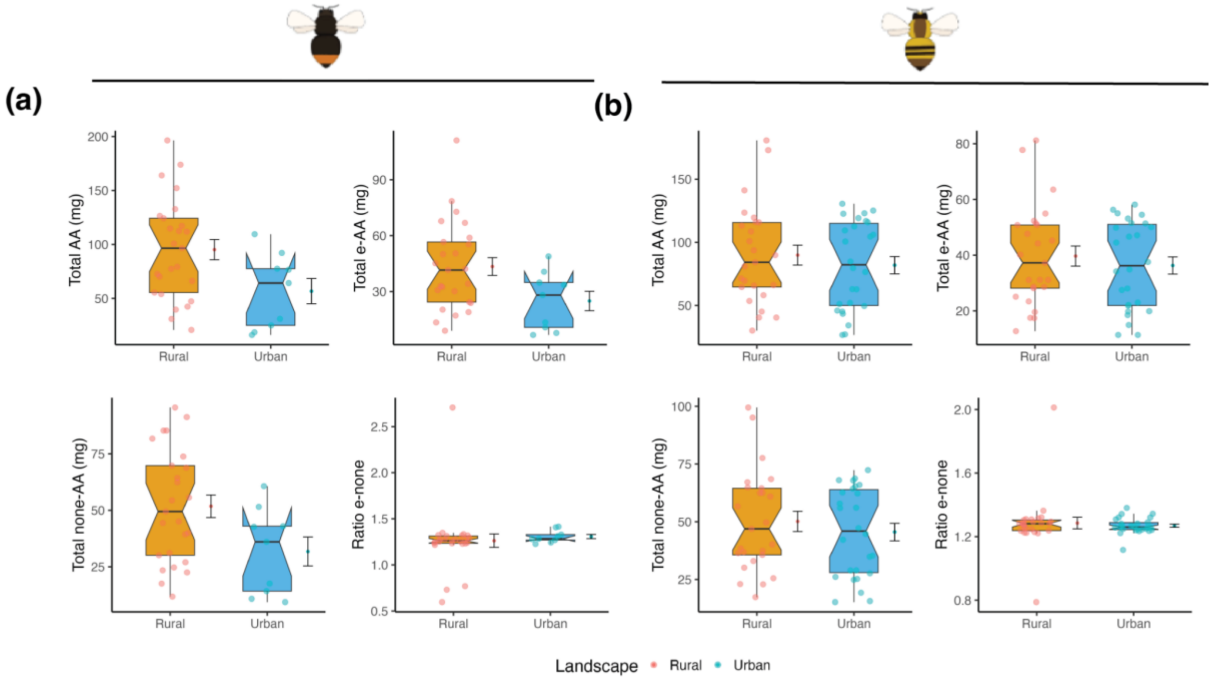


Figure 4. Urban and rural bumblebees have a similar amino acid (AA) intake. Boxplots of the AA composition in the pollen from the leg baskets of urban and rural bumblebees of *Bombus lapidarius* (a) and *Bombus pascuorum* (b). For simplicity, AA have been grouped in four main groups: total AA, total essential AA (Total e-AA), total non-essential AA (Total none-AA) and the ratio between non-essential and essential AA (ratio e-none). Notches indicate the 95% confidence interval of the median. Additionally, on the right side of each boxplot, the mean ± the standard error is also presented. Differences between the means were tested using Generalised Linear Mixed Effects Models with Holm correction for multiple testing.

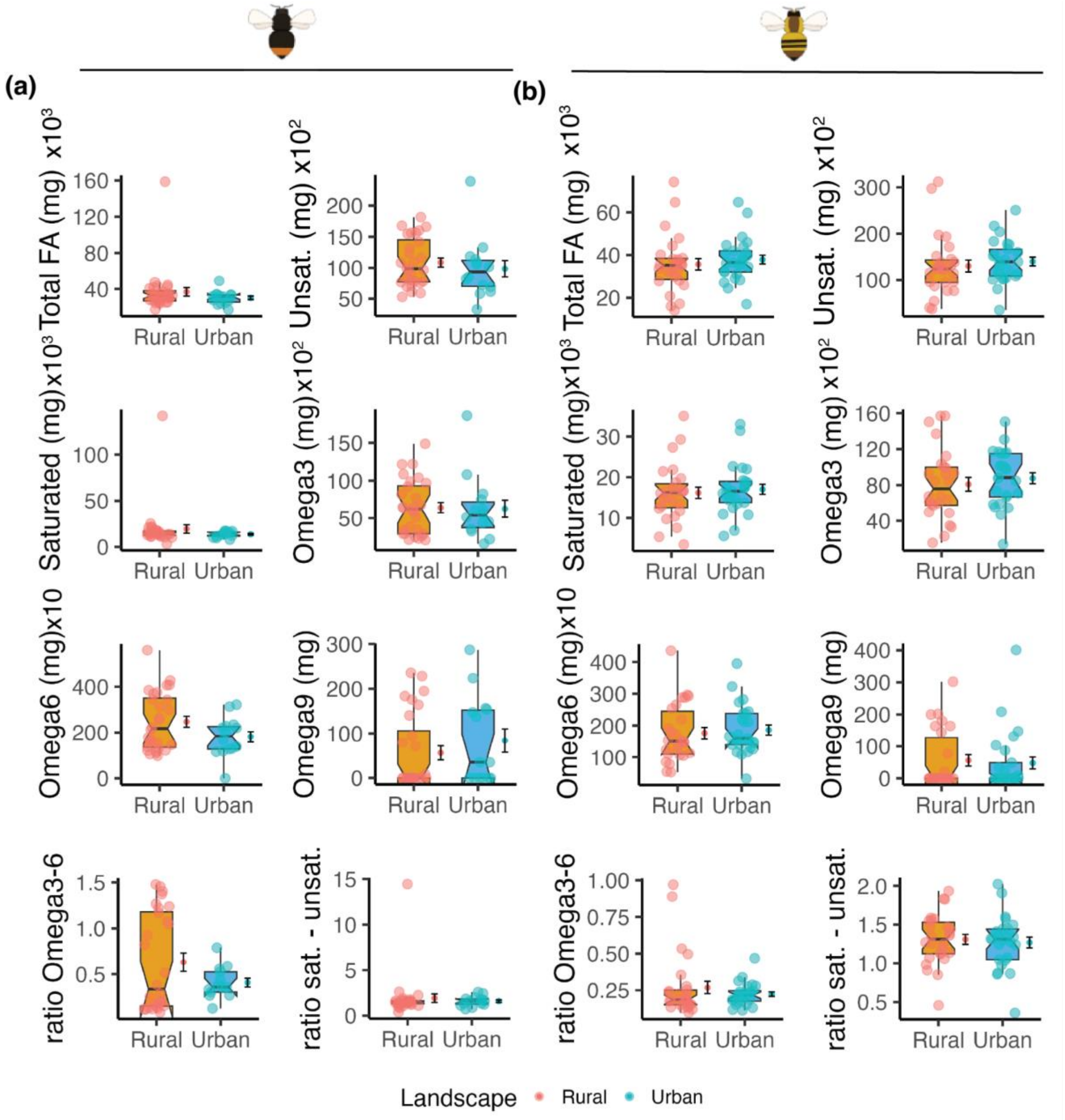


Figure 5 Urban and rural bumblebees have similar fatty acid intake. Boxplots of the fatty acid (FA) composition in the pollen from the leg baskets of urban and rural bumblebees of *Bombus lapidarius* (a) and *Bombus pascuorum* (b). For simplicity, individual FA have been grouped in eight main groups: total FA, total unsaturated FA (Unsat. FA), total saturated FA, Omega-3 FA, Omega-6 FA, Omega-9 FA, the ratio between Omega-3 and Omega-6 FA, and the ratio between saturated and unsaturated FA. Notches indicate the 95% confidence interval of the median. Additionally, on the right side of each boxplot, the mean ± the standard error is also presented. Differences between the means were tested using Generalised Linear Mixed Effects Models with Holm correction for multiple testing.

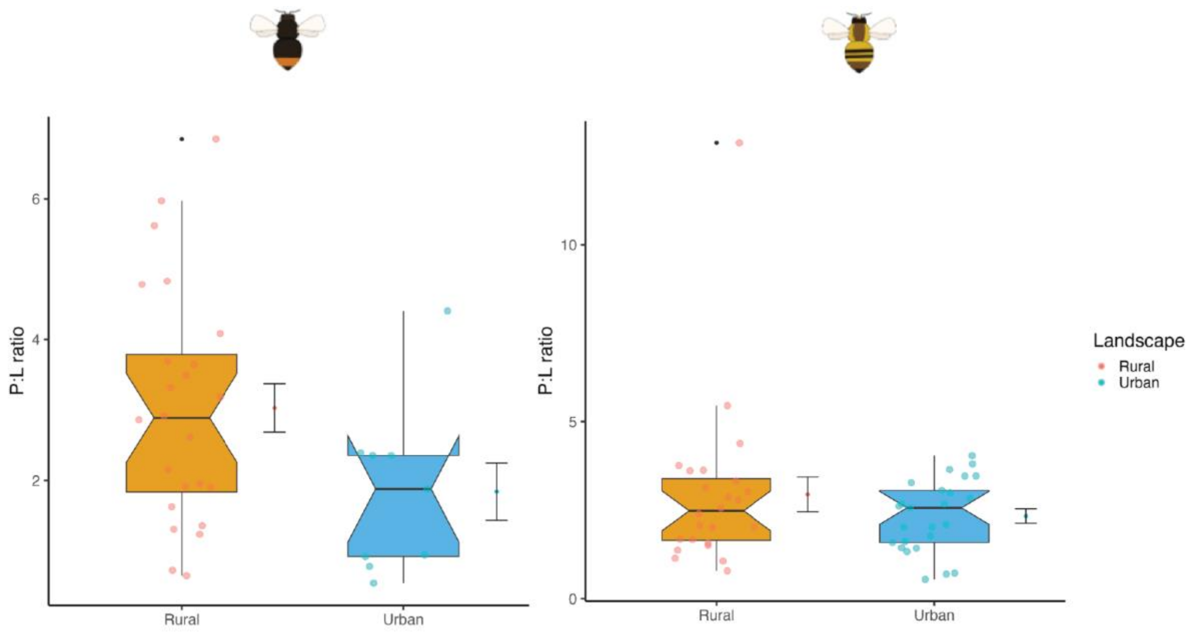


Figure 6. Box plots depicting the differences between the P:L ratio, that is, the ratio of amino acids (P) and fatty acids (L) in urban and rural areas for *Bombus lapidarius* (left) and *B. pascuorum* (right). Moreover the mean ± standard deviation is also provided. Notches indicate the 95% confidence interval of the median.

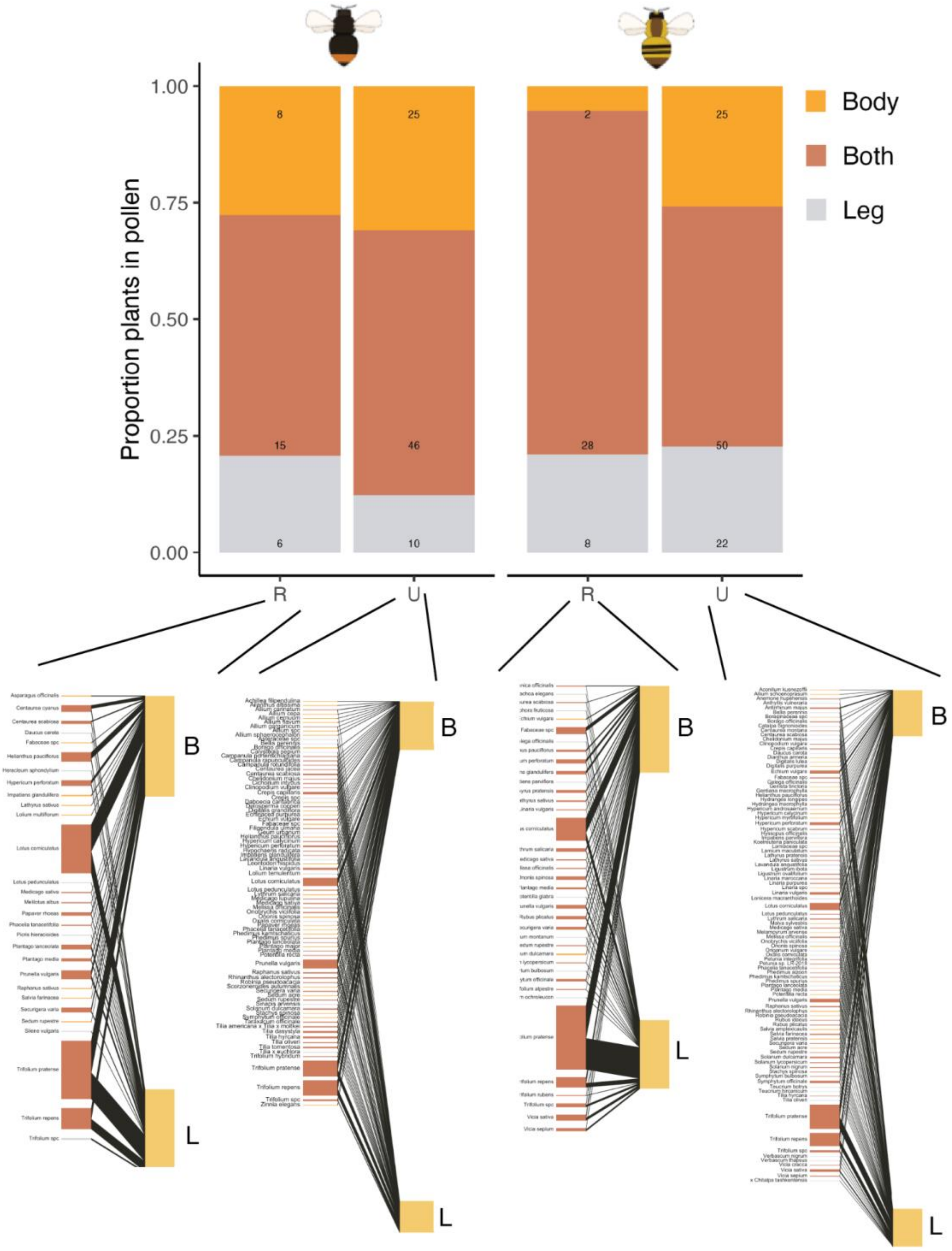


Figure 7. Pollen transportation patterns and networks between pollen transportation structures (Body, B; leg baskets (corbicula), L) , landscapes (urban U; rural, R) The bar plot depicts the proportion of plant species transported only on the body, only on the leg-baskets, or in both transportation structures in urban and rural populations of *B. lapidarius* (left) and *B. pascuorum* (right). The number of plant species is also provided. For each bumblebee species and landscape (i.e. urban and rural), the overall bipartite networks (aggregating all individuals) between plant

species and bumblebee transportation organs (i.e., body and leg) is also provided. Additional information on pollen transportation can be found in Figure S10-S12

Supplementary material for the paper *"Urban bumblebees diversify their foraging strategy to maintain nutrient intake"*

Simonetta Selva, Marco Moretti, Fabian A. Ruedenauer, Alexander Keller, Bertrand Fournier, Sara D. Leonhardt, Helen Eggenberger, Joan Casanelles-Abella.

