REVIEW

Environmental Effects on Bee Microbiota

Phuong N. Nguyen[1] · Sandra M. Rehan[1]

Abstract

Anthropogenic activities and increased land use, which include industrialization, agriculture and urbanization, directly affect pollinators by changing habitats and floral availability, and indirectly by influencing their microbial composition and diversity. Bees form vital symbioses with their microbiota, relying on microorganisms to perform physiological functions and aid in immunity. As altered environments and climate threaten bees and their microbiota, characterizing the microbiome and its complex relationships with its host offers insights into understanding bee health. This review summarizes the role of sociality in microbiota establishment, as well as examines if such factors result in increased susceptibility to altered microbiota due to environmental changes. We characterize the role of geographic distribution, temperature, precipitation, floral resources, agriculture, and urbanization on bee microbiota. Bee microbiota are affected by altered surroundings regardless of sociality. Solitary bees that predominantly acquire their microbiota through the environment are particularly sensitive to such effects. However, the microbiota of obligately eusocial bees are also impacted by environmental changes despite typically well conserved and socially inherited microbiota. We provide an overview of the role of microbiota in plant-pollinator relationships and how bee microbiota play a larger role in urban ecology, offering microbial connections between animals, humans, and the environment. Understanding bee microbiota presents opportunities for sustainable land use restoration and aiding in wildlife conservation.

Keywords Pollinator health · Bacteria · Fungi · Plant-pollinator networks · Urbanization · Agriculture · Sustainability · One Health

Introduction

The microbiome creates complex relationships between a host and its associated bacteria, fungi, protozoa, as well as viruses, leading to networks that contribute to host health and fitness [1]. Commonly found microbial members across many individuals are characterized as part of the species' core microbiota [2], providing key insights into the members that have co-evolved with the host. Characterizing and analyzing the function of microbiota has been an increasing area of study in bees to understand the factors affecting their microbiota and by proxy, bee health. Another key determinant of host health is the surrounding environment, which directly affects an individual's habitat and indirectly influences microbial composition [3]. As cities expand to accommodate a growing population and anthropogenic

activity increases to meet their demand, local environmental changes from land use are inevitable and threaten the landscapes that support the habitats and living conditions of bees and their microbiota. Considering the effects of varying microbial composition alongside the impact of urbanization offers an increasingly relevant perspective as to how bees are responding to changes in land use.

An understanding of how the external surroundings affect bees and microbiota in changing environments will help direct conservation efforts necessary to counteract these human-driven changes and protect pollinators. Bees perform the majority of pollination services and have faced a decrease in population size over the past few decades [4–7]. Due to their important role in both rural and urban agriculture [4, 8], pollinator declines have raised the concerns of food insecurity and ecological collapse [5]. Worsening the issue, human disturbed landscapes that are common in urban areas and result in habitat loss, affect bee abundance and species richness [9–12]. There are arrays of local environmental factors, including plant communities, green space

✉ Sandra M. Rehan
sandra.rehan@gmail.com

1 Department of Biology, York University, Toronto, Canada

availability, microclimate, and types of green space, which filter out certain bee functional traits and alter bee community composition [10, 13, 14]. While studies investigating exactly how anthropogenic activities may be responsible for this global pollinator decline over time are ongoing, considerations need to be made to determine how to protect bees from anthropogenic activities.

Here we review the literature on how bee microbiota is established and is altered by their environment, such as in response to urbanization, agriculture, and microclimate change (Fig. 1). Mainly examining studies using targeted sequencing to characterize bacteria and fungi in bees, we highlight an array of recent and fundamental studies that describe factors influencing beneficial bee microbiota and enable comparisons among bee genera. First, we discuss the role of bee natural history and sociality in determining how microbiota are acquired and maintained. Second, we characterize pollinator susceptibility to changes in their microbiota related to human land use, and further examine which different environmental factors may contribute to altered microbiota. Finally, we examine the role of microbiota in plant-pollinator relationships and how its composition and diversity could illuminate strategies of pollinator conservation. We also highlight the role that microbiota play in urban ecology and the sensitivities of symbiotic relationships to anthropogenic activities. This review offers unique insights into the nature of the bee microbiome to better understand bee declines and the impact of urbanization on wildlife conservation and ecological health.

