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RESEARCH

The Gut Microbial Community of Solitary Bees is Acquired through Host and Location Filtering

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Abstract

Species traits and environmental conditions are among the many factors that shape bee communities. Their effective conservation is currently challenged due to global changes. The gut microbiome likely contributes to bee plasticity and resilience but is largely understudied in solitary bees. A stable core microbiome in social bees has been identified to be important for health and survival in changing environmental conditions, but knowledge on a host-specific core microbiome in solitary bees is very scarce. In the present study, we analyzed the gut bacterial and fungal communities of eight solitary bee species commonly found in apple orchards along a latitudinal gradient throughout Europe. We aimed to understand the intra- and interspecific variations in the gut microbial communities and the extent to which host species and local environment shape the solitary bee gut microbiota. The bacterial community showed strong host effects, with each bee species having a distinct core bacterial community that was mostly stable across locations. The fungal community was most strongly influenced by the local environment, while different environmental variables were responsible for the variation in bacterial and fungal communities. Our study demonstrated that the examined solitary bee species harbor a distinct microbial diversity and composition, which undergoes host- and location-specific filtering.

Keywords Solitary bees · Gut microbiome · Amplicon sequencing · Environmental factors · Host specificity

Introduction

Human-induced environmental changes over the past two centuries have profoundly impacted wildlife, to the extent that the era is frequently referred to as the Anthropocene or the sixth major extinction event [1]. The declines in insect populations match or surpass those observed in plants and vertebrates [2]. Bees play a vital role in plant–pollinator networks where plants and pollinators depend on each other. This interdependence can render bees even more susceptible to environmental changes through declines in plant populations [3]. Moreover, many wild bees have highly specialized diets and nesting behaviors, making them vulnerable to environmental changes that reduce the availability of habitat with adequate floral and nesting resources [4–6]. Plasticity in pollen diet, thermal tolerance, phenology, and/or behavior is an important contributor to the resilience of bee populations to environmental shifts in the Anthropocene [7–9]. In this resiliency, both the host and its associated microbial

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communities contribute to the survival and success of the holobiont [10].

Studies in social bees such as honey bees and bumble bees have demonstrated that the gut microbiota is important for bee health by providing key functionalities such as nutrient acquisition, digestion of macromolecules, and pathogen inhibition [11–13]. However, recent studies questioned how the gut microbiota influences honey bee metabolism and development, as they found no effect of the gut community on honey bee weight gain [14, 15]. Whether the gut microbiota is involved in the adaptability of the host to environmental shifts remains to be elucidated. In solitary bees, the gut microbiota is considered more variable and strongly correlated with the environment due to the solitary lifestyle. Solitary bees are directly exposed to the environment upon emergence, lacking the initial priming of gut symbionts from nest mates as observed in social bee species [16]. Solitary bees may therefore acquire microbes through pollen provisions from the mother and through foraging after emergence [17]. Accordingly, the solitary bee gut reportedly consists of mainly environmental microbes [18–25].

Therefore, environmental conditions are likely to impact the microbial composition of the solitary bee gut. Cohen and colleagues demonstrated that the bacterial community of *Osmia lignaria* varied by environmental parameters, such as bee species richness, floral abundance, and percentage of natural cover [23]. Additionally, Shell and Rehan established that the gut microbiome of *Ceratina australensis* varied across its range in Australia [26]. The *Ceratina calcerata* microbiome varied across urban land use gradients, and different urban environmental variables drove the bacterial and fungal communities [27]. Furthermore, the microbiome of *C. calcerata* differed in urban and rural populations, and environmental parameters such as latitude and precipitation affected the microbial composition [28]. In contrast, other reports demonstrated that some solitary bees harbored a species-specific microbiome [20, 25, 29, 30]. It appeared that certain properties and life history traits of the bee host select for microbes from the environment. The local plant–bee network, filtration mechanisms of microbes by flowers and bees, and the degree of specialization of the bee species can additionally influence which microbes are being transmitted to bees [31]. However, it remains to be elucidated if species-specific microbiomes occur in all solitary bees and whether the host specificity remains unchanged under environmental variations.

In the present study, we characterized the bacterial and fungal communities of eight solitary bee species sampled in apple orchards along a latitudinal gradient in Europe. Closely and distantly related bee species were included in the study to assess the host-specificity of the gut microbiome as well as its diversity and overlap among the analyzed bee species. Environmental parameters, relating to bee community, climate, and landscape, collected at each orchard were used to evaluate how and which local conditions shaped the gut microbiome.

Methods

Sampling, Dissection, and DNA Extraction

Solitary bees were sampled from 46 (organic and nonorganic) apple orchards during the 2019 apple blooming season in the Netherlands, Belgium, France, Spain, and the UK [32]. Closely located sampling locations were grouped into pairs of organic and nonorganic orchards and were between 2 and 15 km apart. A subset of bees from each orchard was immediately frozen at −20 °C upon sampling. For the present study, we selected eight solitary bee species that were well represented along a latitudinal gradient throughout Europe, i.e., *Andrena cineraria, A. dorsata, A. flavipes, A. haemorrhoa, A. nitida, Anthophora plumipes, Osmia bicornis*, and *O. cornuta*. Selection of orchards along the latitudinal gradient with high numbers of each of the eight selected solitary bee species resulted in nine pairs of orchard sites including both northern and southern locations (Fig. S1). Selected bees ($n = 310$) were surface-sterilized using Ummonium38 Medical Spray and dissected under sterile conditions. The gut was extracted and homogenized in 275 µl of STET buffer (8% sucrose, 5% Triton X-100, 50 mM EDTA, and 50 mM Tris–HCl) using sterile micropestles. Total bacterial and fungal DNA was extracted using an adaptation of the phenol–chloroform protocol described earlier [33]. The STET supernatant was collected in a separate 1.5-ml Eppendorf tube after initial centrifugation and was used to resuspend the pellet before the lysis step. DNA quality was assessed with NanoDrop and DNA concentration was measured using a Quantus™ Fluorometer.