Supplementary text

Text S1. Additional information on the study sites and studied bumblebees.

Per region, urban and rural landscapes were separated by a minimum distance of 20 km, to restrict gene flow and interbreeding between urban and rural bumblebee populations. In total, the study covered 16 different sites (Figure S1, Table S1): six in and around Zurich, six in and around Basel, and four in and around Bern. Within each region we selected three non-overlapping sampling sites (radius 800 m) that represented the upper foraging ranges of both selected bumblebee species (Figure S1, Table S1).

The canton of Zurich was the first sampling region, with the city of Zurich as the urban area (47°22′N, 8°33′E) and the lower Töss valley as the rural area (47°21′1″N, 8°56′6″E). The second sampling took place in Basel and its surroundings. Basel-Stadt in the canton Basel-Stadt was the urban zone (47°34′N, 7°36′E) and the Fricktal in the canton Aargau was the rural zone (47°30′21.43″N, 8°3′7.63″E). The third region was in the canton of Bern, with the city of Bern as the urban zone (46°57′N, 7°27′E) and the Bernese Mittelland as the rural zone (46°56′N, 7°12′E). See Figure S1 for visual overview and Table S1.

The two studied bumblebees (*Bombus lapidarius* and *Bombus pascuorum*) are common in the Swiss lowlands, inhabit similar urban and rural environments, have equivalent annual activity patterns and cover comparable distances while foraging. Additionally, while the bumblebee species are generalist, they still have more marked preferences (for the family Fabaceae) than other generalist species, but with a larger degree of variation than specialist bumblebees (Wood et al., 2021). However, *B. pascuorum* has a longer tongue than *Bombus lapidarius* (Wood et al., 2021).

For each 800 m radius sampling site, we collected 30–40 individuals per species, except for one urban site in Bern where only three individuals of B. lapidarius were found. Sampling efforts were standardized across all sites and conducted during peak bumblebee activity hours (09:00–17:00) and under optimal weather conditions (sunny with at least 70% clear sky, temperatures above 15°C, and little to no wind, i.e., 0–2 on the Beaufort scale). To minimize seasonal effects, we collected individuals within a short time frame from July to mid-August 2016, when worker abundances for both species peaked (Von Hagen & Aichhorn, 2014). Sampling dates were randomized between zones within and between regions. Bumblebee individuals were actively captured using sweep-nets and collection tubes while they foraged on blooming flowers. We limited the capture to 5–10 individuals per sampling patch at a time. In cities, foragers were collected in parks, botanical gardens, cemeteries, along planted flower strips, and in private gardens. In rural areas, foragers were primarily found in managed meadows, pasture lands, and along flower strips.

Text S2: Pollen collection laboratory workflow.

Bumblebees were taken from the -20°C compartment for the pollen collection. For the collection of the corbicula pollen, the rear pairs of legs of each bumblebee were separated under the binoculars and the bee’s body placed into the 2 ml Eppendorf tube. If there was pollen in the corbicula, the pollen was carefully separated under the binoculars using tweezers and a needle and transferred to a prepared, well-labeled PCR plate. The storage-vial also got checked if there was pollen stuck on its side. If there was, this pollen was also added to the leg samples. If there was no pollen on the legs or in the vials, this step was skipped.

Text S2 - Figure I. A picture of one of the collected bumblebees (*Bombus lapidarius*) with the pollen load in the curbicula.

For the collection of the body pollen, 500µl H2O Milli-Q was added to the bee with removed corbicula pollen in the Eppendorf tube and centrifuged briefly.After, the bees were placed in an ultrasonic bath for 4 minutes. Next, the Eppendorf tubes with the bees were transferred into a centrifuge and centrifuge at 10000 RCF for 5 minutes. The bee was removed and again stored at –20 °C. The remaining liquid and pollen mixture was then again centrifuged for 1 minute at 10000 RCF to form a nice pellet and the surplus was discarded. The remaining liquid was then again mixed with the pollen pellet and transferred to the PCR plate. The PCR plates with the corbicula and the body pollen were sealed with an airpor tape and stored at -80 °C for at least 1 hour before getting lyophilized overnight. The samples were then ready for metabarcoding and chemical analysis.

Text S3: Pollen metabarcoding laboratory workflow.

DNA was isolated from samples using the Quick-DNA Microprep Plus Kit (Zymo Research), strictly following the manufacturer's instructions. DNA was resuspended in a final volume of 15 μL. A DNA extraction blank (Bex1-16) in each round of the DNA isolation procedure was included, to be treated as regular samples in the next step of the library construction process to check for cross-contamination. The isolated DNA was quantified by fluorimetry with Qubit, using the High-Sensitivity dsDNA Assay (Thermo Fisher Scientific). There was quantifiable DNA from most samples, except from 93 samples. These 93 samples were too low for Qubit quantification detection with the High-Sensitivity dsDNA Assay kit, meaning that the DNA values were below 0.1 ng/μL. Therefore, library construction may be compromised.

For library preparation, a fragment of the ITS2 genomic region (of around 300 bp) was amplified using the primers ITS_S2F (Yao et al., 2010) and ITS4R (White et al., 1990). Illumina sequencing primers were attached to these primers at their 5' ends. Then, PCRs were carried out in a final volume of 12.5 μL, containing 1.25 μL of template DNA, 0.5 μM of the primers, 6.25 μL of Supreme NZYTaq 2x Green Master Mix (NZYTech), and ultrapure water up to 12.5 μL. In the next step, the reaction mixture was incubated as follows: an initial denaturation step at 95 ºC for 5 min, followed by 35 cycles of 95 ºC for 30 s, 51 ºC for 45 s, 72 ºC for 30 s, and a final extension step at 72 ºC for 10 min. The oligonucleotide indices which are required for multiplexing different libraries in the same sequencing pool were attached in a second PCR round with identical conditions but only 5 cycles and 60 ºC as the annealing temperature. A negative control that contained no DNA (BPCR) was included in every PCR round to check for contamination during library preparation. The libraries were then run on 2 % agarose gels stained with GreenSafe (NZYTech), and imaged under UV light to verify the library size.

The libraries were purified using the Mag-Bind RXNPure Plus magnetic beads (Omega Biotek), following the instructions provided by the manufacturer. Then, the libraries were pooled in equimolar amounts according to the quantification data provided by the Qubit dsDNA HS Assay (Thermo Fisher Scientific). This pool also contained a testimonial amount of the corresponding extraction blanks (Bex) and the PCR blanks (BPCR). The pool was sequenced in a fraction of an Illumina NovaSeq PE250 flowcell (Illumina), aiming for a total output of 30 gigabases.

In DNA metabarcoding studies it has been observed that a low percentage of the reads of a library can be assigned to another library. This phenomenon, referred to as mistagging, tag jumping, index hopping, index jumping, etc. is the result of the misassignment of the indices during library preparation, sequencing, and/or demultiplexing steps or cross-contamination (Bartram et al., 2016; Esling et al., 2015; Guardiola et al., 2016; Illumina, 2017). In order to correct for this phenomenon, ASVs occurring at a frequency below 0.01 % in each sample were removed.

Text S3. Additional details on nutritional analyses

For AA, we considered the total amount of AA, the total amount of essential AA (i.e., AA that cannot be synthesized by the bees and need to be acquired only through their food), the total amount of non-essential AA (i.e., AA that can be synthesized by bees), and the proportion between essential and non essential AA. It is worth noting that both essential and non-essential AA are crucial for bee nutrition (ref). Regarding FA, we considered the total amount of FA, the total amount of unsaturated FA, the total amount of saturated FA, the total amount of Omega3,-6 and -9 FA, the ratio of saturated and unsaturated FA and the ratio between Omega3 and Omega6 FA.

Supplementary figures

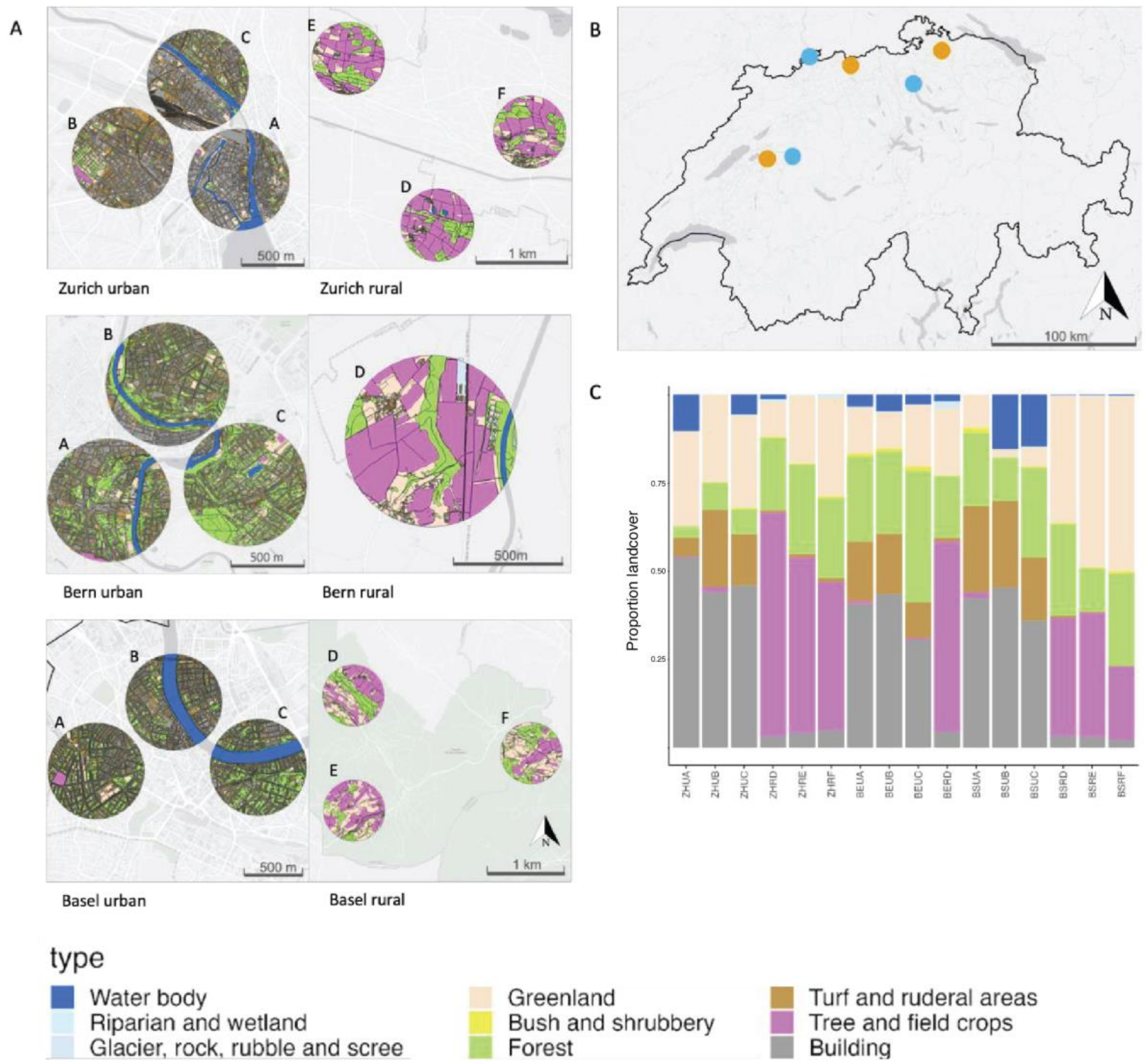


Figure S1. Overview on the sampling sites. A) Configuration of the different habitats of the 16 sites across 6 regions sampled by Eggenberger et al., (2019) using the habitat map of Switzerland V1 (Price et al., 2021) in the cantons of Basel, Bern and Zurich. The 9 habitat tapes are indicated by colour. B) Geographical distribution of the sampling regions in Switzerland (urban - blue, rural - orange). C) Proportion of each habitat per site.

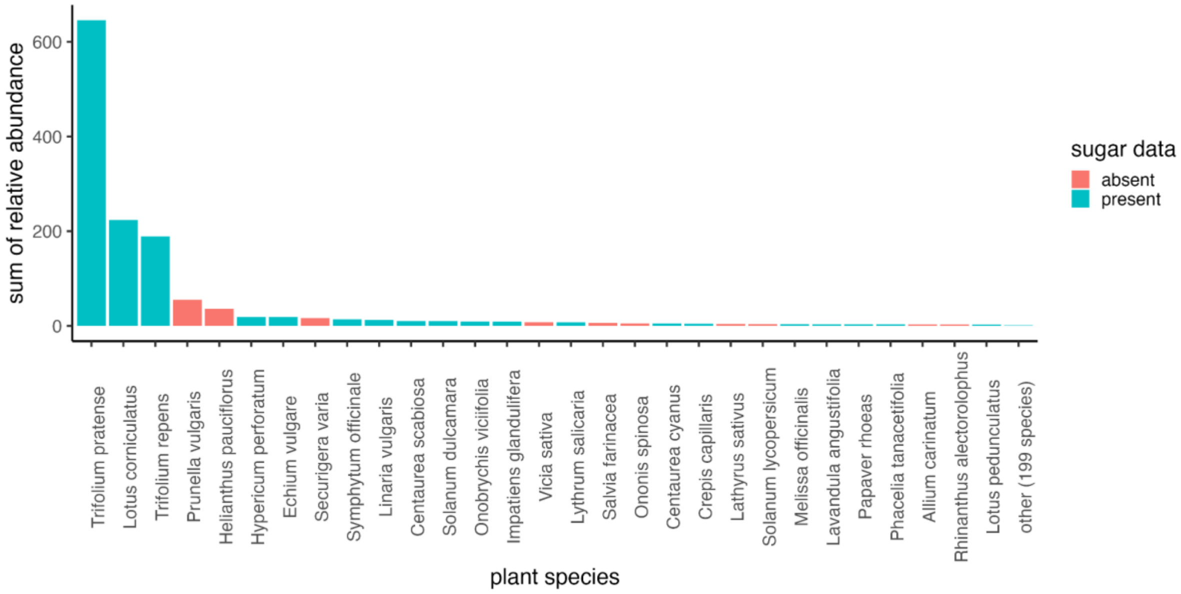


FIGURE S2. Coverage of the sugar data on the most common detected plant species in pollen of the two studied bumblebees. Barplot showing the sum of the relative abundances (y-axis) of the 30 most abundant plant species found in the pollen metabarcoding of the two bumblebee species combined. For each of the 30 species, we indicate if sugar data was available in the existing published databases. The sum of the relative abundance is calculated by adding up all the relative abundances per plant species within one pollen sample. Sugar concentration was obtained from Tew et al., 2021 and Filipiak et al., 2022).