The Honey Bee Microbiota

Studies of bacteria and fungi in *Apis mellifera* have provided foundational descriptions of the microbial communities associated with bees (Table 1, S1). There are relatively few bacterial genera found within the honey bee gut, despite high levels of functional diversity within the core microbiota, offering an array of benefits in pathogen defense, immunity, and nutrient utilization [15, 16]. The core honey bee microbiota consists of five species groups comprising as much as 95–99.9% of honey bee bacterial communities [17, 18] (Fig. 2, Table S2). These microbiota are generally considered to belong to a highly conserved core group within corbiculates, including predominantly social honey bees, bumble bees, and stingless bees [19]. Microbial composition are particularly similar in adult workers, with males and queens displaying more variance in microbial communities due to different social roles [17, 20–22]. Recent studies have gone beyond bacteria to explore the fungal communities within the honey bee, finding that commonly occurring and augmented fungi such as *Saccharomyces* and *Aspergillus* can affect microbial communities, immunity, and physiology [22–24] (Table 1). These characterizations of the honey bee microbiota have provided a baseline for microbial diversity and taxonomic composition within bees and have been a vital stepping stone to exploration of other factors that affect the microbiota (Table 1, S1).
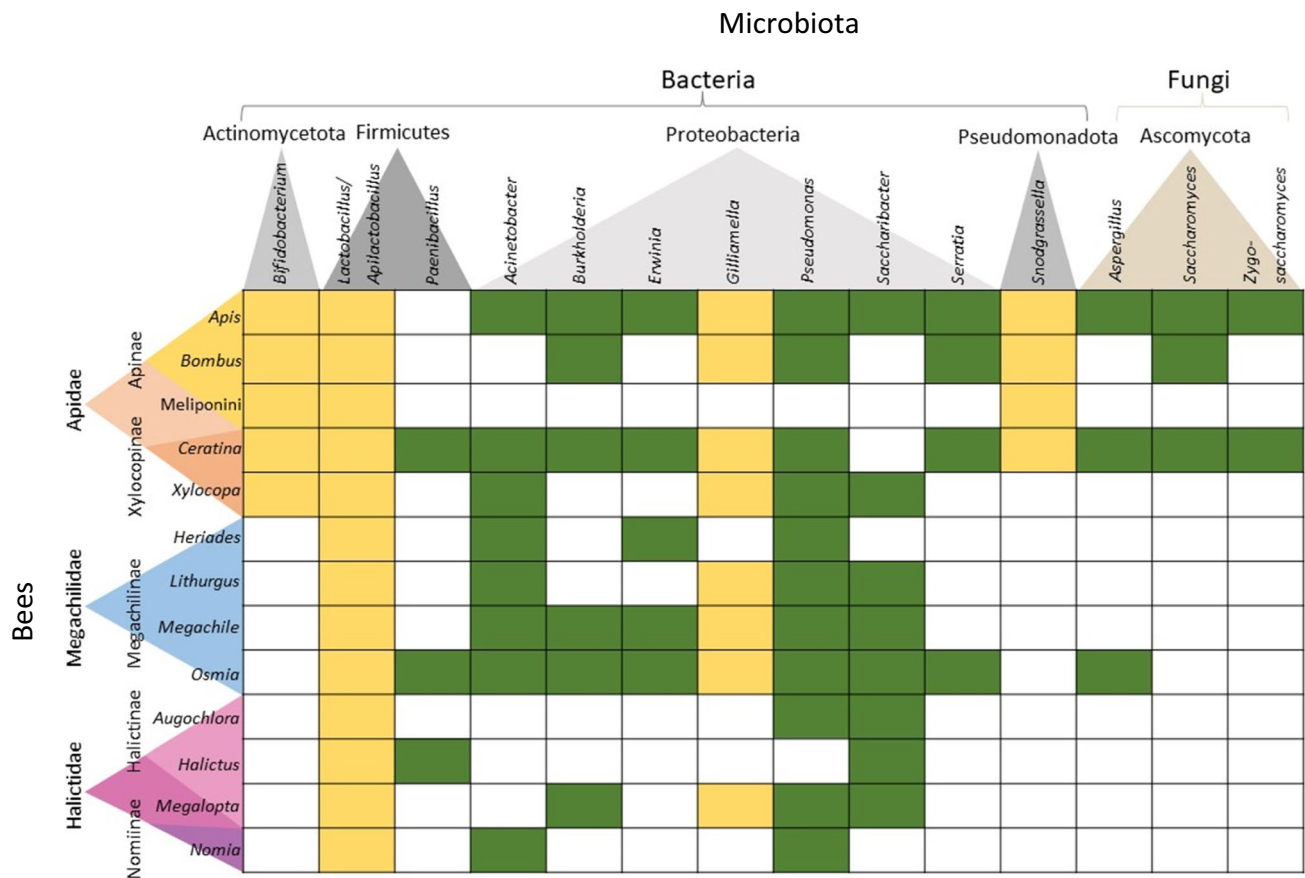
As found mainly in honey bees, microbial communities underscore the role of microbiota in their bee host's physiology and behaviour. Microbiota offer increased immunity by aiding in pathogen defense and protecting against parasites [15, 25, 26], while also inducing expression of antimicrobial peptides crucial in innate immunity [27, 28]. Bee nutrition is benefitted by some microbiota, with functions in carbohydrate breakdown and transport providing detoxification of food components and easily accessible energy sources [15, 19]. Bee microbiota alter olfactory learning and memory, as they affect gene expression in the brain [29] and promote long-term memory retention [30]. While many microbiota are beneficial to bees, pathogens and parasites can harm these pollinators and dominate beneficial microbiota [31].

Fig. 1 Environmental and social effects on the bee microbiome. Thick arrows and lines represent a greater effect and correlation compared to thinner, dashed lines and arrows. Facultatively social and solitary bee microbiomes are more heavily influenced by environmental effects as opposed to social interactions, while obligately eusocial bees tend to share a microbiome socially more distinctly than through environmental acquisition
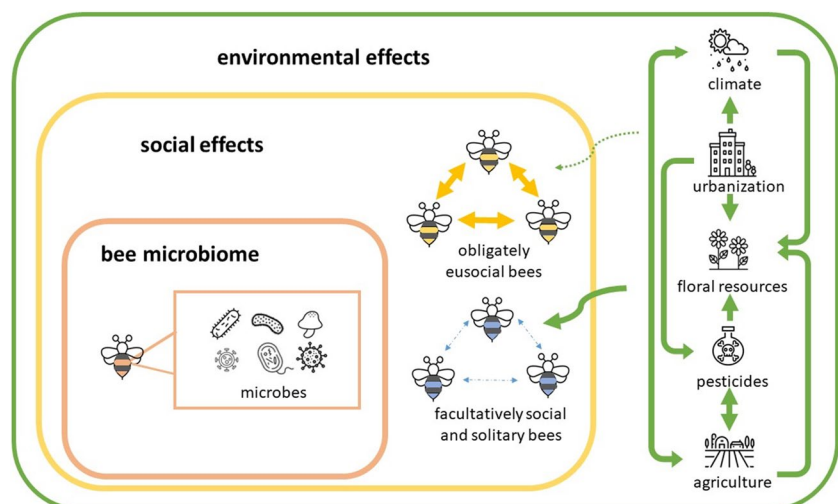
Table 1 Summary of studies characterizing bacteria and fungi within bee genera across different environments and developmental stages. Studies directly comparing bees across developmental stages are noted as a factor affecting the microbiome. Further details can be found in Supplementary Table S1