Amplicon Sequencing of the Bacterial and Fungal Communities

In case the DNA yield was insufficient (< 5 ng/µl) for amplicon sequencing of both the 16S rRNA gene and the eukaryotic internal transcribed spacer 2 (ITS2) region, we prioritized amplicon sequencing of the former. As a result, a total of 310 DNA samples were used for 16S rRNA gene amplicon sequencing and 214 DNA samples for ITS2 amplicon sequencing (Table S1). Library preparation and amplicon sequencing were performed by BaseClear B.V. (Leiden, the Netherlands). The V3–V4 region of the 16S rRNA gene was PCR amplified using primer pair 341 F (5′-CCTACGGGNGGCWGCAG-3′) and 785R (5′-GACTACHVGGGTATCTAATCC-3′), and the ITS2 region was PCR amplified using primer pair ITS3F (5′-GCATCGATGAAGAACGCA

GC-3′) and ITS4R (5′-TCCTCCGCTTATTGATATGC-3′). The libraries were sequenced on an Illumina MiSeq platform generating 300-bp paired-end reads.

16S rRNA and ITS2 raw reads were analyzed separately with DADA2 version 1.16 [34]. Bacterial forward and reverse reads were trimmed to a length of 280 bp and 230 bp, respectively, and primers were removed using the trimLeft parameter. Merged paired reads with a length between 400 and 428 bp were retained for further analyses. ITS2 primers were removed with cutadapt version 3.4 [35] and the rest of the DADA2 pipeline was followed with default parameters. Taxonomy assignment to the final 16S rRNA and ITS2 amplicon sequence variants (ASVs) was performed with the DADA2 formatted training FASTA files from the SILVA SSU database version 138.1 [36] and the General FASTA release files from the UNITE ITS database version 8.3 (all eukaryotes) [37], respectively. ASVs without a taxonomic assignment or assigned to chloroplasts, mitochondria, Archaea, and Eukarya were removed from the 16S rRNA dataset, whereas only fungal ASVs were retained in the ITS2 dataset.

Microbial Community Analyses

All data analyses were performed in R version 4.1.0. Alpha diversity estimates (observed richness and Shannon diversity) were calculated for the bacterial and fungal datasets and plotted using the phyloseq package version 1.40.0 [38]. Coupled organic and nonorganic orchards (Fig. S1) showed no significant difference in alpha diversity estimates in linear regressions with coupled orchards as a random effect and were, therefore, collapsed into a single coupled location (hereafter referred to as ‘location’) for further analyses. Alpha diversity estimates were compared between bee species and bee genera for the whole dataset and at each location, as well as between locations for the whole dataset and for each bee species and genus. Statistical significance was tested using the Kruskal–Wallis test and Wilcoxon rank-sum tests for pairwise comparisons (ggpubr package).

Community dissimilarity was assessed via PERMANOVA tests (‘adonis2’ function with 9,999 permutations) on Bray–Curtis dissimilarity matrices using the vegan package [39]. Coupled organic and non-organic orchards (Fig. S1) showed no significant difference in microbial community composition in a PERMANOVA analysis and were, again, collapsed into a single location (hereafter referred to as ‘location’) for further beta diversity analyses. To assess the effect of bee host and environment on the gut bacterial and fungal communities, PERMANOVA analyses were performed with bee genus, bee species, and location, where bee species was nested within bee genus and location as independent factors. Dispersion of the data was analyzed via the ‘betadisper’ function (vegan package). Community dissimilarity was visualized via principal coordinates analysis (PCoA) plots of Bray–Curtis distances.

Environmental variables collected by Weekers et al., 2022 [32] were used to evaluate correlations with the microbial communities (Table S2). We divided the variables into three groups (i.e., bee community, landscape, and climate) and performed Pearson’s correlation tests within groups to identify variables that were highly correlated ($p < 0.05$) (Table S3). We retained the variables that were correlated with most other variables within the group and least correlated with the remaining variables to avoid colinearity between variables in further analyses. We selected two bee community variables (bee abundance and bee Shannon index), four landscape variables computed at a 1-km radius around each orchard (urban cover, seminatural cover, land Shannon index, and orchard size), and two climate variables (latitude and average seasonal precipitation). Separate dissimilarity matrices were calculated for each of the three variable groups (bee community, landscape, and climate) using Euclidean distances. Haversine distances were used to create a distance matrix with the physical geographical distance between sampling sites. We then compared the Bray–Curtis dissimilarity in bacterial and fungal communities to the dissimilarity in bee community, landscape, climate, and geographical distance matrices using Mantel tests (Spearman’s correlation coefficient with 9999 permutations). For a more fine-scale analysis of how each environmental variable was related to the variation in microbial community between sampling sites, we used a redundancy analysis to select for variables that could best explain the variation in the microbial community. To this end, a forward selection of variables was performed on Hellinger transformed ASV datasets using the ordiR2step function (vegan package), with final selection of variables based on Monte Carlo permutation tests with 9999 permutations and Benjamini–Hochberg corrected p values. An ordination diagram was constructed that fitted the selected variables as vectors onto the ordination.

Core bacterial and fungal ASVs were determined for each bee species, based on the definition that a core ASV should have at least 50% prevalence and 1% relative read abundance [20]. Per bee species, core ASVs were also determined at each coupled location with more than four bee specimens to evaluate variations in core community along the latitudinal gradient.