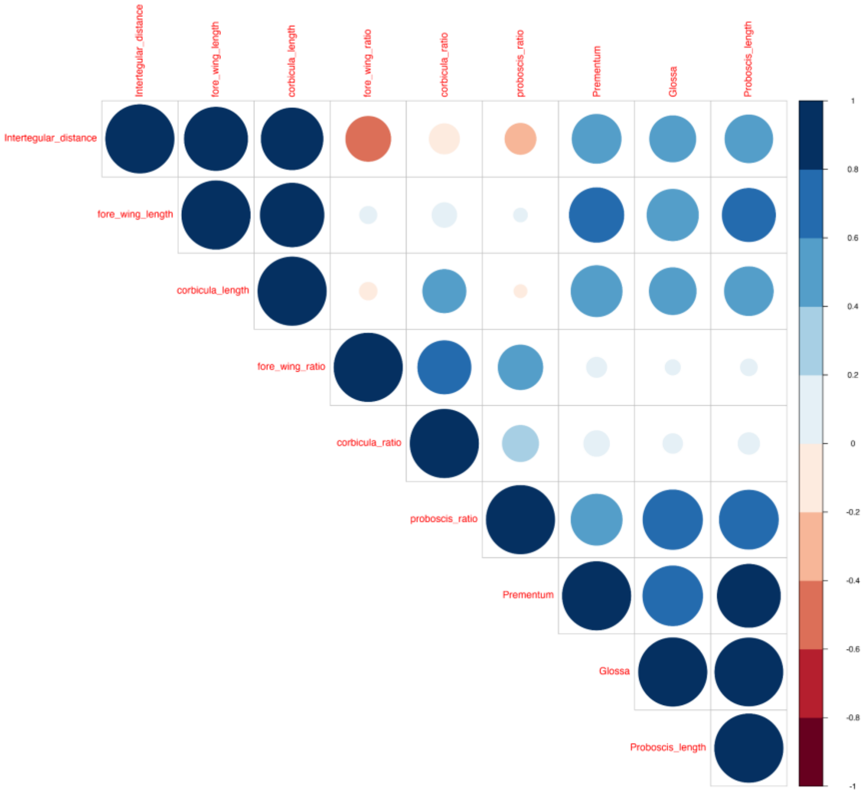


Figure S3. Correlation among morphological traits.

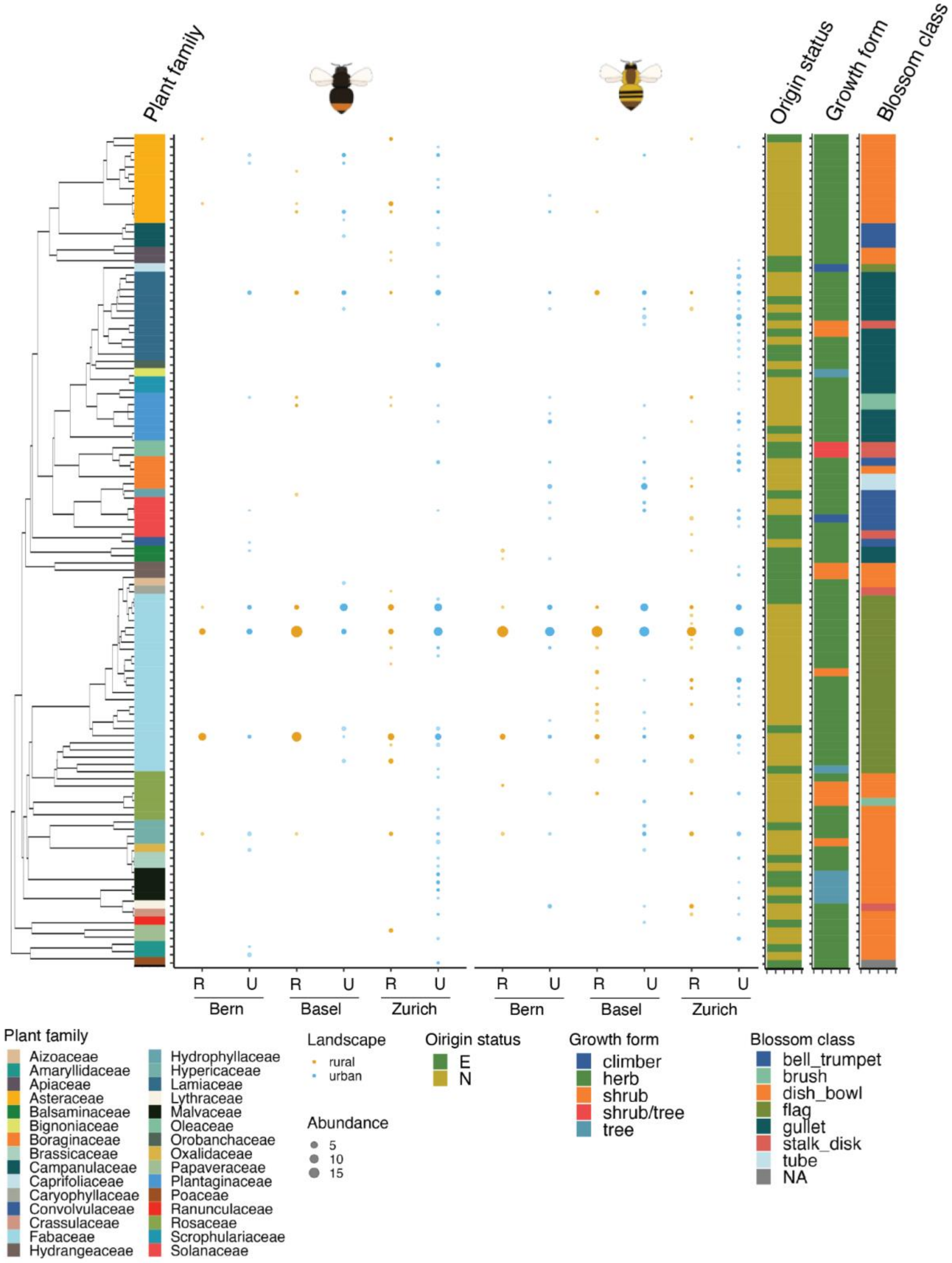


Figure S4. Differences in the diet breadth (i.e., plant composition) of the pollen collected from the leg baskets of rural and urban bumblebee individuals. The plot shows the relative abundance of the pollen of bumblebee individuals collected in rural (orange) and urban (blue) areas of the three studied regions (Bern, Basel, Zurich) for *B. lapidarius* (left) and *B. pascuorum* (right). Dot size reflects the abundance of pollen species. Plant species are classified in families (depicted in different colors), and sorted according to their phylogeny. The dendrogram on the left was done with the package V.PhyloMaker2 (Jin and Qian 2022).

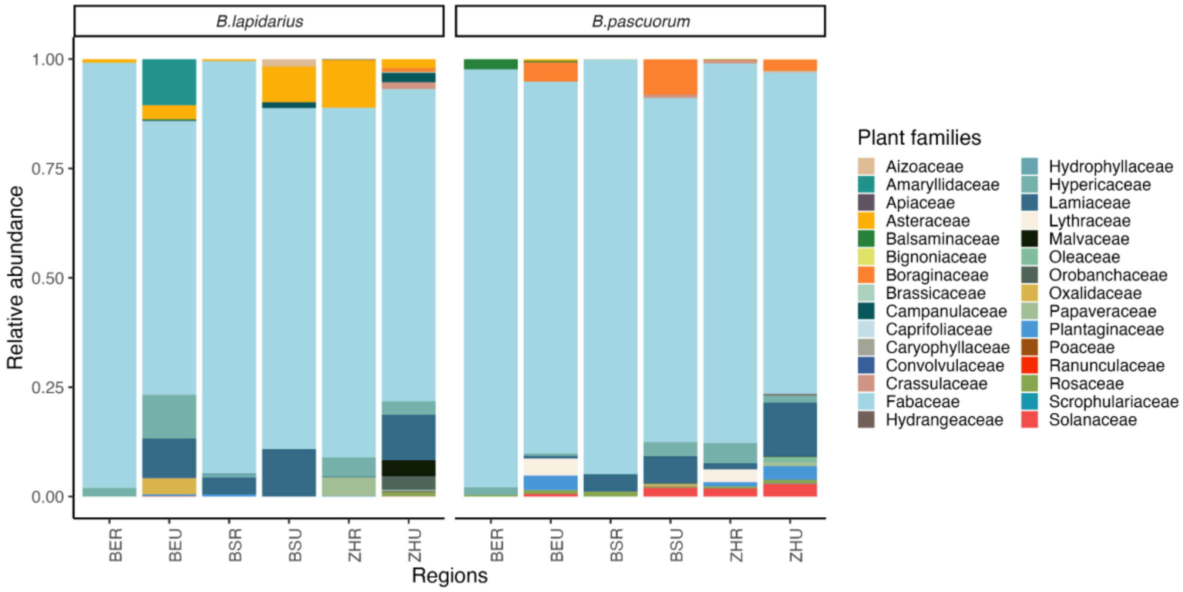


Figure S5. Composition of plant families in urban and rural sites. Relative proportion of the plant family composition in the diet of *B. lapidarius* (left) and *B. pascuorum* (right) (see legend) in the studied rural (R) and urban (U) areas of the six regions (Bern BE; Basel BS; Zurich ZH. Example: BER= Bern-Rural; ZHU= Zurich-Urban). The families were identified using DNA metabarcoding. The results of the corresponding X^2 tests are summarised in Table S6.

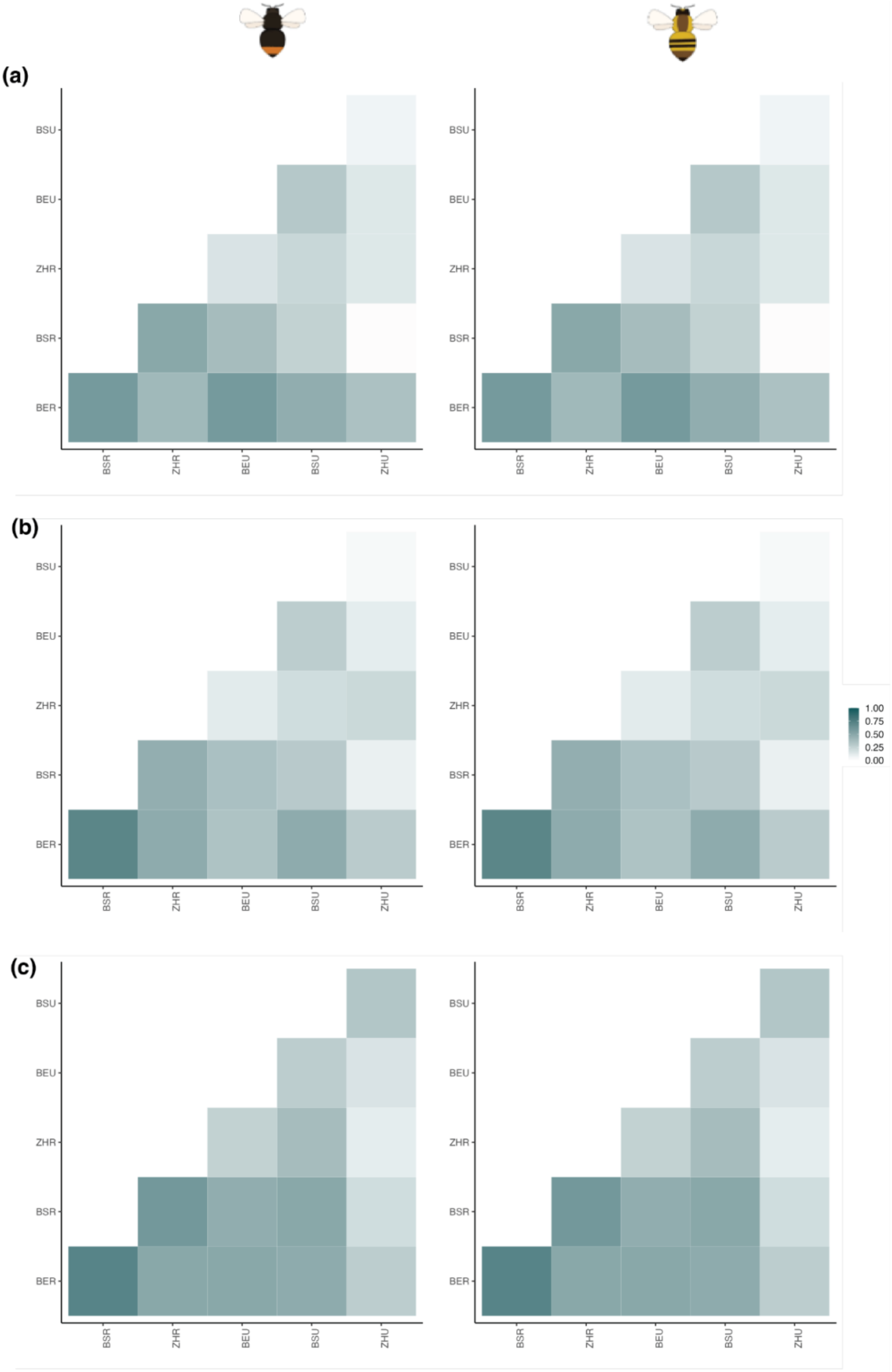


Figure S6. Diet consistency. Pairwise correlations of the plant composition in the pollen from the bumblebee leg baskets among urban and rural sites in the three studied regions (Zurich, ZH; Basel, BS; Bern, Be). For *Bombus lapidarius* (left) and *Bombus pascurourm (right)*, the site pairwise correlations of the collected plant taxa are shown at the family (a), genus (b) and species (c) levels. The colour of the squares indicates the value of the correlation. Note that the correlation values are expressed as absolute values.