Bee Family	Bee Genera	Loci	Developmental Stage				Factors Affecting Microbiome					References
			Larvae	Pupae	Callow	Adult	Host Species	Development	Behaviour	Environment	Diet	
Corbiculate	Apidae	Apis	16S ITS	X	X	X	X	X	X	X	X	X	Mohr and Tebbe, 2006; Martinson et al., 2012; Moran et al., 2012; Tarpy et al., 2015; Yun et al., 2018; Tauber et al., 2019; Muñoz-Colmenero et al., 2020; Vernier et al., 2020; Callegari et al., 2021; Li et al., 2022
	Bombus	16S ITS	X			X	X	X	X	X		Mohr and Tebbe, 2006; Martinson et al., 2011; Bosmans et al., 2018; Li et al., 2021
	Melipona, Plebeia, Partamona, Scaptotrigona, Scaura, Trigona, Tetragonisca	16S				X	X				X	Figueroa et al., 2021
Non-Corbiculate	Ceratina	16S ITS	X	X	X	X	X	X		X	X	Graystock et al., 2017; Nguyen and Rehan, 2022ab; Shell and Rehan, 2022
	Xylocopa	16S				X	X					Handy et al., 2022; Holley et al., 2022
	Halictidae	Augochlora, Halictus, Megalopta	16S	X	X		X	X				X	McFrederick et al., 2012; McFrederick et al., 2014; Graystock et al., 2017
	Nomia	16S	X	X	X	X		X				Kapheim et al., 2021
	Megachilidae	Heriades, Lithurgus, Megachile	16S ITS	X			X	X				X	McFrederick et al., 2017; Voulgari-Kokota et al., 2019
	Osmia	16S ITS	X			X	X			X	X	Mohr and Tebbe, 2006; McFrederick et al., 2017; Voulgari-Kokota et al., 2019; Cohen et al., 2020

Fig. 2 Bacterial and fungal genera found in bee genera microbiota. Corbiculate bees and bacteria forming their core are highlighted in yellow. Microbiota not specific to the corbiculate core tend to be environmentally acquired and are shown in green. Bacteria are highlighted in grey, while fungal genera are shown in brown. Additional list and references in Supplementary Table S2

Some fungal members of the genus *Aspergillus* are opportunistic pathogens that result in stonebrood, or the mummification of honey bee brood [32, 33]. However, other *Aspergillus* species do not create toxins and hydrolytic enzymes, rather being mutualistic to bees and competing with other pathogens [32]. As microbiota affect host health in a variety of avenues, functional characterizations of both bacteria and fungi remain ongoing in efforts to understand how changes to microbial communities go on to affect bee health.

In particular, ubiquitous microbial taxa such as *Apilactobacillus* or *Lactobacillus* have been examined in honey bees. *Lactobaillus kunkeei* acquired within the honey bee nest offer antimicrobial properties due to its ability to outcompete harmful microorganisms and protect against pathogens that lead to honey and bee bread spoilage [28]. Bee bread is fermented pollen and nectar that uses the low pH environment caused by lactic acid bacteria to allow *Saccharomyces* yeasts to ferment and preserve this dietary source for honey bees [34]. Lactic acid bacteria also form biofilms, which create extracellular polymeric substances that help host colonization and cellular recognition, and are predicted to benefit honey bee health [28]. *Lactobacillus* also aid in amino acid digestion by regulating tryptophan metabolism, thereby changing neurological processes within the bee [29]. These bacteria even provide resistance against agrochemicals and pathogen adhesion, encouraging their use as probiotics [35, 36]. As such, loss of critical microbiota can negatively affect both immunity and host fitness, going so far as to delay development in both immature managed and wild bees [37, 38], leading to weakened immunity and higher mortality [19]. The documented importance of the microbiota highlights the need for a deeper understanding to provide key insights to better support pollinator health.

Contextual and situational analyses of the honey bee microbiota have uncovered that many different factors affect microbial diversity, composition, and abundance (Table 1). Following honey bee queens throughout development has revealed changes in microbial abundance and composition, with queens being more variable in early life and sharing more similarities with the worker microbiota upon queen maturity [20]. Honey bees of different social status possess striking differences in fungal communities. While nurse

worker bees are dominated by *Saccharomyces*, foraging workers and queen bees maintain more diversity and reveal an overrepresentation of *Zygosaccharomyces* [22]. External environments have also been considered, with landscape types such as agriculture shifting the relative abundances of the core microbiota, such that opportunistic bacteria metabolizing insecticides are overrepresented compared to beneficial bacteria [39, 40]. Even factors such as diet adjustments due to seasonal climate have been shown to change microbial composition, leading to the overrepresentation of beneficial microbiota such as *Bartonella* that aid in metabolism during winters [41]. In these contexts, studies in honey bees continue to expand on the sensitivity of microbiota to different factors and explore their role in maintaining bee and hive health.

The Microbiota of Wild Bees

Although honey bees are a popular pollinator of study, the thousands of wild bee species also offer crucial pollination services to both wild and agricultural plants [42, 43]. Microbial characterizations of some wild bees have suggested vastly different microbiota from honey bees (Table 1, S1; Fig. 2), exacerbating the need for more studies on other bees so that the breadth of microbial diversity can be fully understood [44]. Research questions regarding how wild bee microbiota are shaped, maintained, and affect bee health are ongoing, with emerging studies examining the role of important factors that affect more than just the honey bee. Developmental stages reveal alterations in microbial diversity as bees grow from larvae to pupae and adults in *Nomia melanderi* and *Ceratina calcarata* [45, 46]. Natural habitat and access to floral resources have been characterized around *Osmia lignaria* to evaluate microbiota in urban bees, finding an association between landscape features and important bacteria [47]. Similarly, habitat also influences bumble bee microbiota and the prevalence of pathogens [48]. As more bee genera are studied and technological advancements make it easier to conduct these microbial analyses, gaps in knowledge surrounding pollinator microbiota are being filled at a rapid pace and confirm that wild bee microbiota are labile and fluctuate over time.