Results

The gut bacterial communities of 310 bees representing eight solitary bee species and the gut fungal communities of 214 bees representing six solitary bee species were determined via marker-gene amplicon sequencing (Table S1). Raw data were analyzed with the DADA2 pipeline, and data cleaning retained 3,476,422 16S rRNA V3-V4 and 2255,138 ITS2 high-quality reads attributed to 4804 and 880 ASVs, respectively. Rarefaction curves of the bacterial ASVs showed that a sufficient sequencing depth was reached for most samples, except for many *A. haemorrhoa* samples and for some samples of other *Andrena* species (Fig. S2). Fungal rarefaction curves exhibited greater variability in sequencing depth, with many *A. haemorrhoa* and *A. cineraria* samples not reaching a plateau (Fig. S3). The bacterial and fungal community composition of each bee species based on the 20 most abundant ASVs is shown in Fig. 1. The most predominant bacterial genera were identified as *Acidocella*, *Lactococcus*, *Wolbachia*, and *Sodalis*, whereas *Metschnikowia*, *Cladosporium*, and *Starmerella* were the most predominant fungal genera. The relative abundance of the predominant bacterial genera excluding endosymbiont ASVs (i.e., *Wolbachia*, *Sodalis*, *Arsenophonus*, and *Spiroplasma* ASVs) is shown in Fig. S4.

Effect of Host on the Microbial Community

Bee genus explained most of the detectable variation in the bacterial community (PERMANOVA, $R^2 = 0.202$, $p < 0.001$, betadisper $p < 0.001$), whereas it had less explanatory power over the fungal community (PERMANOVA, $R^2 = 0.077$, $p < 0.001$, betadisper $p = 4.2\text{e-}03$). Bee species explained 13% of the variation in the bacterial community (PERMANOVA, $R^2 = 0.129$, $p < 0.001$, betadisper $p < 0.001$) but had no significant effect on the fungal community (PERMANOVA, $R^2 = 0.016$, $p = 0.09$, betadisper $p = 4.5\text{e-}02$). Clustering according to bee genus and bee species could be observed on the PCoA ordinations for the bacterial communities of the full dataset (Fig. 2a) and of the subsets per location (Fig. S5). No clear clustering according to host was observed on the PCoA ordinations for the fungal communities of the full dataset (Fig. 2b) and of the regional subsets (Fig. S6). Both microbial communities, however, were significantly different by bee species and genus at most locations (Figs. S5 and S6). PERMANOVA analyses performed on the bacterial dataset excluding endosymbiont ASVs showed that bee genus and species still had a significant effect on the bacterial community (p values < 0.001). However, the variation explained by bee genus (3.9%) was considerably lower than that of bee species (12.5%).

Observed richness and Shannon diversity estimates of the overall bacterial and fungal datasets were significantly

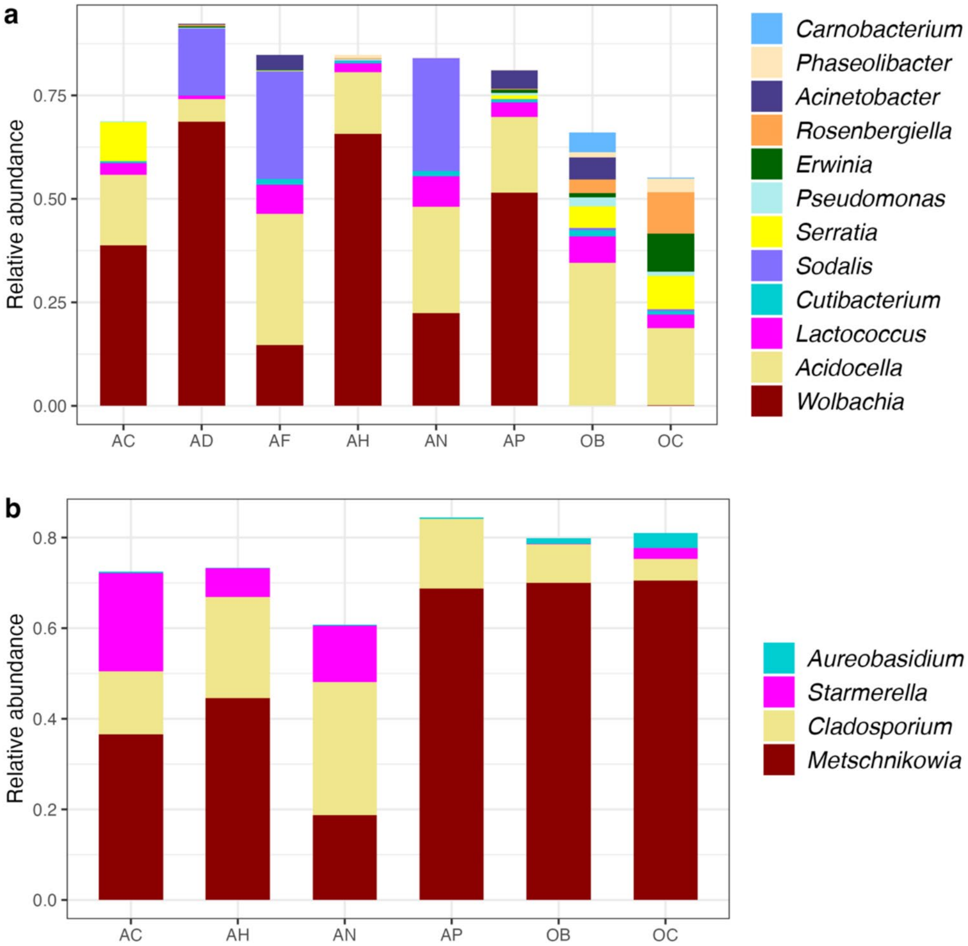


Fig. 1 Relative abundance of the 20 most abundant bacterial **a** and fungal **b** ASVs summarized at genus level and averaged per bee species. AC, *Andrena cineraria*; AD, *Andrena dorsata*; AF, *Andrena flavipes*; AH, *Andrena haemorrhoa*; AN, *Andrena nitida*; AP, *Anthophora plumipes*; OB, *Osmia bicornis*; OC, *Osmia cornuta*