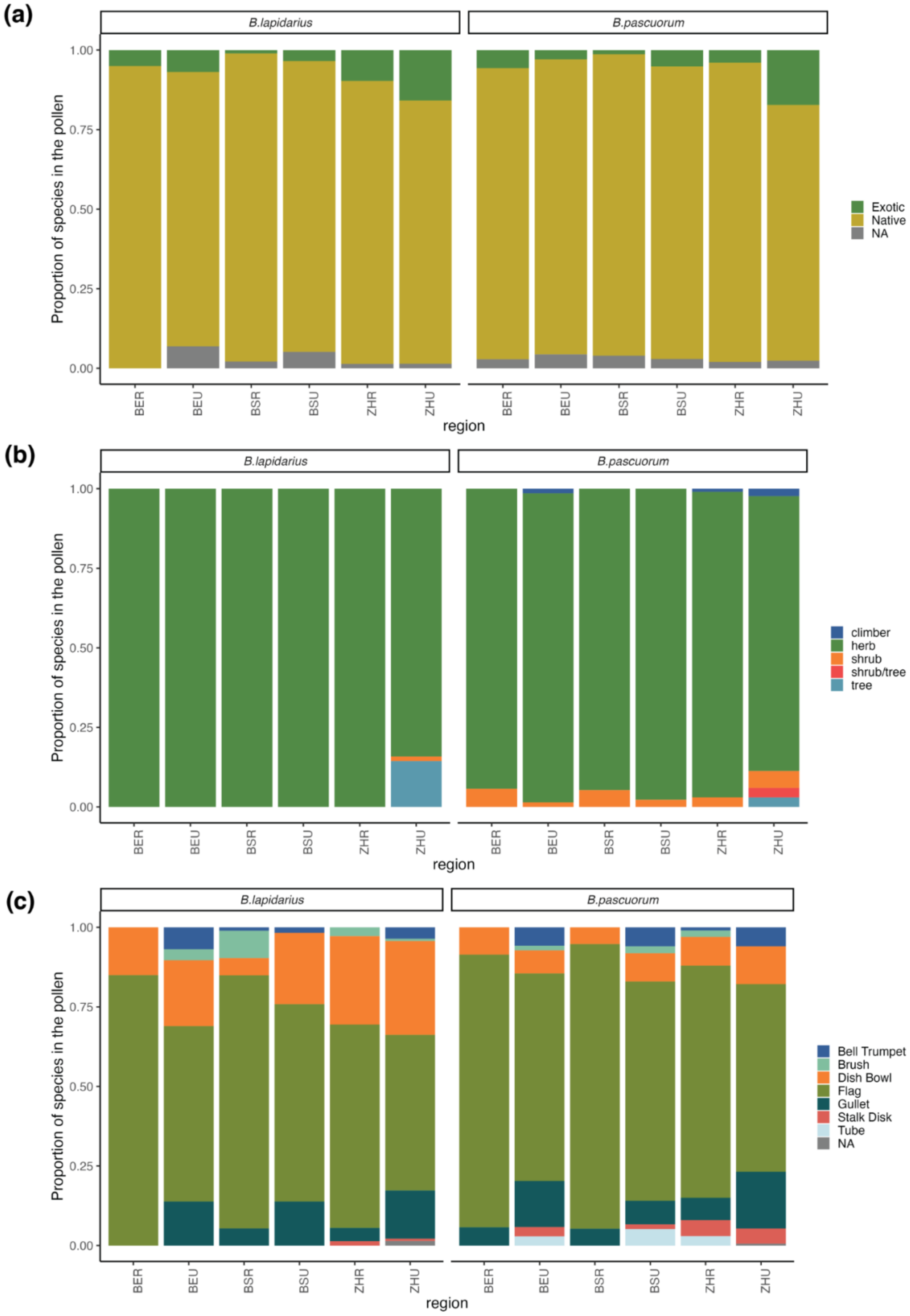

(a)
B.lapidarius
B.pascuorum
Proportion of species in the pollen
1.00
0.75
0.50
0.25
0.00
BER
BEU
BSR
BSU
ZHR
ZHU
region
Exotic
Native
NA
(b)
B.lapidarius
B.pascuorum
Proportion of species in the pollen
1.00
0.75
0.50
0.25
0.00
BER
BEU
BSR
BSU
ZHR
ZHU
region
climber
herb
shrub
shrub/tree
tree
(c)
B.lapidarius
B.pascuorum
Proportion of species in the pollen
1.00
0.75
0.50
0.25
0.00
BER
BEU
BSR
BSU
ZHR
ZHU
region
Bell Trumpet
Brush
Dish Bowl
Flag
Gullet
Stalk Disk
Tube
NA

Figure S7. Composition of plant traits in urban and rural sites. Relative proportions of plant traits (y-axis) for *B. lapidarius* (left) and *B. pascuorum* (right) in the studied rural (R) and urban (U) areas of the six regions (x-axis: Bern BE; Basel BS; Zurich ZH. Example: BER= Bern-Rural; ZHU= Zurich-Urban). **a)** Origin status of visited plants: The difference between landscapes was significant for *B.lapidarius*, with more exotic species being visited in urban areas (*B.lapidarius*: X^2 (1, N = 2751) = 18.744, p < .001). For *B.pascuroum* there was no significant association between landscape and origin status (*B.pascuorum:* X^2 (1, N = 2718) = 1.735, p = 0.188). **b)** Growth form of visited plants: By far the most visited plants were herbaceous. Again, urban bumblebees of both species visited significantly more types of growth forms (*B.lapidarius*: X^2 (4, N = 2751) = 22.003, p < .001, *B.pascuorum:* X^2 (4, N = 2718) = 67.324, p < .001). **c)** Flower shape of visited plants: Flag-shaped flowers were visited the most by the bumblebees. The differences between landscapes were statistically significant, with urban bumblebees having visited more diverse flower shapes (*B.lapidarius*: X^2 (6, N = 2751) = 128.619, p < .001, *B.pascuorum:* X^2 (6, N = 2718) = 102.661, p < .001). The results of the corresponding X^2 tests are summarised in Table S6.

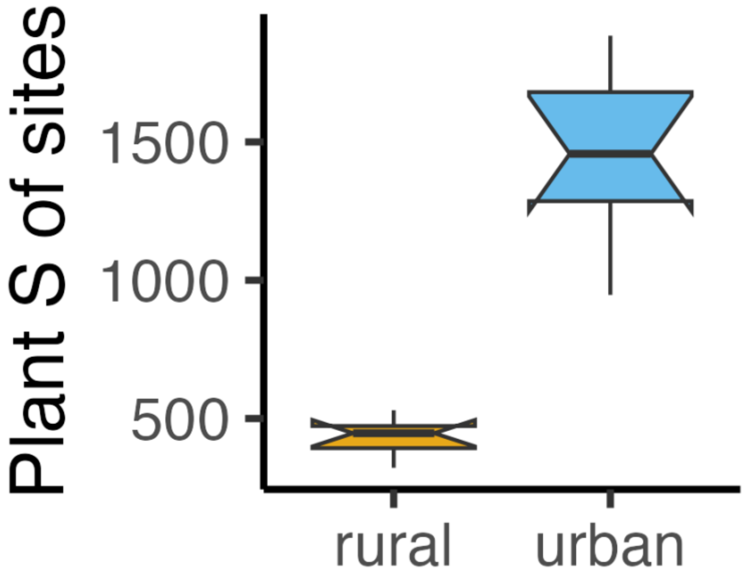


Figure S8. Difference in the plant species richness between the studied urban and rural areas. Plant species richness was calculated around 1500 m radius in each of the study sites. Plant species were extracted from GBIF and InfoFlora (see methods).

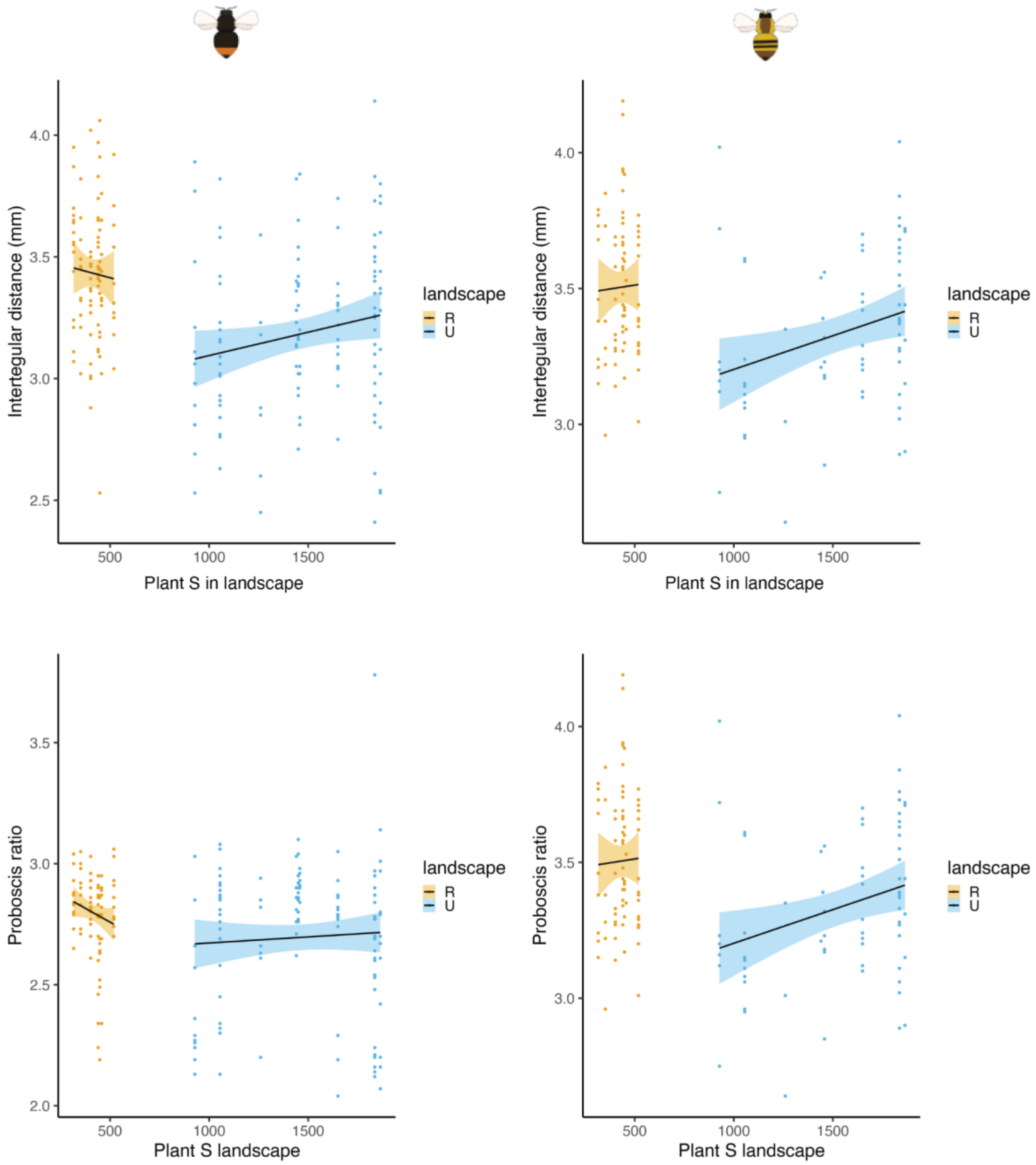


Figure S9. Linear models depicting the relationship between the intertegular distance and the probiscis ratio of bumblebee individuals and the plant species richness (S) in the landscape (within 1500 m radius) for *Bombus lapidarius* (left) and *Bombus pascuroum* (right). Colors indicate individuals from rural (R) sites and urban (U) sites. Points indicate the raw measurements. Colored bands indicate the 95% confidence interval.

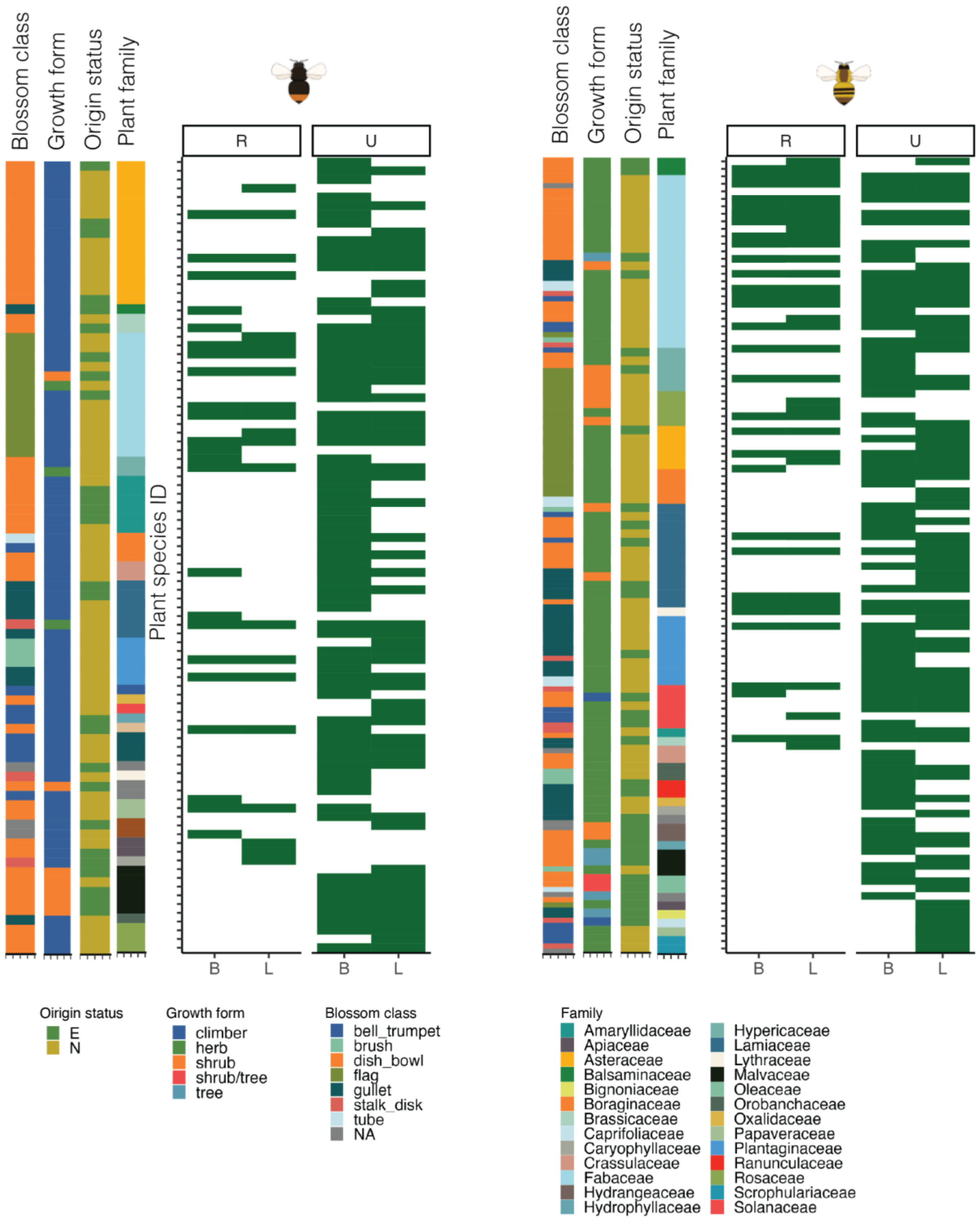


Figure S10. Plant composition according to the pollen transport structure, that is, in the leg baskets (L) and the body (B) for *Bombus lapidarius* (left) and *Bombus pascuorum* (right). Green squares represent occurrences. Plant species are sorted according to their families. Every division in th Y-axis represents a plant species. For every species, the information on their origin status, growth form and blossom class is also provided.