Many questions remain in wild bee microbiota research, and new techniques and integrative methods are leading to deeper insights into the complexities of bee microbiota. Microbiota of bees in the family Apidae and, to a lesser extent the Megachilidae, have been particularly well classified, amidst the wealth of studies examining the corbiculate honey and bumble bees (Table 1, S1). Detailed characterization of microbiomes from other wild bees, such as those in the understudied bee families Halictidae, Colletidae and Andrenidae, promises a better overall understanding of the

role of microbiota in bee health (Table 1, S1). This also relies on functional descriptions of the key microbial members within the microbiome, such as the non-core microbiota and fungi observed in wild bee species. Most microbiome studies target a locus that classifies reads from the targeted region as bacterial or fungal taxa. These techniques have been and continue to be the standard in microbiome research. While the 16S rRNA region is common for bacteria (Table 1), fewer works have implemented targeting of other regions, such as the ITS for fungal reads. Recent studies on bumble bees showcase the importance of characterizing the diversity of fungi and non-core bacteria in different landscapes [48]. Fungi were almost exclusively found in queens from forested habitats and not detected in urban sites. Furthermore, those bumble bees from forested sites harboured more environmental bacteria not included in the bee-specific core, predicting that natural environments may lead to increased microbial diversity [48]. Studies like this reveal that limiting comparisons to the five core bacterial groups over a small group of bees hinders a necessary appreciation of the microbial diversity among wild bees.

Extending analyses towards characterizing the composition of additional microbial taxa and plants is an evolving method of understanding the entire bee microbiome and its interactions with floral resources. Examining the relationship between bees and their associated plants can provide additional ecological insights due to the important role of flowers as pollinator habitat, diet source, and microbial reservoir. For example, the rbcL region has classified an array of pollen types used for brood provisions in small carpenter bee and megachilid provisions, revealing differences in floral usage across landscapes and varying plant associations with bees [49–52]. Future work characterizing the plant composition directly on and within bees will contribute to an understanding of how these plants and associated microbiota become established within the microbiome.

More recent characterizations of all reads unaligned with the host reference genome eliminate the need for targeting regions and allows shotgun metagenomic studies to uncover diverse microbial genes across different taxa such as bacteria, fungi, viruses, plants, arachnids, and protists. Thus, metagenomics opens new avenues to exploring environmental associations between the bee and any organism with which it interacts, without the limitations of deciding upon one or two loci a priori [53]. Metagenomic analyses in *Apis* bees and their honey have initiated studies of the prevalence of viral, fungal, protozoan, and metazoan species in bees, highlighting the diversity of interactions between bacterial and eukaryotic microbiota and potential applications of environmental DNA [54–56]. *Ceratina* small carpenter bees have also revealed that bee species associated microbiota are affected by their local environment and can be affected by plant pathogens [57]. Implementing these strategies and

exploring an array of taxa promises a more complete understanding of bee interactions and its complex role in extensive ecological networks.

Social Maintenance of Microbiota

Solitary bees and eusocial corbiculate bees acquire their microbiota in different ways based on their lifestyle (Fig. 1). Unlike obligately social bee species, solitary and facultatively social bees often maintain microbiota shaped by their environment because they lack the social interactions that reinforce the consistent core microbiota among nestmates [45, 49, 52, 58, 59]. Pollen and nectar that nourish bees are also important sources of microbiota [49, 51, 52, 60], and can become affected by the pollinators visiting them [61]. Studies on *Ceratina* small carpenter bees and *Osmia* mason bees have highlighted the importance of environmental acquisition of microbiota from flowering plants and transfer to bee pollen provisions [49, 52, 62] (Table 1). With landscape and local environments likely being directly responsible for changes in microbial communities in bees due to the availability of flowers and green space, looking at wild bee habitats offers new opportunities to understand their microbial communities. The importance of understanding how urbanization, land use change, temperature, and pesticide usage affect plant and microbial communities are crucial due to these sensitivities [44], raising concerns for how wild bees are forced to re-establish their microbiota as anthropogenic activities increase.

Obligately eusocial corbiculate bees such as *Apis* and *Bombus* maintain a core microbiota through vertical transmission and social interaction between colony members [17, 20, 63–66]. Obligate eusociality is not strictly required in order to maintain core microbiota, as non-corbiculate, facultatively social and gregarious bees that live in shared nesting sites, such as *Xylocopa* species, are also dominated by members of the core noted in honey and bumble bees, such as *Bombilactobacillus*, *Bombiscardovia*, and *Bifidobacterium* [67, 68] (Fig. 2, Table S2). However, these large carpenter bees do interact socially at shared nesting sites and may have experienced a phylogenetic inheritance of common bacteria widespread in the bee family Apidae and also associated with the corbiculate honey and bumble bees [67] (Fig. 2). Interestingly, honey bee genotypes affect which bacterial strains are passed through social transmission, highlighting the importance of ancestry in shaping the microbiota [66]. Sharing core microbiota is a trait consistent within obligately eusocial bees. Core microbiota such as *Lactobacillus* may then affect social behaviours that potentially reinforce microbiota establishment amongst nestmates through social interactions [21, 69]. Microbial colonization increases the rate of head-to-head interactions between bees

as a result of increased metabolites and amino acids that affect synaptic transmission and encourage social contact, potentially increasing the chances of social interactions [69]. Not only are adult honey bee roles affecting bacterial composition, colony-specific microbiota can also dictate colony membership [21]. Shared strain-specific bacteria, particularly the honey bee specific symbiont *Gilliamella apicola*, within colonies can alter individual cuticular hydrocarbon profiles, leading to pheromones and chemical signatures that affect bee nestmate recognition [21]. These social interactions and behaviour have thus created pathways for sharing of microbiota that make up the most commonly established microbiome.

Because eusocial corbiculate bees such as honey bees tend to rely on social transmission to acquire their microbiome, it is relatively easy to describe core microbiota [19]. However, the idea of a core microbiome has been challenged due to inconsistent definitions and the lack of consensus on the standardized metrics such as exact occurrence and abundance cut-off values used to quantify the core microbiota [70–72]. As the focus has traditionally been on these dominating microbiota, determining the roles of relatively less abundant microbiota may reveal neglected but important microbial functions. Life stages and social role impart vulnerability to changes in microbial exposure. Studies comparing the microbial composition across honey bee workers [73] and queens [20, 74] have found compositional differences based on behavioural tasks. Conversely, microbiota can define social group membership in colonies, highlighting the correlation between social status and microbial composition [21, 75] (Table 1). Despite very consistent core microbiota described in adult honey bees, larval microbiota are initially variable and can even lead to differing larval gene expression when there is different bacterial composition [76]. Obligately eusocial stingless bee genera *Trigona*, *Melipona*, and *Partamona* maintain the corbiculate core microbiota, but diet has been shown to add new associations with environmentally acquired acidophilic bacteria that are important in bee nutrition [60]. Thus, these bacteria may supplement and sustain these bees' lifestyle and cyclically allow them to make better use of these environments. Although the idea of a core microbiota remains established within social bees more often than for solitary bees, all bees seem to be subject to external factors that can contribute to altered microbiota and dysbiosis.