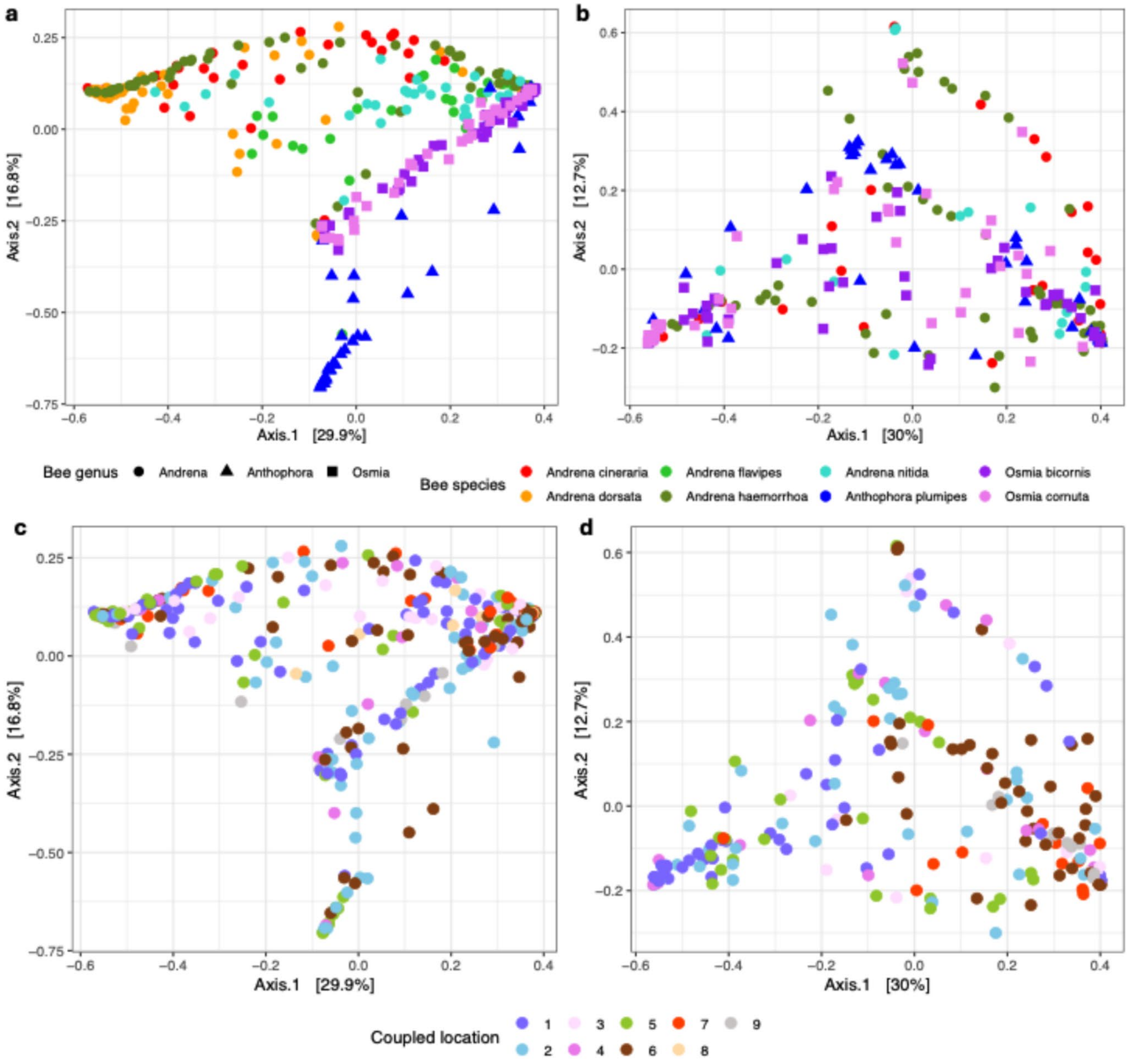


Fig. 2 Principal coordinates analysis plots based on Bray–Curtis dissimilarity matrices of the bacterial **a**, **c** and fungal communities **b**, **d**

different between most bee genera and species (Figs. S7 and S8, respectively). Significant differences in alpha diversity between hosts were also detected at certain locations (Table S4). Differences in alpha diversity estimates were most notable between bee genera (Fig. S7). Bacterial and fungal richness was the lowest for the genus *Andrena* and highest for the genus *Osmia*. *Anthophora plumipes* showed the lowest bacterial Shannon diversity, whereas *Andrena* showed the lowest fungal Shannon diversity. The highest bacterial and fungal Shannon diversity was observed for the genus *Osmia*.

Each bee species harbored a distinct microbial core composition (Table S5). Some core ASVs were detected in all bee species, namely *Acidocella facilis* ASV2 and *Cladosporium austrohemisphaericum* ASV3. Similarities in core composition could also be observed among *Andrena* and *Osmia* species. All *Andrena* species additionally shared the bacterial *Wolbachia* ASV3, whereas the two *Osmia* species shared one bacterial ASV assigned to *Lactococcus* and two fungal ASVs assigned to *Metschnikowia pulcherrima*. Aside from *Wolbachia* ASV3, some *Andrena* species harbored additional core ASVs assigned to *Wolbachia* and/or

Sodalis, another endosymbiont. A distinct *Wolbachia* ASV was detected in the core of *An. plumipes* compared to those present in *Andrena* species. Core ASVs representing endosymbionts were not detected in the *Osmia* species.