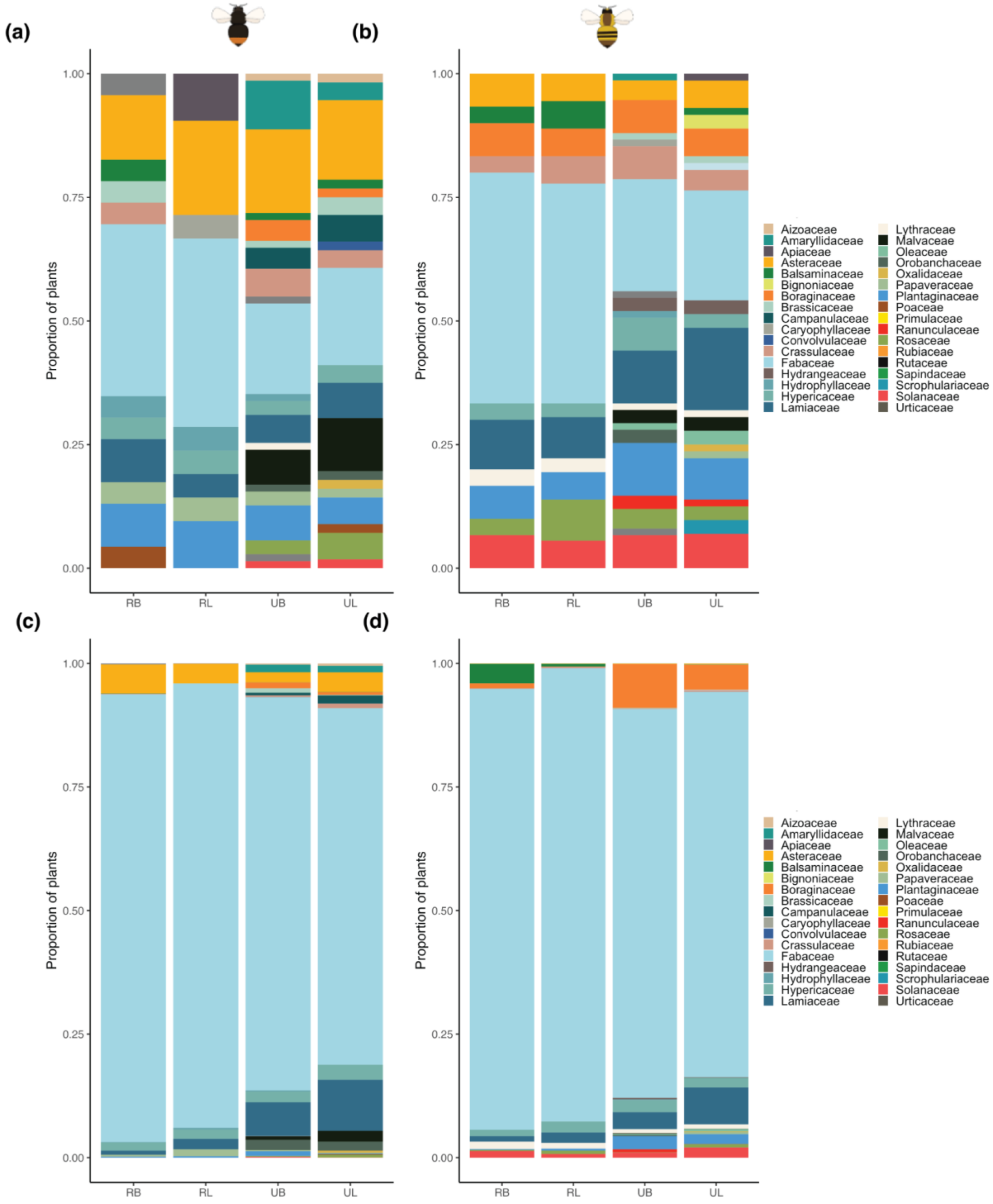


Figure S11. Family composition according to the pollen transport structure, that is, in the leg baskets (L) and the body (B) for *Bombus lapidarius* (left) and *Bombus pascuorum* (right).

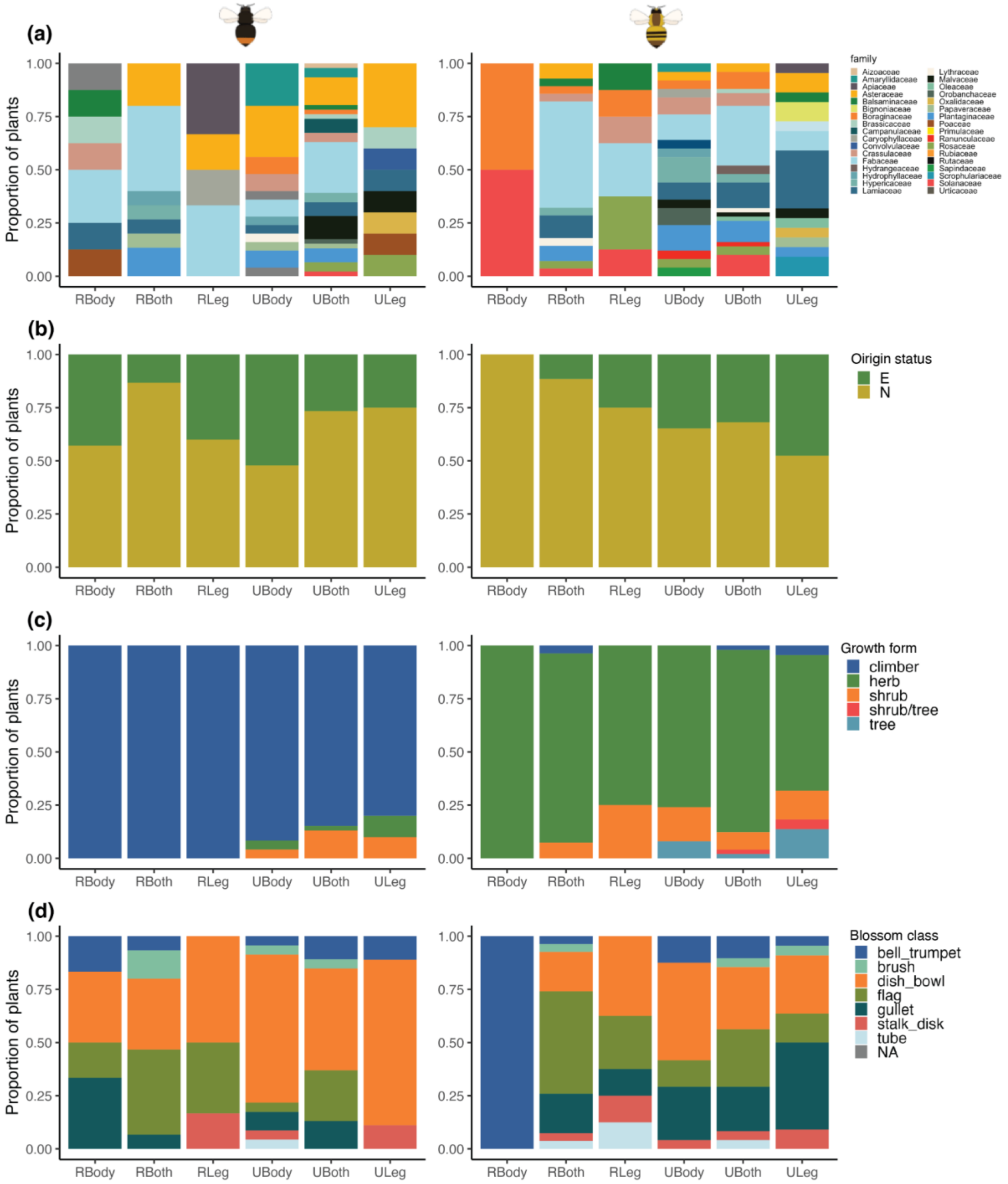
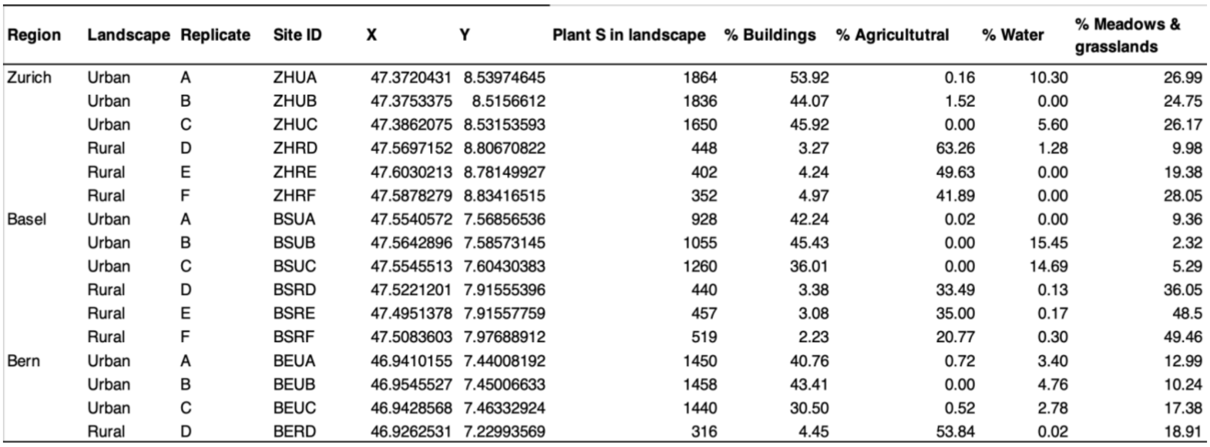


Figure S12. Composition of plant species according to the transportation structure (body and leg baskets) classified according to (a) families, (b) origin status, (c) growth form, and (d) structural blossom class for *Bombus lapidarius* (left) and *Bombus pascuroum* (right).

Supplementary tables

Table S1. Description of the study sites. For each study site, we provide the region (canton), landscape (urban/rural), the replicate, the full ID, the coordinates, the plant species (S) richness at the landscape measured within 1500 m radius, and the % of buildings, agricultural areas, waters and meadows/grasslands as calculated in Eggenberger et al. (2019). Coordinates represent the center of the 800 m radius, as in Eggenberger et al. (2019).

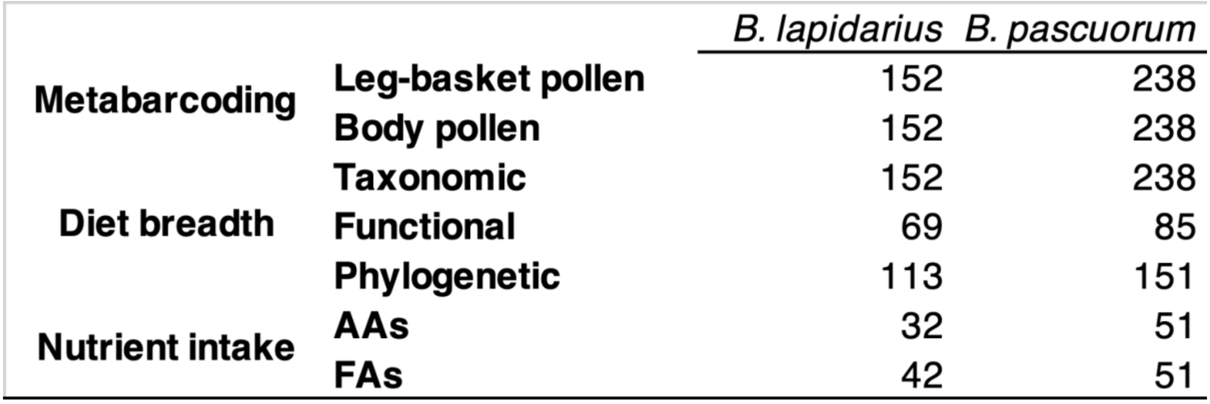


Region	Landscape	Replicate	Site ID	X	Y	Plant S in landscape	% Buildings	% Agricultutral	% Water	% Meadows & grasslands
Zurich	Urban	A	ZHUA	47.3720431	8.53974645	1864	53.92	0.16	10.30	26.99
	Urban	B	ZHUB	47.3753375	8.5156612	1836	44.07	1.52	0.00	24.75
	Urban	C	ZHUC	47.3862075	8.53153593	1650	45.92	0.00	5.60	26.17
	Rural	D	ZHRD	47.5697152	8.80670822	448	3.27	63.26	1.28	9.98
	Rural	E	ZHRE	47.6030213	8.78149927	402	4.24	49.63	0.00	19.38
	Rural	F	ZHRF	47.5878279	8.83416515	352	4.97	41.89	0.00	28.05
Basel	Urban	A	BSUA	47.5540572	7.56856536	928	42.24	0.02	0.00	9.36
	Urban	B	BSUB	47.5642896	7.58573145	1055	45.43	0.00	15.45	2.32
	Urban	C	BSUC	47.5545513	7.60430383	1260	36.01	0.00	14.69	5.29
	Rural	D	BSRD	47.5221201	7.91555396	440	3.38	33.49	0.13	36.05
	Rural	E	BSRE	47.4951378	7.91557759	457	3.08	35.00	0.17	48.5
	Rural	F	BSRF	47.5083603	7.97688912	519	2.23	20.77	0.30	49.46
Bern	Urban	A	BEUA	46.9410155	7.44008192	1450	40.76	0.72	3.40	12.99
	Urban	B	BEUB	46.9545527	7.45006633	1458	43.41	0.00	4.76	10.24
	Urban	C	BEUC	46.9428568	7.46332924	1440	30.50	0.52	2.78	17.38
	Rural	D	BERD	46.9262531	7.22993569	316	4.45	53.84	0.02	18.91

Table S2. Total number of bumblebee individuals used per study site (Site ID). Total *Bombus lapidarius:* 152, total *B. pascuroum:* 238.