Environmental Influences on Microbial Acquisition

Examining bees from different geographic locations offers a preliminary evaluation of how environment shapes microbiota broadly (Fig. 1). Given the consistency across the

corbiculate core microbiota, microbial changes are less evident in honey bees. Geographic location was not the biggest factor affecting the *Apis* microbiota, even though location may still explain why microbial composition changed over time [77]. Geographical distribution was also a poorer predictor of antibiotic resistance genes in honey bee gut bacteria, although there was a geographic pattern of resistance distribution [78]. This contradictory pattern of local landscape effects has been seen in wild bee microbiota as well, with solitary megachilid bee microbiota associating with host bee species rather than location [62]. Small carpenter bees seemingly maintain different microbiota across geographic distributions and locations [57, 79], although there has not yet been directly comparable and longitudinal studies of a bee genus within a demographic and across different local landscapes. However, metagenomic analysis of small carpenter bees from around the world has revealed associations with host bee species that are strongly affected by local environmental features. For example, population comparisons of *Ceratina australensis* revealed that bacteria favouring saline and marine environments were associated with bees located in beach dunes [57]. As microbiome research expands across geographic locations, environmental contexts and bee species, these comparisons among regions will become more accessible. Future studies focusing on specific environmental factors will encourage more detailed comparisons across landscapes and hold great promise for biodiversity conservation.

Climatic variables and environmental conditions such as regional precipitation and temperature have been shown to affect bee microbiomes as well. *Ceratina* from different climatic zones across Australia vary in community composition, with species richness higher in subtropical zones than in temperate zones or grasslands [50]. Microbial diversity and co-occurrences also vary across areas with different mean annual temperature and annual precipitation. Temperature can predict the relative abundances of bacterial and fungal taxa, and vital bacteria such as *Apilactobacillus* are overrepresented in areas with lower annual temperatures [79]. Precipitation is associated with fungal beta diversity, resulting in areas with higher precipitation having less relatively abundant fungi like *Alternaria* [79]. Even in honey bees, low temperatures during winters lead to dietary shifts that are associated with the seasonal dominance of the non-core bacteria *Bartonella* [41]. These bacteria are capable of metabolizing different energy substrates that may benefit the bee host through producing essential amino acids during restricted diets [41]. Honey bees have been shown to display changes in microbial composition in fall and spring time, accommodating the overwintering period in northern temperate climates [80]. Thus, environmental factors associated with climate play a role in determining which microbiota are easily accessible and can benefit the host when established.

Access to flowering plants is an important consideration for bee habitats. Floral resources contributing to diet are important factors influencing the bee microbiota because of the availability of microbiota offered in plant-pollinator relationships [49, 52, 61, 81–84]. For example, non-native tropical milkweed in urban landscapes, mainly visited by honey bees and several solitary bees, harboured bacteria attributable to differences in microbial diversity among floral visitors and the environment [85]. In environments where access to floral resources changes or provisioning requirements becomes more limited, both plant and bee microbiota may experience dysbiosis and a harmful reduction in important microbiota [83, 86]. Visiting an array of plants may also allow bees to become exposed to diverse bacteria and result in improved larval developmental success, as is the case with *Osmia lignaria* pollen provisions with higher bacterial diversity [87]. However, this matter is worsened when considering that pollen and nectar in wild flowers can harbour pesticides, which pollinators bring back to their nests, both in agricultural and urban landscapes [88, 89]. Therefore, the indirect effects of human activities on bee habitats and flower availability raise additional concerns for pollinator health.

While pollinators, and especially wild bees, are exposed to increased microbial diversity in the form of pollen-associated microbiota, wild bees also play a role in determining microbial composition in the plants they visit. For example, nectar robbing by carpenter bees increases the abundance of Acetobacteraceae in nectar more than pollination by hummingbirds, also resulting in functional enrichment in the bacterial genomes indicating different amino acid and saccharide utilization pathways [83]. Acetobacteraceae are commonly found in both nectar and bees, playing an important role in pollen germination of nectar and in honey production [77, 86]. Even yeasts in pollen and nectar have been shown to increase the nutritional value for pollinators [90]. Thus, this mutualistic relationship between plants, bees, and their microbiota suggest that important co-occurrences may be a sign of co-evolution and adaptation and highlights the need to consider plant-pollinator relationships when examining microbial composition and diversity in bees.

Managed bees in agriculture and wild bees in natural habitats house different microbiota, with agrochemicals and pesticides likely acting as a stressor on both bees and their microbiomes [91–94]. As seen in honey bees, beneficial bacteria are more abundant in wild bees from less anthropized environments, while bees from farms harbour more microbiota capable of metabolizing insecticides [36, 39, 92]. Microbiota of hives sprayed with common pesticides can face altered structural and functional potential, such as how genes for oxidative phosphorylation increased while those responsible for sugar metabolism decreased among bacteria in bees exposed to chlorothalonil [93]. Glyphosate, a common herbicide for

weed control, unexpectedly harms bees due to its ability to change the relative abundances of important bacterial symbionts that protect against opportunistic pathogens [95]. The effects of pesticides on microbiota are likely specific to hosts. Imidacloprid has been shown to change the digestive physiology and microbiota of *Drosophila melanogaster* larvae and leads to increased mortality and susceptibility to pathogens in honey bees [96]. However, this pesticide does not affect gut bacteria in honey bees which cannot metabolize imidacloprid [96]. Although it cannot yet be determined that agriculture directly harms bee microbiota, abiotic and chemical factors need to be studied to understand how they interact with the microbiome. While introducing probiotic bacteria can be used to mitigate environmental effects [97, 98], careful consideration needs to be given to microbial manipulation and inoculation as a remedy.