Effect of Local Environment of the Microbial Community

PERMANOVA tests demonstrated that location explained most of the detected variation in the fungal community ($R^2 = 0.142$, $p < 0.001$, betadisper $p < 0.001$), whereas it explained only a minor fraction of the variation in the bacterial community ($R^2 = 0.057$, $p < 0.001$, betadisper $p < 0.001$) compared to the variation explained by bee genus or species. When the PERMANOVA analysis was performed on the bacterial dataset excluding endosymbiont ASVs, variation explained by location increased to 7.5%. No clear clustering according to location could be observed on the PCoA ordinations of the bacterial and fungal communities (Fig. 2c and d, respectively). Yet, microbial communities were significantly different between locations for each bee genus and most bee species (Table S6). The alpha diversity estimates, observed richness, and Shannon diversity, only differed significantly between a few locations for the overall bacterial and fungal datasets (Fig. S9) and subsets per bee species and genus (Table S7).

To evaluate if location also influenced the core microbial community of the bee species, we determined location-specific core ASVs for each bee species (only including locations with four or more samples). The bacterial core ASVs of the bee species changed across locations, although some core ASVs were consistently present in all locations (Fig. S10). Only *A. haemorrhoa* and *A. nitida* showed bacterial core ASVs consistently present across locations, with only some variation in relative abundance. The fungal core ASV compositions of the bee species were more variable between the locations than the bacterial core compositions (Fig. S10). Only the *Andrena* species harbored one fungal core ASV that was consistently present in all included locations (i.e., *Cladosporium austrohemisphaericum* ASV3).

The observed location effects were further assessed by performing Mantel tests to find correlations between the microbial community dissimilarity matrices and the environmental dissimilarity matrices. The dissimilarity in the bacterial community of all bee species was significantly correlated with the dissimilarity in bee community and landscape parameters, whereas the fungal community dissimilarity was significantly correlated to geographic distance and dissimilarity in the bee community (Fig. 3). At the level of individual bee species and genera, geographic distance and bee community dissimilarity were most frequently correlated to the dissimilarity in bacterial communities. Only the genus *Andrena* showed significant correlations between bacterial community dissimilarity and all four environmental dissimilarity matrices. The fungal communities of *An. plumipes*, *O. cornuta*, and genus *Osmia* correlated significantly with all environmental dissimilarity matrices, in high contrast to the fungal communities of *Andrena* species and genus (Fig. 3).

We analyzed how the eight selected environmental variables recorded at the sampling sites (Table S3) related to the variation observed in the microbial communities. Seven environmental variables with a significant effect on the bacterial community variation were selected by the redundancy analysis (Fig. 4). Together they explained 4.8% of the variation in the model. Urban cover and Shannon's landscape diversity index explained the majority of the variation ($R^2 = 0.01$ each) out of the seven variables. All eight variables were selected for the fungal community and together explained 10.7% of the variation. Latitude explained most of the variation ($R^2 = 0.06$) in the fungal community.

Discussion

In the present study, we characterized the gut microbial communities of eight solitary bee species, representing three bee families (Andrenidae, Apidae, and Megachilidae) and three genera (*Andrena, Anthophora*, and *Osmia*), sampled in apple orchards along a latitudinal gradient in Europe. The effect of host, location, and environmental conditions on the composition and diversity of the bacterial and fungal communities was evaluated, to identify parameters involved in shaping the solitary bees' gut microbial communities. We detected that microbial communities were shaped by host species (with the strongest signal in the bacterial community) and by the local environment (with the strongest signal in the fungal community).

Solitary Bee Species Harbor Host-Specific Microbial Communities

PERMANOVA analyses demonstrated that host species and genus explained 33% of the variation in the bacterial community (including endosymbiont ASVs) and 9% of the variation for the fungal community, demonstrating that the bacterial community was more host-specific than the fungal community. The significant effect of the host was furthermore observed in the difference in alpha diversity estimates (Figs. S7 and S8), and the composition of the identified core ASVs (Table S5). These results showed that each of the eight solitary bees examined was able to establish and maintain a distinctive microbial community, demonstrating a strong host influence in establishing the bees' gut microbiota, such as through the selection of environmental microbes based on life history traits of the host [31].