	Region	**Landscape**	**Replicate**	**Site ID**	**N. individuals**
Bombus lapidarius	Bern	Rural	D	BERD	11
	Bern	Urban	A	BEUA	1
	Bern	Urban	B	BEUB	6
	Bern	Urban	C	BEUC	2
	Basel	Rural	D	BSRD	22
	Basel	Rural	E	BSRE	2
	Basel	Rural	F	BSRF	17
	Basel	Urban	A	BSUA	7
	Basel	Urban	B	BSUB	11
	Basel	Urban	C	BSUC	3
	Zurich	Rural	D	ZHRD	8
	Zurich	Rural	E	ZHRE	12
	Zurich	Rural	F	ZHRF	6
	Zurich	Urban	A	ZHUA	6
	Zurich	Urban	B	ZHUB	25
	Zurich	Urban	C	ZHUC	12
Bombus pascuorum	Bern	Rural	D	BERD	20
	Bern	Urban	A	BEUA	14
	Bern	Urban	B	BEUB	6
	Bern	Urban	C	BEUC	8
	Basel	Rural	D	BSRD	19
	Basel	Rural	E	BSRE	8
	Basel	Rural	F	BSRF	10
	Basel	Urban	A	BSUA	14
	Basel	Urban	B	BSUB	27
	Basel	Urban	C	BSUC	9
	Zurich	Rural	D	ZHRD	12
	Zurich	Rural	E	ZHRE	16
	Zurich	Rural	F	ZHRF	13
	Zurich	Urban	A	ZHUA	14
	Zurich	Urban	B	ZHUB	24
	Zurich	Urban	C	ZHUC	16

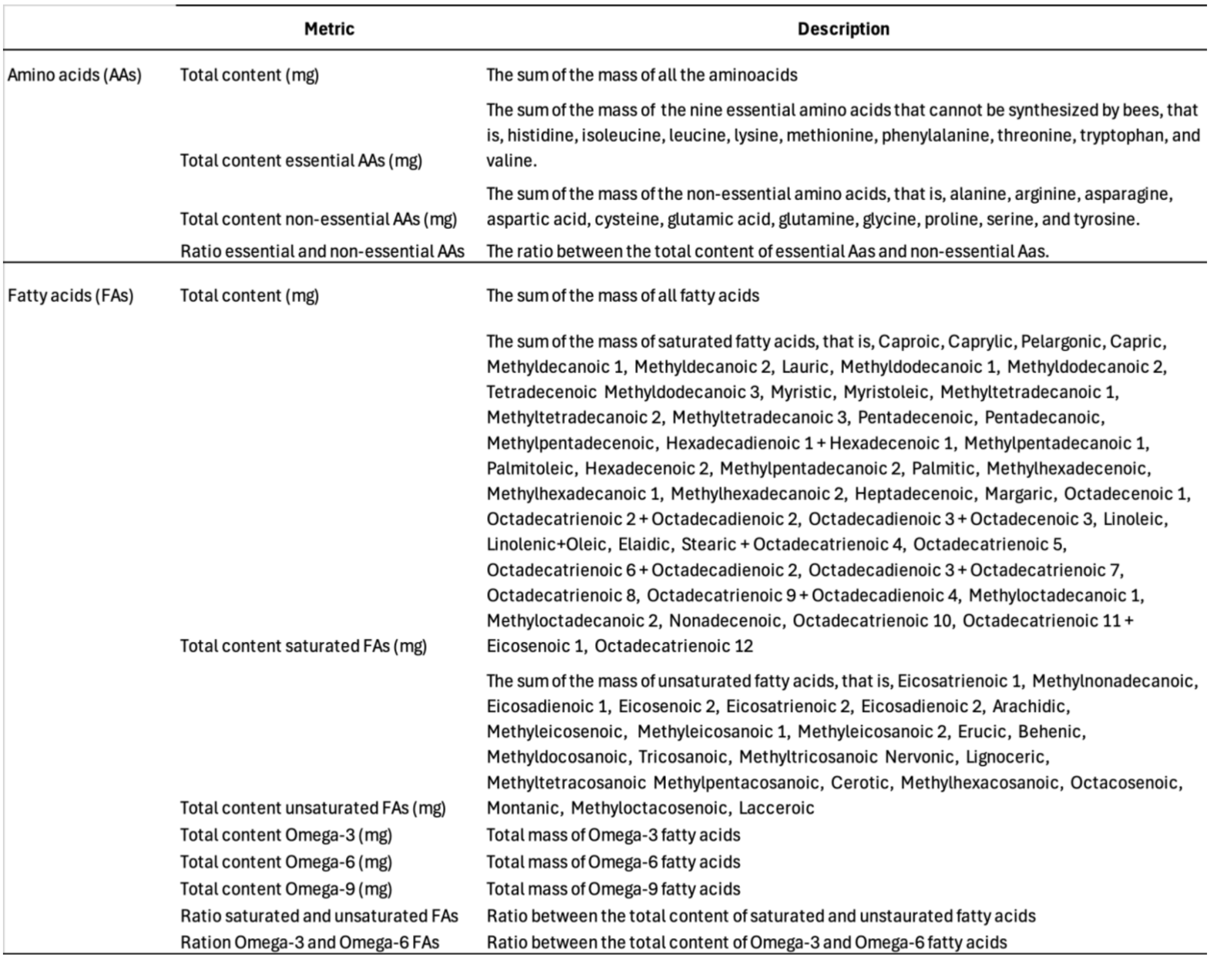
Table S3. Overview samples. For each bumblebee species, the number of individuals (metabarcoding and diet breadth) or aggregated samples (nutrient intake) used in the different analyses is provided.



		B. lapidarius	*B. pascuorum*
Metabarcoding	**Leg-basket pollen**	152	238
	Body pollen	152	238
Diet breadth	**Taxonomic**	152	238
	Functional	69	85
	Phylogenetic	113	151
Nutrient intake	**AAs**	32	51
	FAs	42	51

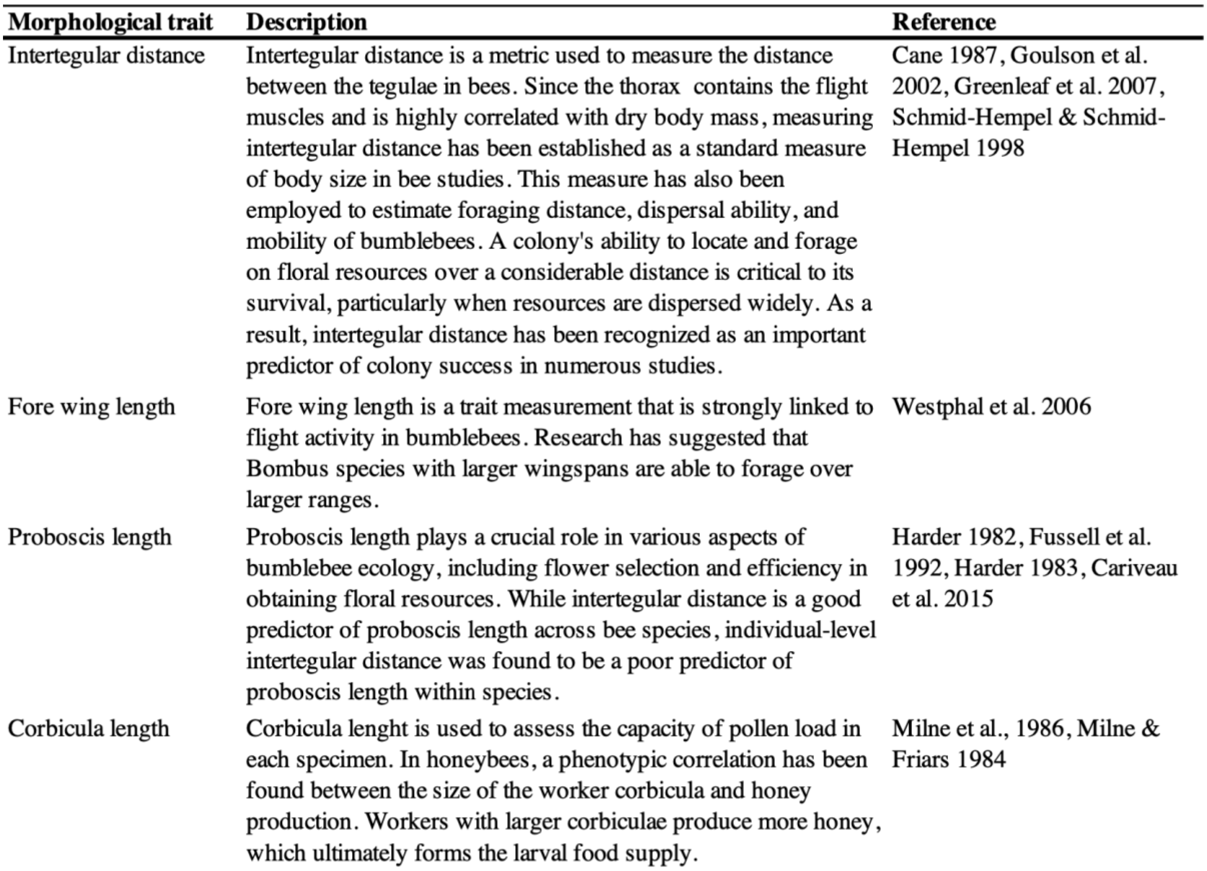
AAs = amino acids; FAs = fatty acids

Table S4. Metrics used to study the intake of amino acids and fatty acids.



	Metric	Description
Amino acids (AAs)	Total content (mg)	The sum of the mass of all the aminoacids
	Total content essential AAs (mg)	The sum of the mass of the nine essential amino acids that cannot be synthesized by bees, that is, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine.
	Total content non-essential AAs (mg)	The sum of the mass of the non-essential amino acids, that is, alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, proline, serine, and tyrosine.
	Ratio essential and non-essential AAs	The ratio between the total content of essential Aas and non-essential Aas.
Fatty acids (FAs)	Total content (mg)	The sum of the mass of all fatty acids
	Total content saturated FAs (mg)	The sum of the mass of saturated fatty acids, that is, Caproic, Caprylic, Pelargonic, Capric, Methyldecanoic 1, Methyldecanoic 2, Lauric, Methyldodecanoic 1, Methyldodecanoic 2, Tetradecenoic Methyldodecanoic 3, Myristic, Myristoleic, Methyltetradecanoic 1, Methyltetradecanoic 2, Methyltetradecanoic 3, Pentadecenoic, Pentadecanoic, Methylpentadecenoic, Hexadecadienoic 1 + Hexadecenoic 1, Methylpentadecanoic 1, Palmitoleic, Hexadecenoic 2, Methylpentadecanoic 2, Palmitic, Methylhexadecenoic, Methylhexadecanoic 1, Methylhexadecanoic 2, Heptadecenoic, Margaric, Octadecenoic 1, Octadecatrienoic 2 + Octadecadienoic 2, Octadecadienoic 3 + Octadecenoic 3, Linoleic, Linolenic+Oleic, Elaidic, Stearic + Octadecatrienoic 4, Octadecatrienoic 5, Octadecatrienoic 6 + Octadecadienoic 2, Octadecadienoic 3 + Octadecatrienoic 7, Octadecatrienoic 8, Octadecatrienoic 9 + Octadecadienoic 4, Methyloctadecanoic 1, Methyloctadecanoic 2, Nonadecenoic, Octadecatrienoic 10, Octadecatrienoic 11 + Eicosenoic 1, Octadecatrienoic 12
	Total content unsaturated FAs (mg)	The sum of the mass of unsaturated fatty acids, that is, Eicosatrienoic 1, Methylnonadecanoic, Eicosadienoic 1, Eicosenoic 2, Eicosatrienoic 2, Eicosadienoic 2, Arachidic, Methyleicosenoic, Methyleicosanoic 1, Methyleicosanoic 2, Erucic, Behenic, Methyldocosanoic, Tricosanoic, Methyltricosanoic Nervonic, Lignoceric, Methyltetracosanoic Methylpentacosanoic, Cerotic, Methylhexacosanoic, Octacosenoic, Montanic, Methyloctacosenoic, Lacceroic
	Total content Omega-3 (mg)	Total mass of Omega-3 fatty acids
	Total content Omega-6 (mg)	Total mass of Omega-6 fatty acids
	Total content Omega-9 (mg)	Total mass of Omega-9 fatty acids
	Ratio saturated and unsaturated FAs	Ratio between the total content of saturated and unstaurated fatty acids
	Ration Omega-3 and Omega-6 FAs	Ratio between the total content of Omega-3 and Omega-6 fatty acids

Table S5. Summary of the morphological bumblebee traits, measured by Eggenberger et al. (2019). We provide a description and references for it.