Examining the effects of urbanization on bee microbiota has been relatively limited but indicate anthropogenic activities may lead to dysbiosis. Studies on *O. lignaria* have indicated that natural habitat, floral resources, bee community species richness in the area, and garden size are all factors that positively influence the abundance of important bacteria, such as *Lactobacillus* [47, 99]. Likewise, studies on *Bombus terrestris*, comparing forest and urban queens revealed potentially beneficial diversity of environmentally acquired bacteria and fungi exclusive to queens from forests, and a more standard core microbiota in urban bees [48]. Furthermore, studies on small carpenter bees across an urbanization gradient found that important bacteria such as *Acinetobacter* and *Apilactobactillus* were overrepresented in sites with low impervious surface and increased green space availability [79]. Bees from areas with low land use development also had more plant associated microbiota, whereas microbiota from high land use development areas may be harboured due to their functions in bee development and digestion [79]. Although urbanization has not been shown to be detrimental to bee health, growing cities do threaten microbial compositions containing known beneficial symbionts. Future studies conducting direct comparisons between urban and rural sites will prove valuable in characterizing the effects of human activities on the established microbial relationships.

The environmental microbiome has changed with anthropogenic activity. Many wild animals such as birds, reptiles, and mammals have begun to develop microbiomes increasingly similar to those of humans, hinting that spillover may be affecting animals in cities [100]. Changing microbial composition does not always lead to positive associations and increases in diversity may potentially lead to dysbiosis. Ants have lost symbiotically beneficial microbiota when transitioning from forested to urban areas, potentially harming host colonies [101]. Thus, in considering how anthropogenic activities have come to change the bee microbiome,

it may prove useful to account for broader perspectives to determine how microbiomes are changing throughout the surrounding environment and across wildlife. Examining microbial content within the environment, such as in soil and floral resources, will be necessary to characterize bee exposures to microbiota. Specifically, looking at nests and pollen provision microbiota across anthropized landscapes has been unexplored in most species. This would offer insights into the environmental and social transmission of microbiota, potentially opening avenues for discovering how wildlife must adjust to changing environments and microbiota.

Placing bees in a larger ecological context, these pollinators may act as an intermediary carrier of microbiota between plants and other animals due to their role in the ecosystem [102, 103]. As bees deposit their microbiota on flowers and these plants then offer their collection of bacteria and fungi to other plant visitors [61, 81, 84], the spread of both beneficial and harmful microbiota can occur to other bee species, other pollinators, and other animals. This research ties directly to the One Health concept, which examines the connections between humans, animals, and the environment across disciplines and fields of study to examine the consequences of animal-human-ecosystem networks [104, 105]. This can be applied towards both pathogenic and beneficial microbiota and their impact on bees and their habitats [106]. Environmental and wildlife microbiota have been shown to affect human health, reinforcing how changes such as urbanization can lead to cascading effects impacting all organisms [106]. With a broader perspective into the important role that bees play within ecosystems and their pollination activities, promoting bee health has implications far beyond this one group of insects. Using bees as a study model, particularly wild bees that may be more sensitive to environmental factors, offers both a unique perspective and a practical avenue to exploring the role of microbiota in One Health.

Future Directions

While changing environments have been shown to affect both solitary and social bee microbiota composition, challenges remain in furthering our understanding of how the environment affects bee microbiota and the degree of harm to bees. In some instances, bees from anthropogenic environments face decreased microbial diversity, although whether this is beneficial or harmful to bees in the long term is still unclear. Wild and captive animal microbiota tend to differ [107, 108] and the impact of environmental changes differs greatly between bee genera and habitats, leading to difficulties in extrapolating from experimentation in lab and requiring a more robust survey of wild bees. Therefore, establishing a baseline characterization of microbiota

across bees to compare and contrast how evolving environmental changes are affecting microbiota will require additional resources and long-term observations. To answer the vast array of questions remaining as to how environmental features are affecting the way microbiota are acquired and maintained, both functional and experimental work need to be performed across a broader range of bee species. Experiments quantitatively altering the proportion of environmental features, whether through the addition of pesticides, manipulating temperature conditions, or changing access to floral resources, are necessary to characterize changing microbiota effects on their host. Functional research describing the role of microbiota in symbioses is also much needed, particularly due to the fast pace of characterizing new taxa with no known function. This is also true for pathogens, where it is largely unknown how environmental factors and land use contribute to their presence and abundance. These goals have become more accessible with the recent ease of exploring a wider range of microbiota within the metagenome using shotgun metagenomics and environmental DNA sequencing approaches, including fungi, plants, and viruses. Megabarcoding opens even more avenues for understanding metagenomics, using increasingly affordable high throughput sequencing technologies such as MinION or PacBio for upscaling [109]. This will expedite characterization of the bee and its microbiota and will highlight numerous new taxa and functional associations to be studied.

An understanding of the role the microbiota plays in maintaining bee health and how it changes with differing land use offers opportunities for protection efforts by unraveling the cascade of effects that environmental changes have on bees and their microbiota. The use of DNA-based techniques, such as metagenomics or megabarcoding, can be extrapolated for use in monitoring pollinators [109]. Direct changes through habitat restoration or indirectly through habitat augmentation may be used to supplement floral resources, increase green space availability, and foster better habitats for pollinators [4, 110]. Increasing gardens and green spaces is one way to support plant-pollinator networks and their associated microbiota. Probiotics have also been a consideration for bees, as they can provide crucial bacteria and fungi to bees that may increase immunity. Offering probiotic Lactobacilli in a nutrient patty to honey bee hives has provided colony resistance against American foulbrood [111]. However, this should be proceeded with caution, as the previously demonstrated probiotic *Parasacharribacter apium* [112] in practice did not protect bees from European Foul Brood disease caused by the bacteria *Melissococcus plutonius* [113]. Thus, supplementing the microbiota and further manipulating bee habitats requires careful implementation in order to prevent dysbiosis within these and other pollinators.