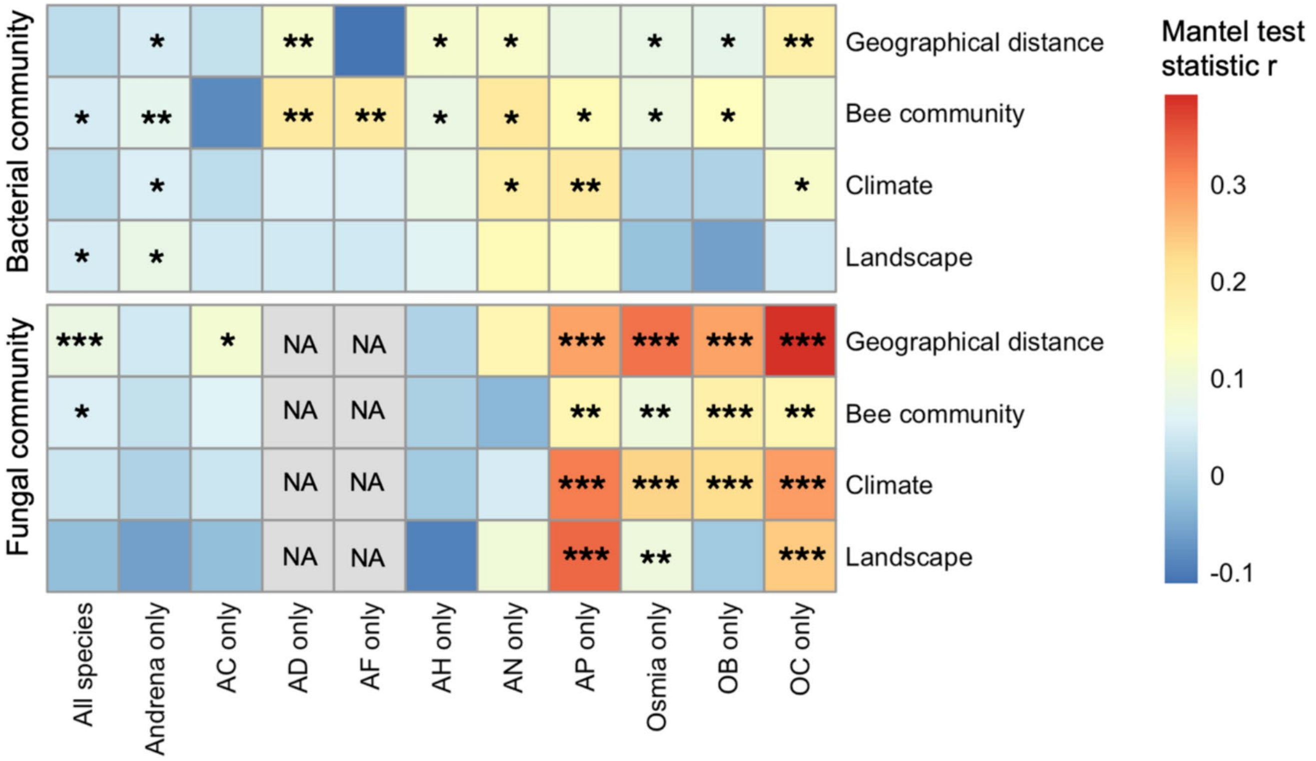
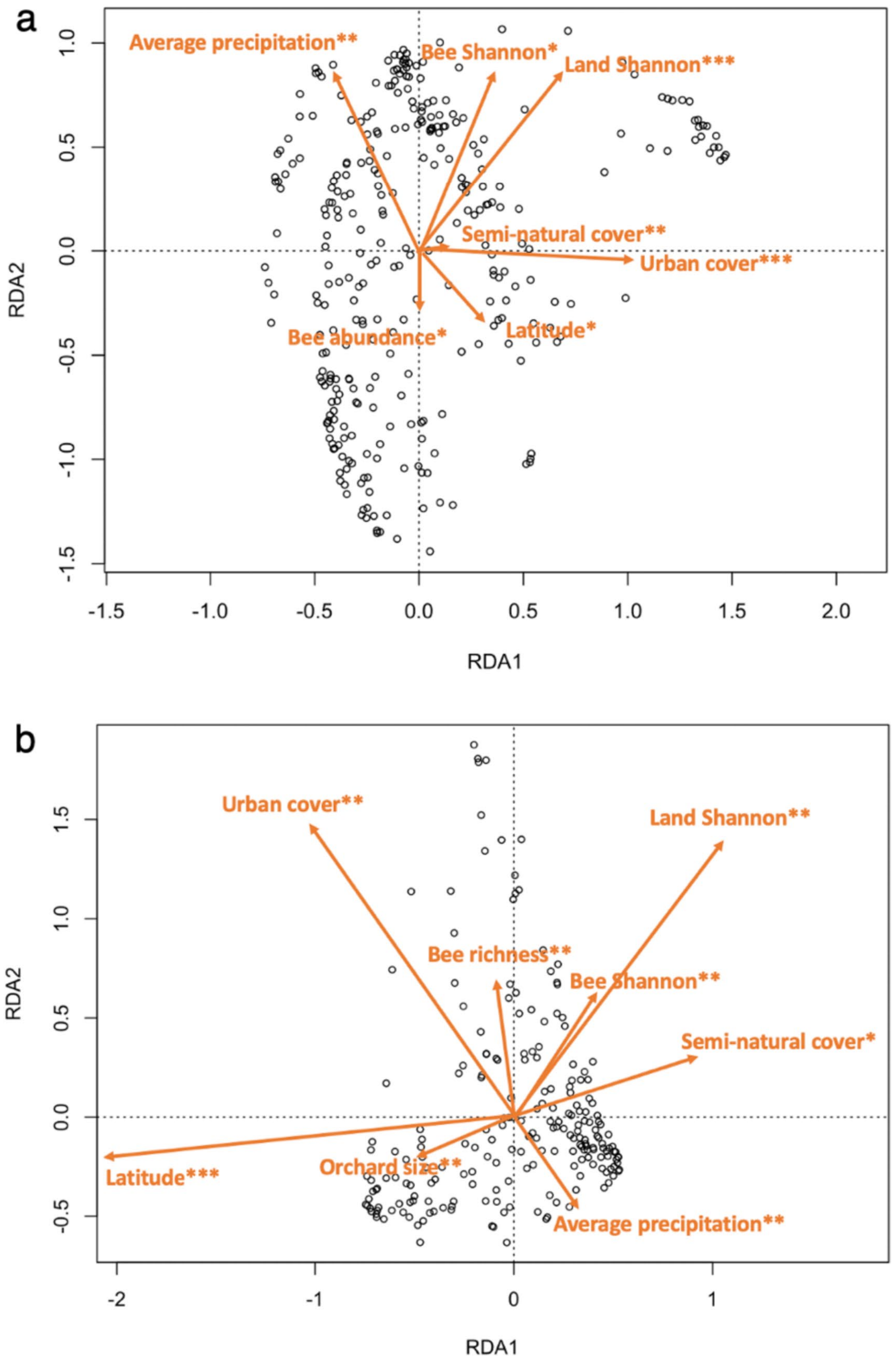