Morphological trait	Description	Reference
Intertegular distance	Intertegular distance is a metric used to measure the distance between the tegulae in bees. Since the thorax contains the flight muscles and is highly correlated with dry body mass, measuring intertegular distance has been established as a standard measure of body size in bee studies. This measure has also been employed to estimate foraging distance, dispersal ability, and mobility of bumblebees. A colony's ability to locate and forage on floral resources over a considerable distance is critical to its survival, particularly when resources are dispersed widely. As a result, intertegular distance has been recognized as an important predictor of colony success in numerous studies.	Cane 1987, Goulson et al. 2002, Greenleaf et al. 2007, Schmid-Hempel & Schmid-Hempel 1998
Fore wing length	Fore wing length is a trait measurement that is strongly linked to flight activity in bumblebees. Research has suggested that Bombus species with larger wingspans are able to forage over larger ranges.	Westphal et al. 2006
Proboscis length	Proboscis length plays a crucial role in various aspects of bumblebee ecology, including flower selection and efficiency in obtaining floral resources. While intertegular distance is a good predictor of proboscis length across bee species, individual-level intertegular distance was found to be a poor predictor of proboscis length within species.	Harder 1982, Fussell et al. 1992, Harder 1983, Cariveau et al. 2015
Corbicula length	Corbicula lenght is used to assess the capacity of pollen load in each specimen. In honeybees, a phenotypic correlation has been found between the size of the worker corbicula and honey production. Workers with larger corbiculae produce more honey, which ultimately forms the larval food supply.	Milne et al., 1986, Milne & Friars 1984

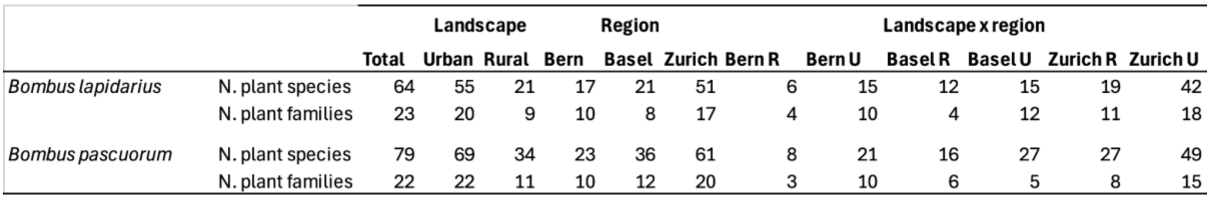
Table S6. Description of the 10 plant traits used. For each trait, we provide a description of the trait regarding plant-bee interactions, a description of the levels considered and the references used. See also Casanelles-Abella et al. (2021)

Trait	Description	Level	References
Origin status	Not a functional trait itself but a key feature to assess the importance of the social investment (e.g. gardening and other horticultural activities) in wild bee diet.	A species was considered native if its origin was Europe and exotic if it originated elsewhere.	Casanelles-Abella et al., 2022
Pollination mode		Pollination can be classified as either biotic or abiotic. Biotic pollination involves the transfer of pollen by a living organism, such as bees or moths, while abiotic pollination involves the transfer of pollen by non-living factors such as wind or water.	Ackerman, 2000
Flowering duration	Duration of flowering, or flowering period, is the length of time during which a plant produces flowers.	numeric	
Flowering start	Month in the year (1-12) in which the flowering starts	numeric	
Growth form	A trait related to the accessibility of the flowers considering the height where flowers occur	Four broad categories were defined: tree, shrub, herb and climber. - Trees included woody species typically classified as phanerophytes, including species described as small trees or tall shrubs. - Shrubs included mostly chamerophytes	Casanelles-Abella et al., 2022

Trait	Description	Level	References
		- Herbs included all herbaceous plants regardless of their height or growth form. - Herbs included all herbaceous plants regardless of their height or growth form.	
Plant height	Height of plant in meters	numeric	
Inflorescence	An inflorescence is a group or cluster of flowers arranged on a stem that is composed of branches, each of which has flowers attached to it.	presence of an inflorescence: 0 - not present; 1 - present	Prusinkiewicz et al., 2007
Structural blossom class	A trait related to accessibility of the flowers considering their morphology	Seven general blossom classes were defined according to the accessibility of the floral rewards. - Dish-bowl - Stalk-dish - Bell-trumpet - Brush - Gullet - Flag - Tube	Faegri & Van der Pijl, 1979
Symmetry	A trait related to accessibility of the flowers considering their morphology. Flower symmetry refers to the balanced arrangement of floral structures around a central axis, which can be actinomorph or sigomorph.	3 levels are defined: - actinomorph (radial) - sigomorph (bilateral) - no symmetry	

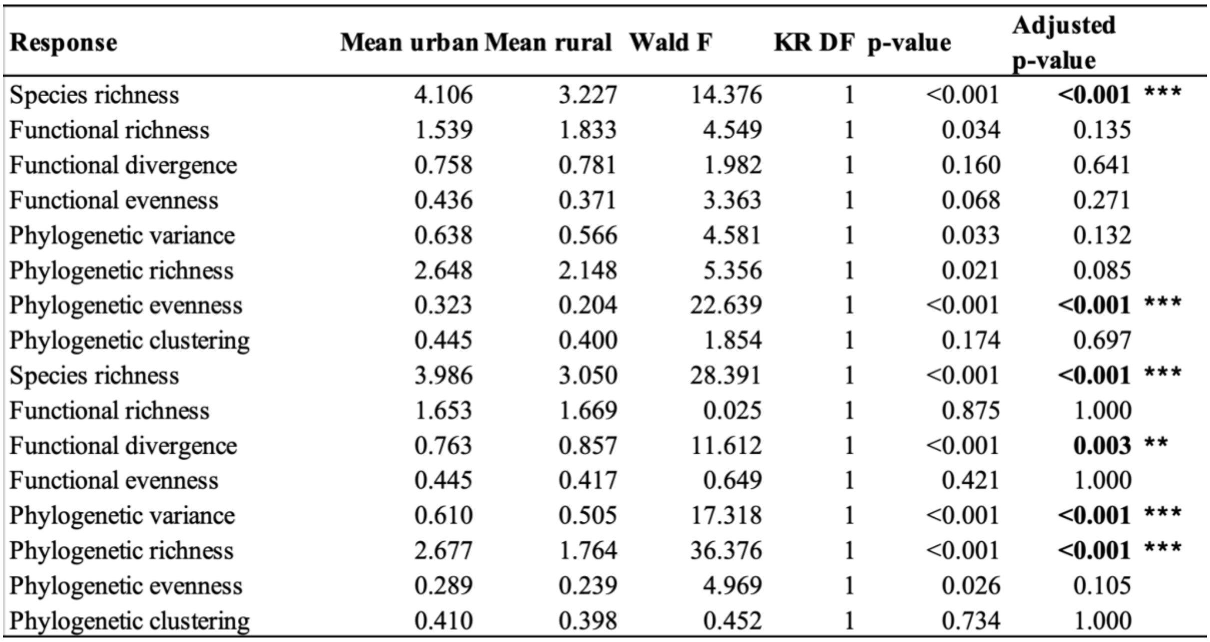
Trait	Description	Level	References
Sugar concentration	Concentration of the sugar in the pollen in µg per flower	numeric	Filipiak et al. 2022, Tew et al. 2021

Table S7. Numbers of plant species and plant families collected per landscape type (urban and rural), region (Swiss canton) and their intersections.



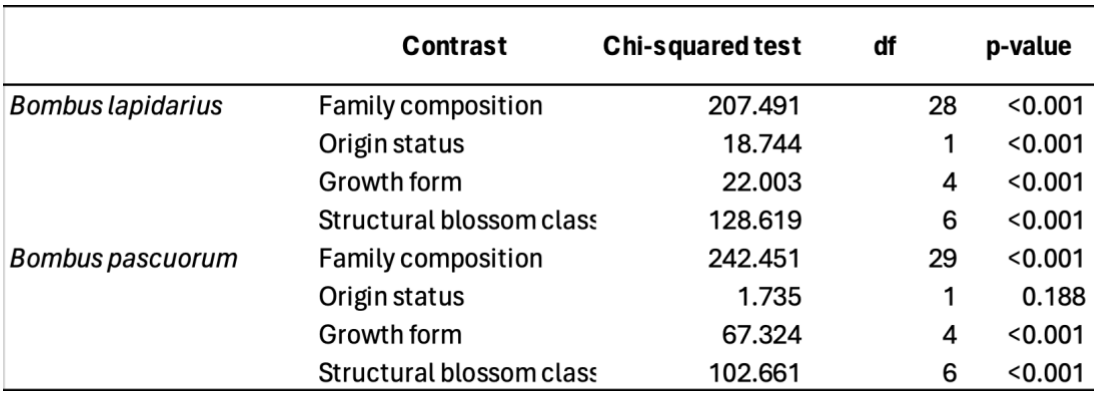
			Landscape		Region			Landscape x region					
		Total	**Urban**	**Rural**	**Bern**	**Basel**	**Zurich**	**Bern R**	**Bern U**	**Basel R**	**Basel U**	**Zurich R**	**Zurich U**
Bombus lapidarius	N. plant species	64	55	21	17	21	51	6	15	12	15	19	42
	N. plant families	23	20	9	10	8	17	4	10	4	12	11	18
Bombus pascuorum	N. plant species	79	69	34	23	36	61	8	21	16	27	27	49
	N. plant families	22	22	11	10	12	20	3	10	6	5	8	15

Table S8. Results of the effects of landscape on the taxonomic, functional and phylogenetic diversity metrics between urban and rural bumblebee populations. The table depicts the analysis of deviance using Wald F test and Kenward-Roger degrees of freedom. Adjusted p-values are corrected with Holm correction for multiple testing.



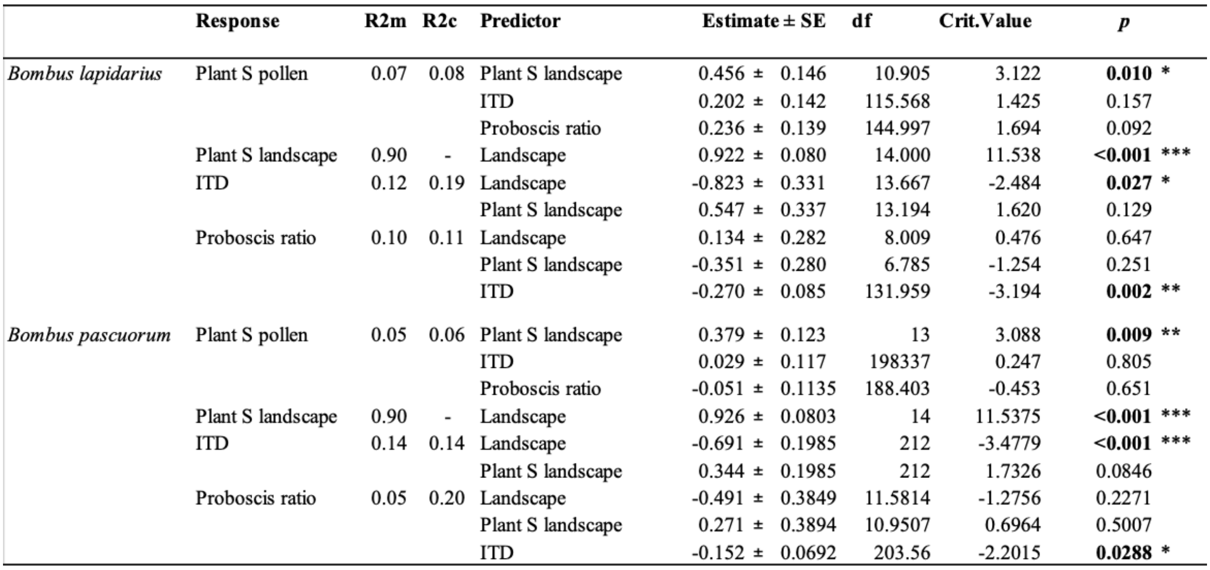
Response	**Mean urban**	**Mean rural**	**Wald F**	**KR DF**	**p-value**	**Adjusted p-value**	
Species richness	4.106	3.227	14.376	1	<0.001	**<0.001**	***
Functional richness	1.539	1.833	4.549	1	0.034	0.135	
Functional divergence	0.758	0.781	1.982	1	0.160	0.641	
Functional evenness	0.436	0.371	3.363	1	0.068	0.271	
Phylogenetic variance	0.638	0.566	4.581	1	0.033	0.132	
Phylogenetic richness	2.648	2.148	5.356	1	0.021	0.085	
Phylogenetic evenness	0.323	0.204	22.639	1	<0.001	**<0.001**	***
Phylogenetic clustering	0.445	0.400	1.854	1	0.174	0.697	
Species richness	3.986	3.050	28.391	1	<0.001	**<0.001**	***
Functional richness	1.653	1.669	0.025	1	0.875	1.000	
Functional divergence	0.763	0.857	11.612	1	<0.001	**0.003**	**
Functional evenness	0.445	0.417	0.649	1	0.421	1.000	
Phylogenetic variance	0.610	0.505	17.318	1	<0.001	**<0.001**	***
Phylogenetic richness	2.677	1.764	36.376	1	<0.001	**<0.001**	***
Phylogenetic evenness	0.289	0.239	4.969	1	0.026	0.105	
Phylogenetic clustering	0.410	0.398	0.452	1	0.734	1.000	

Table S9. Results of the Chi-squared test between urban and rural bumblebee populations.



	Contrast	Chi-squared test	df	p-value
Bombus lapidarius	Family composition	207.491	28	<0.001
	Origin status	18.744	1	<0.001
	Growth form	22.003	4	<0.001
	Structural blossom class	128.619	6	<0.001
Bombus pascuorum	Family composition	242.451	29	<0.001
	Origin status	1.735	1	0.188
	Growth form	67.324	4	<0.001
	Structural blossom class	102.661	6	<0.001

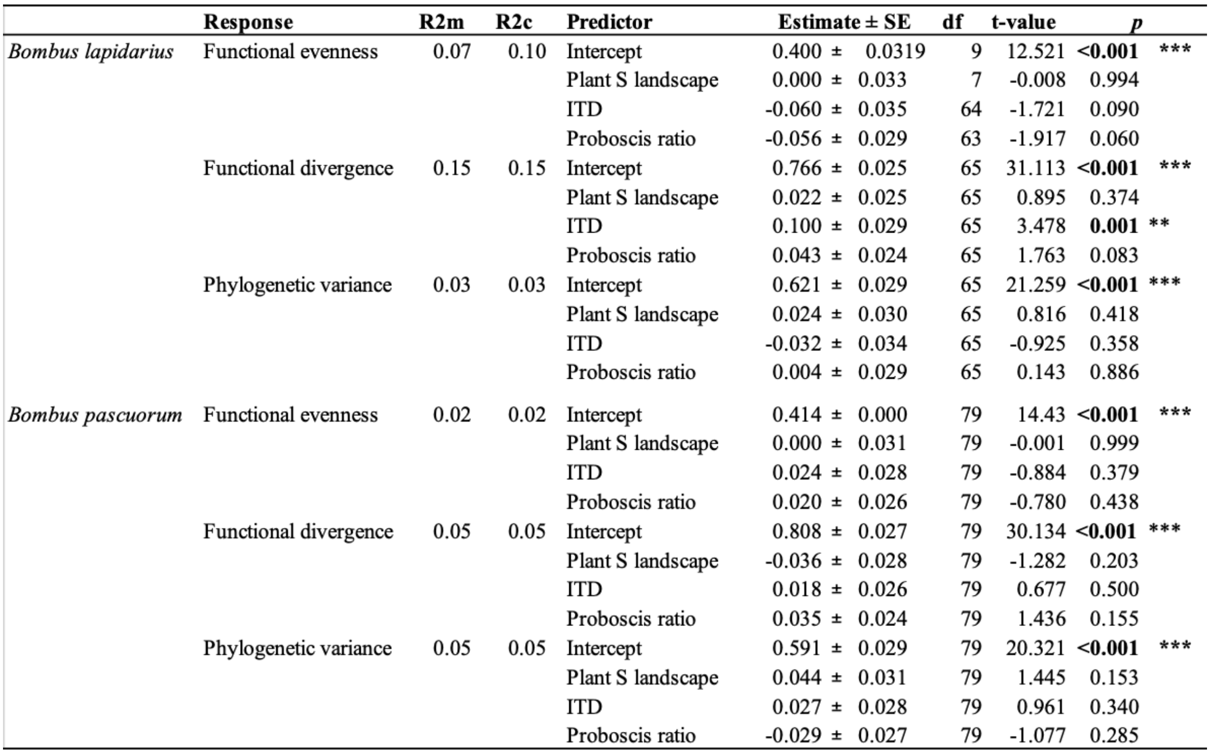
Table S10. Multilevel SEM of direct and indirect effects of landscape type (urban vs. rural), plant richness at the landscape (Plant S landscape) and bumblebee individual morphological traits (intertegular distance, ITD, and proboscis ratio) on the plant species richness found in the pollen collected from the bumblebee leg baskets for *Bombus lapidarius* (Fisher's C = 0.363, df = 2, p-value = 0.834) and *Bombus pascuorum* (Fisher's C = 1.653, df = 2, p-value = 0.438).