To conclude, environmental features broadly affect microbiota through a variety of avenues, especially solitary bees that largely acquire microbiota from their surroundings and diet. Different regions, environmental temperature, precipitation, pesticide usage, floral resources, and human urbanization are just some factors that change the microbial communities within bees and hosts widely. This generates questions as to how host health is affected in changing environments and inspires studies examining the functional effects of microbiota. As these altered habitats continue to threaten bees, reducing harmful anthropogenic activities and supplementing microbiota with probiotics may be opportunities to protect these important pollinators. Using bees as model organisms will also further provide insights into how a wider range of animals are experiencing changes in their microbiome because of environmental stressors, land use and changes in microclimate. Thus, this field of research has foundational implications for understanding and maintaining the web of interactions that support the overall health of bees, pollinators, wildlife, humans, and the environment.

Declarations

References

1. Rosenberg E, Koren O, Reshef L et al (2007) The role of microorganisms in coral health, disease and evolution. Nat Rev Microbiol 5:355–362. https://doi.org/10.1038/nrmicro1635
2. Turnbaugh PJ, Gordon JI (2009) The core gut microbiome, energy balance and obesity. J Physiol 587:4153–4158. https://doi.org/10.1113/JPHYSIOL.2009.174136
3. Teeling H, Glöckner FO (2012) Current opportunities and challenges in microbial metagenome analysis—a bioinformatic perspective. Brief Bioinform 13:728–742. https://doi.org/10.1093/BIB/BBS039
4. Winfree R (2010) The conservation and restoration of wild bees. Ann N Y Acad Sci 1195:169–197. https://doi.org/10.1111/J.1749-6632.2010.05449.X
5. Marshman J, Blay-Palmer A, Landman K (2019) Anthropocene crisis: climate change, pollinators, and food security.

Environments 6:22. https://doi.org/10.3390/ENVIRONMEN TS6020022

6. Kremen C, Williams NM, Thorp RW (2002) Crop pollination from native bees at risk from agricultural intensification. Proc Natl Acad Sci U S A 99:16812–16816. https://doi.org/10.1073/PNAS.262413599

7. Hung KLJ, Kingston JM, Albrecht M et al (2018) The worldwide importance of honey bees as pollinators in natural habitats. Proc R Soc B Biol Sci 285:1870. https://doi.org/10.1098/RSPB.2017.2140

8. Potter A, LeBuhn G (2015) Pollination service to urban agriculture in San Francisco, CA. Urban Ecosyst 18:885–893. https://doi.org/10.1007/S11252-015-0435-Y

9. Winfree R, Aguilar R, Vázquez DP et al (2009) A meta-analysis of bees' responses to anthropogenic disturbance. Ecology 90:2068–2076. https://doi.org/10.1890/08-1245.1

10. Ayers AC, Rehan SM (2021) Supporting bees in cities: how bees are influenced by local and landscape features. Insects 12:128. https://doi.org/10.3390/insects12020128

11. McKinney ML (2008) Effects of urbanization on species richness: a review of plants and animals. Urban Ecosyst 11:161–176. https://doi.org/10.1007/S11252-007-0045-4

12. Kelemen EP, Rehan SM (2021) Opposing pressures of climate and land-use change on a native bee. Glob Chang Biol 27:1017–1026. https://doi.org/10.1111/GCB.15468

13. Mathiasson ME, Rehan SM (2020) Wild bee declines linked to plant-pollinator network changes and plant species introductions. Insect Conserv Divers 13:595–605. https://doi.org/10.1111/ICAD.12429

14. Theodorou P, Radzevičiūtė R, Lentendu G et al (2020) Urban areas as hotspots for bees and pollination but not a panacea for all insects. Nat Commun 11:576. https://doi.org/10.1038/S41467-020-14496-6

15. Engel P, Martinson VG, Moran NA (2012) Functional diversity within the simple gut microbiota of the honey bee. PNAS 109:11002–11007. https://doi.org/10.1073/pnas.1202970109

16. Martinson VG, Danforth BN, Minckley RL et al (2011) A simple and distinctive microbiota associated with honey bees and bumble bees. Mol Ecol 20:619–628. https://doi.org/10.1111/J.1365-294X.2010.04959.X

17. Kwong WK, Moran NA (2016) Gut microbial communities of social bees. Nat Rev Microbiol 14:374–384. https://doi.org/10.1038/nrmicro.2016.43

18. Moran NA, Hansen AK, Powell JE, Sabree ZL (2012) Distinctive gut microbiota of honey bees assessed using deep sampling from individual worker bees. PLoS One 7:e36393. https://doi.org/10.1371/JOURNAL.PONE.0036393

19. Raymann K, Moran NA (2018) The role of the gut microbiome in health and disease of adult honey bee workers. Curr Opin Insect Sci 26:97–104. https://doi.org/10.1016/J.COIS.2018.02.012

20. Tarpy DR, Mattila HR, Newton ILG (2015) Development of the honey bee gut microbiome throughout the queen-rearing process. Appl Environ Microbiol 81:3182–3191. https://doi.org/10.1128/AEM.00307-15

21. Vernier CL, Chin IM, Adu-Oppong B et al (2020) The gut microbiome defines social group membership in honey bee colonies. Sci Adv 6:3431. https://doi.org/10.1126/SCIADV.ABD3431

22. Yun JH, Jung MJ, Kim PS, Bae JW (2018) Social status shapes the bacterial and fungal gut communities of the honey bee. Sci Rep 8:2019. https://doi.org/10.1038/s41598-018-19860-7

23. Callegari M, Crotti E, Fusi M et al (2021) Compartmentalization of bacterial and fungal microbiomes in the gut of adult honeybees. npj Biofilms Microbiomes 7:42. https://doi.org/10.1038/s41522-021-00212-9

24. Tauber JP, Nguyen V, Lopez D, Evans JD (2019) Effects of a resident yeast from the honeybee gut on immunity, microbiota, and *Nosema* disease. Insects 10:296. https://doi.org/10.3390/insects10090296