Fig. 3 Mantel correlations between Bray–Curtis distance matrices of the bacterial and fungal communities and the Haversine distance matrix of the geographical distances and the Euclidean distance matrices of different sets of environmental parameters. Significant correlations are marked with * for $p < 0.05$, ** for $p < 0.01$, and *** for $p < 0.001$. Cells were marked with 'NA' for bee species whose fungal community was not analyzed. AC, *Andrena cineraria*; AD, *Andrena dorsata*; AF, *Andrena flavipes*; AH, *Andrena haemorrhoa*; AN, *Andrena nitida*; AP, *Anthophora plumipes*; OB, *Osmia bicornis*; OC, *Osmia cornuta*

The solitary bee species included in the present study are polylectic and floral visitors of apple orchards, yet they exhibit different affinities towards pollen sources and plant families [40–42]. The closely related *O. bicornis* and *O. cornuta* are pollen generalist bees but develop differently on pollen from the same plant species [43] and show specific fitness responses based on the available pollen resources [44]. In addition, *O. bicornis* avoids Asteraceae pollen during foraging, while Asteraceae pollen composed a large part of the diet of related species [45]. Bee tongue length and body size can influence their foraging behavior as they play a crucial role in accessing food rewards offered by flowers [46, 47]. As a result, specific plant-pollinator networks arise based on the dietary preference of the host, and foraging can act as a transmission route for floral bacteria to enter the solitary bee gut [21]. Different plant species or families are likely associated with distinct microbial communities depending on the chemical and nutritional properties of pollen and nectar and other floral characteristics [48]. In addition to dietary preferences, the bee species included in the present study exhibit different nesting behaviors. *Andrena* species nest underground, and *Osmia* species utilize aboveground cavities, whereas *An. plumipes* nests in both above- and underground resources [49]. Different microbes will be encountered by bees based on their nesting behavior and substrates used for nest construction, which further can contribute to a host-specific microbiota. Additional host traits, such as morphological and physiological characteristics, immune system, and gut conditions, are likely involved in this host-specific microbe selection process as well [31, 50]. As certain characteristics are shared between closely related bee species, it was not surprising that bee species of the same genus harbored a more similar microbial community. Indeed, the bacterial communities clustered together according to bee genus on the PCoA ordinations (Fig. 2) and core ASVs were also more similar among species of the same genus (Table S5). Besides the life history traits of the host, microorganisms may also aid in host specificity. They may have gained adaptations or properties that allow them to colonize the gut or recognize their host species [50]. Finally, solitary bees may have established transmission pathways for the transfer of symbionts between generations. Endosymbiotic bacteria, such as *Wolbachia* and *Sodalis*, can be vertically transmitted from mother to offspring, whereas the nest can serve as an initial source of microbes for the newly emerged adult bee [31, 50]. Microbes likely occur on the


a
Average precipitation**
Bee Shannon*
Land Shannon***
Semi-natural cover**
Urban cover***
Latitude*
Bee abundance*
RDA2
1.0
0.5
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-0.5
-1.0
-1.5
-1.5
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0.5
1.0
1.5
2.0
RDA1
b
Urban cover**
Land Shannon**
Bee richness**
Bee Shannon**
Semi-natural cover*
Latitude***
Orchard size**
Average precipitation**
RDA2
1.5
1.0
0.5
0.0
-0.5
-2
-1
0
1
RDA1

◂**Fig. 4** Transformation-based redundancy analysis ordinations of the bacterial **a** and fungal **b** communities. The selected environmental variables that best explain the variation in community composition were fitted onto the ordinations as vectors. Significant values were determined by Monte Carlo permutation tests with 9,999 permutations (* for $p < 0.05$, ** for $p < 0.01$, and *** for $p < 0.001$)

brood provision, on the brood cell walls, and in feces left by the mother or the larvae and can be gathered by the emerging adult bee.

Although it is frequently hypothesized or reported that solitary bees harbor a highly variable microbiome, we detected microbial taxa that were abundantly present in the majority of replicates of a given species. This allowed us to define the bacterial and fungal core ASVs for each bee species, and each species was characterized by a distinct core microbiome (Table S5). Several of the core ASVs could be assigned to taxa commonly found on flowers, such as *Rosenbergiella* [51], *Starmerella* [52] and *Metschnikowia* [53]. Others, such as *Pseudomonas* [54], *Lactococcus* [55], and *Cladosporium* [56] occur in many environmental sources. Core ASVs assigned to the insect endosymbionts *Wolbachia* and *Sodalis* were also detected. *Wolbachia* was abundantly present in the core microbiome of *Andrena* spp. and *An. plumipes*, across all locations, demonstrating a tight host relationship (Fig. S10). Moreover, a different *Wolbachia* ASV was consistently associated with the *Andrena* spp. compared to *An. plumipes*, as was previously reported [25]. After removal of the endosymbiont ASVs, bee genus explained a considerably lower variation in the PERMANOVA analysis, indicating that these *Wolbachia* ASVs contributed significantly to the genus effect. *Sodalis* ASVs were also detected in the core of several *Andrena* species, where each species was associated with a distinct *Sodalis* ASV (Table S5). Some core ASVs occurred consistently across all bee species and were assigned to *Acidocella facilis*, *Lactococcus*, and *Cladosporium austrohemisphaericum*. These microbes likely contribute to essential functionalities within solitary bees, regardless of the host species. *Cladosporium austrohemisphaericum* was shown to extend the life span of honey bees [57], whereas *Lactococcus* can degrade pollen pectin and other plant polymers, and can thus act as an important fermenter of carbohydrates [58]. *Acidocella facilis* is an acidophile of the family Acetobacteriaceae [59]. It might, like other acidophilic bacteria, aid in gut acidification and defense against pathogens [60].