	Response	**R2m**	**R2c**	**Predictor**	**Estimate ± SE**	**df**	**Crit.Value**	***p***
Bombus lapidarius	Plant S pollen	0.07	0.08	Plant S landscape	0.456 ± 0.146	10.905	3.122	**0.010** *
				ITD	0.202 ± 0.142	115.568	1.425	0.157
				Proboscis ratio	0.236 ± 0.139	144.997	1.694	0.092
	Plant S landscape	0.90	-	Landscape	0.922 ± 0.080	14.000	11.538	**<0.001** ***
	ITD	0.12	0.19	Landscape	-0.823 ± 0.331	13.667	-2.484	**0.027** *
				Plant S landscape	0.547 ± 0.337	13.194	1.620	0.129
	Proboscis ratio	0.10	0.11	Landscape	0.134 ± 0.282	8.009	0.476	0.647
				Plant S landscape	-0.351 ± 0.280	6.785	-1.254	0.251
				ITD	-0.270 ± 0.085	131.959	-3.194	**0.002** **
Bombus pascuorum	Plant S pollen	0.05	0.06	Plant S landscape	0.379 ± 0.123	13	3.088	**0.009** **
				ITD	0.029 ± 0.117	198337	0.247	0.805
				Proboscis ratio	-0.051 ± 0.1135	188.403	-0.453	0.651
	Plant S landscape	0.90	-	Landscape	0.926 ± 0.0803	14	11.5375	**<0.001** ***
	ITD	0.14	0.14	Landscape	-0.691 ± 0.1985	212	-3.4779	**<0.001** ***
				Plant S landscape	0.344 ± 0.1985	212	1.7326	0.0846
	Proboscis ratio	0.05	0.20	Landscape	-0.491 ± 0.3849	11.5814	-1.2756	0.2271
				Plant S landscape	0.271 ± 0.3894	10.9507	0.6964	0.5007
				ITD	-0.152 ± 0.0692	203.56	-2.2015	**0.0288** *

Note: For each response variable, th eR2 is provided. ***p<0.001; **p< 0.01; *p< 0.05.Abbreviations: AICc, corrected Akaike information criterion; df, degrees of freedom; SE, standard error.

Table S11. Results on the generalised linears mixed effects models GLMMs testing the influence of plant species richness and morphological traits on the selected functional and phylogenetic diversity metrics (metrics having interrcorrelations < 0.7).



	Response	**R2m**	**R2c**	**Predictor**	**Estimate ± SE**	**df**	**t-value**	***p***	
Bombus lapidarius	Functional evenness	0.07	0.10	Intercept	0.400 ± 0.0319	9	12.521	**<0.001**	***
				Plant S landscape	0.000 ± 0.033	7	-0.008	0.994	
				ITD	-0.060 ± 0.035	64	-1.721	0.090	
				Proboscis ratio	-0.056 ± 0.029	63	-1.917	0.060	
	Functional divergence	0.15	0.15	Intercept	0.766 ± 0.025	65	31.113	**<0.001**	***
				Plant S landscape	0.022 ± 0.025	65	0.895	0.374	
				ITD	0.100 ± 0.029	65	3.478	**0.001**	**
				Proboscis ratio	0.043 ± 0.024	65	1.763	0.083	
	Phylogenetic variance	0.03	0.03	Intercept	0.621 ± 0.029	65	21.259	**<0.001**	***
				Plant S landscape	0.024 ± 0.030	65	0.816	0.418	
				ITD	-0.032 ± 0.034	65	-0.925	0.358	
				Proboscis ratio	0.004 ± 0.029	65	0.143	0.886	
Bombus pascuorum	Functional evenness	0.02	0.02	Intercept	0.414 ± 0.000	79	14.43	**<0.001**	***
				Plant S landscape	0.000 ± 0.031	79	-0.001	0.999	
				ITD	0.024 ± 0.028	79	-0.884	0.379	
				Proboscis ratio	0.020 ± 0.026	79	-0.780	0.438	
	Functional divergence	0.05	0.05	Intercept	0.808 ± 0.027	79	30.134	**<0.001**	***
				Plant S landscape	-0.036 ± 0.028	79	-1.282	0.203	
				ITD	0.018 ± 0.026	79	0.677	0.500	
				Proboscis ratio	0.035 ± 0.024	79	1.436	0.155	
	Phylogenetic variance	0.05	0.05	Intercept	0.591 ± 0.029	79	20.321	**<0.001**	***
				Plant S landscape	0.044 ± 0.031	79	1.445	0.153	
				ITD	0.027 ± 0.028	79	0.961	0.340	
				Proboscis ratio	-0.029 ± 0.027	79	-1.077	0.285	

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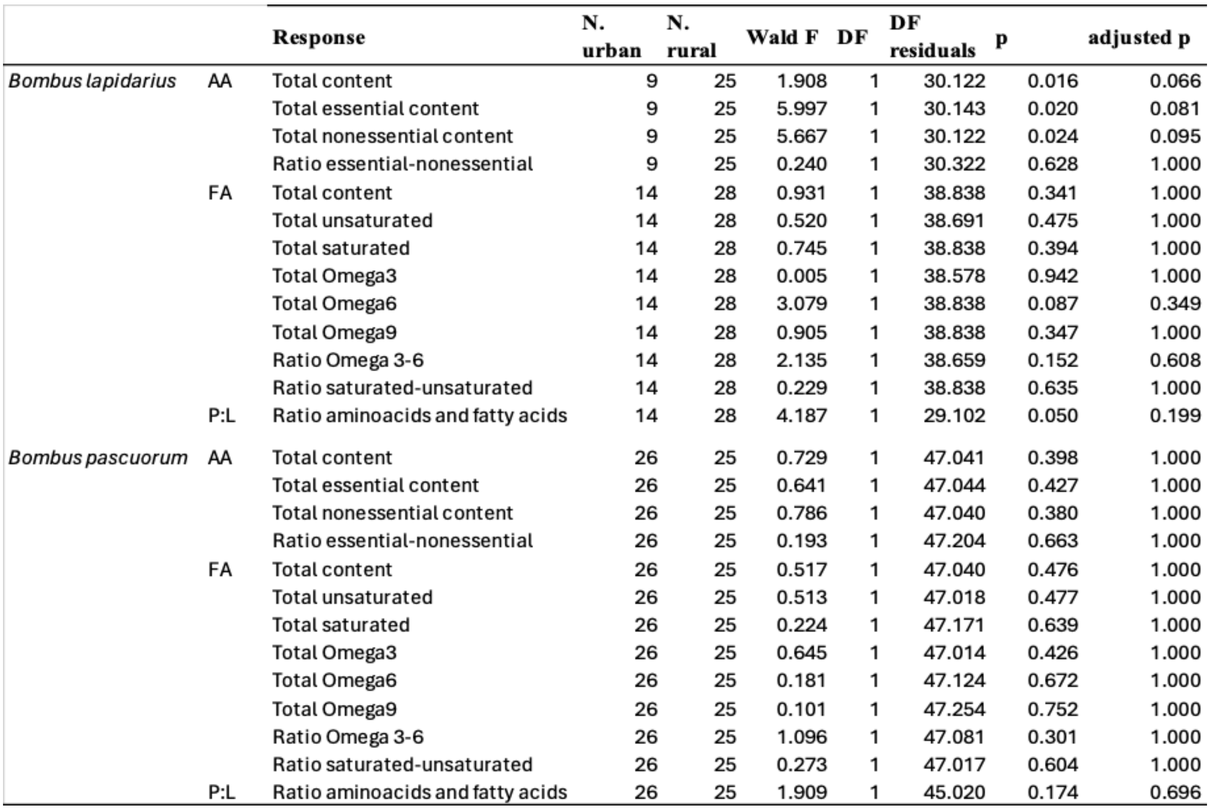
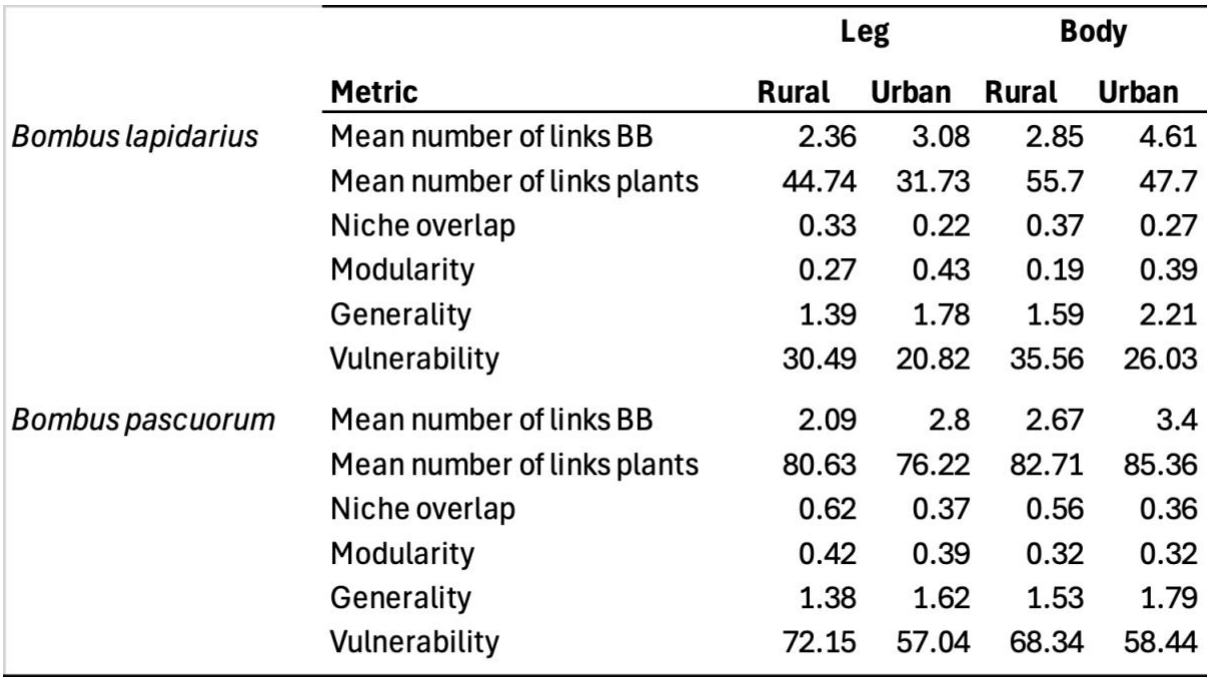
Table S12. Contrasts on the macronutrients studied as proxies of nutrient intake , that is, aminoacids and fatty acids, between urban and rural bumblebee individuals. Each response represents a specific macronutrient or ratio, organized as aminoacid (AA), fatty acid (FA) and the ratio between aminoacids and fatty acids (P:L). The table shows the analysis of deviance from linear mixed effects models (LMMs), using Wald F tests and Kenward-Roger degrees of freedom (KR DF).



		Response	**N. urban**	**N. rural**	**Wald F**	**DF**	**DF residuals**	**p**	**adjusted p**
Bombus lapidarius	AA	Total content	9	25	1.908	1	30.122	0.016	0.066
		Total essential content	9	25	5.997	1	30.143	0.020	0.081
		Total nonessential content	9	25	5.667	1	30.122	0.024	0.095
		Ratio essential-nonessential	9	25	0.240	1	30.322	0.628	1.000
	FA	Total content	14	28	0.931	1	38.838	0.341	1.000
		Total unsaturated	14	28	0.520	1	38.691	0.475	1.000
		Total saturated	14	28	0.745	1	38.838	0.394	1.000
		Total Omega3	14	28	0.005	1	38.578	0.942	1.000
		Total Omega6	14	28	3.079	1	38.838	0.087	0.349
		Total Omega9	14	28	0.905	1	38.838	0.347	1.000
		Ratio Omega 3-6	14	28	2.135	1	38.659	0.152	0.608
		Ratio saturated-unsaturated	14	28	0.229	1	38.838	0.635	1.000
	P:L	Ratio aminoacids and fatty acids	14	28	4.187	1	29.102	0.050	0.199
Bombus pascuorum	AA	Total content	26	25	0.729	1	47.041	0.398	1.000
		Total essential content	26	25	0.641	1	47.044	0.427	1.000
		Total nonessential content	26	25	0.786	1	47.040	0.380	1.000
		Ratio essential-nonessential	26	25	0.193	1	47.204	0.663	1.000
	FA	Total content	26	25	0.517	1	47.040	0.476	1.000
		Total unsaturated	26	25	0.513	1	47.018	0.477	1.000
		Total saturated	26	25	0.224	1	47.171	0.639	1.000
		Total Omega3	26	25	0.645	1	47.014	0.426	1.000
		Total Omega6	26	25	0.181	1	47.124	0.672	1.000
		Total Omega9	26	25	0.101	1	47.254	0.752	1.000
		Ratio Omega 3-6	26	25	1.096	1	47.081	0.301	1.000
		Ratio saturated-unsaturated	26	25	0.273	1	47.017	0.604	1.000
	P:L	Ratio aminoacids and fatty acids	26	25	1.909	1	45.020	0.174	0.696

Table S13. Interaction network metrics per pollen transportation structure (leg-baskets, body) and landscape type (urban, rural). BB = bumblebees



		Leg		Body	
	Metric	**Rural**	**Urban**	**Rural**	**Urban**
Bombus lapidarius	Mean number of links BB	2.36	3.08	2.85	4.61
	Mean number of links plants	44.74	31.73	55.7	47.7
	Niche overlap	0.33	0.22	0.37	0.27
	Modularity	0.27	0.43	0.19	0.39
	Generality	1.39	1.78	1.59	2.21
	Vulnerability	30.49	20.82	35.56	26.03
Bombus pascuorum	Mean number of links BB	2.09	2.8	2.67	3.4
	Mean number of links plants	80.63	76.22	82.71	85.36
	Niche overlap	0.62	0.37	0.56	0.36
	Modularity	0.42	0.39	0.32	0.32
	Generality	1.38	1.62	1.53	1.79
	Vulnerability	72.15	57.04	68.34	58.44

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