Solitary Bee Gut Microbial Communities Are Influenced by the Local Environment

The local environment influenced the microbial composition of solitary bee species. PERMANOVA analysis demonstrated that the fungal community of the bee species was more strongly influenced by location ($R^2 = 0.142$) than the bacterial community ($R^2 = 0.057$). This showed that the gut microbial communities of solitary bees were influenced by the local environmental conditions at each location. Although all bees were collected in nominally the same habitat type (apple orchards), the local bee community and broader landscape composition were unique to each site, whereas the local climate varied along the latitudinal gradient. We included neighboring organic and non-organic orchards to assess the effect of management type on the microbiota (Fig. S1). However, the microbial communities of bees were not affected by management type in the present study. Orchards where conventional practices and integrated pest management were employed were grouped into a single category (i.e., nonorganic orchards) as they displayed similar management intensity [32]. However, the distinction between different management types is not always clear, and the practices implemented can be highly heterogeneous [32], which might explain why we did not detect an effect of management type on the bee microbial communities. The effect of the local environment was also visible in the core ASVs, as the bacterial core ASVs differed slightly across locations while the fungal core ASV compositions were more variable, except for *A. haemorrhoa* and *A. nitida* that contained the same bacterial core ASVs across the analyzed locations (Fig. S10). Although this evaluation was useful to assess changes in the core ASV composition along the latitudinal gradient, low sample numbers and missing specimens at several locations hampered robust core ASV calculations and investigation of the core ASV dynamics.

We first used Mantel tests to assess if variations in the microbial community were related to variations in the environment at a broader scale, then used redundancy analysis to examine the individual impact of the eight selected environmental variables. The bacterial community dissimilarity matrices were most frequently correlated with the bee community dissimilarity matrices and geographical distance between orchards (Fig. 3) demonstrating that the bee bacterial communities were more similar in orchards with similar bee community parameters and at a closer distance. Although the variation in the bacterial community explained by all environmental variables together was less than 5% in the redundancy analysis, urban cover and Shannon's landscape diversity index (two landscape variables) explained most of the detected variation (Fig. 4). Both variables were computed at a 1-km radius around each orchard and represent the proportion of urbanization and the heterogeneity of the landscape within that area. These findings corroborated previous work that demonstrated that bee community and landscape variables are important drivers of the wild bee bacterial community [23, 27, 28]. The bee community and landscape variables determine the structure of the local plant-pollinator network, which in turn is associated with the transmission of microbes between plants, solitary bees, and other pollinators [31]. Together, this demonstrated that the different plant and bee species involved in the local plant–pollinator networks contributed to host-specific filtering mechanisms in solitary bees and determined which microbes are transmitted upon bee-bee and bee-plant interactions. Although a large part of the variation detected in the bacterial community of the present study can be attributed to the host (33%), future studies would benefit from including additional environmental parameters, specifically those focusing on the plant-pollinator network. As it was recently shown that plant-pollinator network structure alone cannot explain microbiome variability at the individual level in wild bees, other relevant sources of microbes must be considered as well [61].

The fungal community dissimilarity matrices were strongly correlated with the geographical distance between orchards, while *An. plumipes* and *Osmia* fungal communities showed strong correlations with all environmental dissimilarity matrices (Fig. 3). Latitude (a climate variable) explained most of the detected variation in the redundancy analysis (6%, Fig. 4). As the apple orchards included in the present study were located on a latitudinal gradient throughout Europe which represented a climate gradient, these results indicate that fungal communities in solitary bees were strongly influenced by climate and differed by geographical distance between orchards and along the climate gradient. Latitude was highly correlated with the average temperature and solar radiation at the sampling sites (–0.94 and –0.97, respectively, Table S3). These two environmental variables together with average precipitation, the second included climate variable (Fig. 4), likely influenced the bee fungal communities through their effect on the presence of environmental fungi in foraging and nesting resources. Temperature and precipitation were also identified to influence the fungal microbiome in *Ceratina calcerata* [27]. Climate variables may also impact the physiology of the host (for example, as a response to heat stress or heavy precipitation events) resulting in an altered gut microbial community.

Conclusion

The present study demonstrated that the gut microbial composition of eight solitary bee species sampled along a latitudinal gradient throughout Europe was shaped by both bee hosts and the local environment. The bacterial community was more host-specific, whereas the fungal community was more strongly influenced by the local environment. Different environmental variables were responsible for shaping the bacterial and fungal communities in solitary bees. Parameters characterizing the local plant-pollinator network and climate were likely involved in the environmental occurrence of microbes and the host-specific filtering mechanism, resulting in a host-specific gut microbiome composed of environmental microorganisms. This host specificity is further enforced by the existence of a core microbial community for each bee species.

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Author Contributions A.H., G.S., and P.V. conceptualized the study. N.J.V., D.M., G.S., and P.V. acquired the funding. T.W., L.M., N.L., T.J.W., D.C., M.G., N.J.V., and D.M. provided the study material. A.H. and T.T. performed the experiments. A.H., M.J., and T.T. performed the data analysis. A.H. and P.V. wrote the original draft of the manuscript. All authors reviewed and contributed to the final version of the manuscript.

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Data Availability The 16S rRNA gene and ITS2 amplicon sequencing data generated for the present study are archived at the European Nucleotide Archive and are accessible through BioProject accession numbers PRJEB78945 and PRJEB78946, respectively.

Declarations

Ethics Approval No approval of research ethics committees was required.

Competing interests The authors declare no competing interests.

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