25. Mockler BK, Kwong WK, Moran NA, Koch H (2018) Microbiome structure influences infection by the parasite *Crithidia bombi* in bumble bees. Appl Environ Microbiol 84:2335–2352. https://doi.org/10.1128/AEM.02335-17

26. Rubanov A, Russell KA, Rothman JA et al (2019) Intensity of *Nosema ceranae* infection is associated with specific honey bee gut bacteria and weakly associated with gut microbiome structure. Sci Rep 9:1–8. https://doi.org/10.1038/s41598-019-40347-6

27. Kwong WK, Mancenido AL, Moran NA (2017) Immune system stimulation by the native gut microbiota of honey bees. R Soc Open Sci 4:170003. https://doi.org/10.1098/rsos.170003

28. Vásquez A, Forsgren E, Fries I et al (2012) Symbionts as major modulators of insect health: lactic acid bacteria and honeybees. PLoS One 7:e33188. https://doi.org/10.1371/JOURNAL.PONE.0033188

29. Zhang Z, Mu X, Cao Q et al (2022) Honeybee gut *Lactobacillus* modulates host learning and memory behaviors via regulating tryptophan metabolism. Nat Commun 13:2307. https://doi.org/10.1038/s41467-022-29760-0

30. Li L, Solvi C, Zhang F et al (2021) Gut microbiome drives individual memory variation in bumblebees. Nat Commun 12:1–10. https://doi.org/10.1038/s41467-021-26833-4

31. Fünfhaus A, Ebeling J, Genersch E (2018) Bacterial pathogens of bees. Curr Opin insect Sci 26:89–96. https://doi.org/10.1016/J.COIS.2018.02.008

32. Becchimanzi A, Nicoletti R (2022) *Aspergillus*-bees: a dynamic symbiotic association. Front Microbiol 13:968963. https://doi.org/10.3389/FMICB.2022.968963

33. Foley K, Fazio G, Jensen AB, Hughes WOH (2014) The distribution of *Aspergillus* spp. opportunistic parasites in hives and their pathogenicity to honey bees. Vet Microbiol 169:203–210. https://doi.org/10.1016/J.VETMIC.2013.11.029

34. López-Uribe M, Lawrence B (2021) The biology of the bread that bees make. In: Fermentology. NC State University Libraries. https://doi.org/10.52750/340675

35. Pachla A, Ptaszyńska AA, Wicha M et al (2021) Insight into probiotic properties of lactic acid bacterial endosymbionts of *Apis mellifera* L. derived from the Polish apiary. Saudi J Biol Sci 28:1890–1899. https://doi.org/10.1016/J.SJBS.2020.12.040

36. Tlais AZA, Polo A, Filannino P et al (2022) Biofilm formation as an extra gear for *Apilactobacillus kunkeei* to counter the threat of agrochemicals in honeybee crop. Microb Biotechnol 15:2160. https://doi.org/10.1111/1751-7915.14051

37. Dharampal PS, Danforth BN, Steffan SA (2022) Exosymbiotic microbes within fermented pollen provisions are as important for the development of solitary bees as the pollen itself. Ecol Evol 12:e8788. https://doi.org/10.1002/ECE3.8788

38. Daisley BA, Chmiel JA, Pitek AP et al (2020) Missing microbes in bees: how systematic depletion of key symbionts erodes immunity. Trends Microbiol 28:1010–1021. https://doi.org/10.1016/J.TIM.2020.06.006

39. Muñoz-Colmenero M, Baroja-Careaga I, Kovačić M et al (2020) Differences in honey bee bacterial diversity and composition in agricultural and pristine environments – a field study. Apidologie 51:1018–1037. https://doi.org/10.1007/s13592-020-00779-w

40. Jones JC, Fruciano C, Hildebrand F et al (2018) Gut microbiota composition is associated with environmental landscape in honey bees. Ecol Evol 8:441–451. https://doi.org/10.1002/ECE3.3597

41. Li C, Tang M, Li X, Zhou X (2022) Community dynamics in structure and function of honey bee gut bacteria in response to winter dietary shift. MBio 13:e01131-e1222. https://doi.org/10.1128/MBIO.01131-22

42. Mallinger RE, Gratton C (2015) Species richness of wild bees, but not the use of managed honeybees, increases fruit set of a pollinator-dependent crop. J Appl Ecol 52:323–330. https://doi.org/10.1111/1365-2664.12377

43. Demeter I, Balog A, Sárospataki M (2021) Variation of small and large wild bee communities under honeybee pressure in highly diverse natural habitats. Front Ecol Evol 9:846. https://doi.org/10.3389/FEVO.2021.750236

44. Voulgari-Kokota A, McFrederick QS, Steffan-Dewenter I, Keller A (2019) Drivers, diversity, and functions of the solitary-bee microbiota. Trends Microbiol 27:1034–1044. https://doi.org/10.1016/J.TIM.2019.07.011

45. Kapheim KM, Johnson MM, Jolley M (2021) Composition and acquisition of the microbiome in solitary, ground-nesting alkali bees. Sci Rep 11:2993. https://doi.org/10.1038/s41598-021-82573-x

46. Nguyen PN, Rehan SM (2022) Developmental microbiome of the small carpenter bee, *Ceratina calcarata*. Environ DNA 4:808–819. https://doi.org/10.1002/EDN3.291

47. Cohen H, McFrederick QS, Philpott SM (2020) Environment shapes the microbiome of the blue orchard bee, *Osmia lignaria*. Microb Ecol 80:897–907. https://doi.org/10.1007/S00248-020-01549-Y

48. Bosmans L, Pozo MI, Verreth C et al (2018) Habitat-specific variation in gut microbial communities and pathogen prevalence in bumblebee queens (*Bombus terrestris*). PLoS One 13:e0204612. https://doi.org/10.1371/JOURNAL.PONE.0204612

49. Dew RM, McFrederick QS, Rehan SM (2020) Diverse diets with consistent core microbiome in wild bee pollen provisions. Insects 11:E499. https://doi.org/10.3390/insects11080499

50. McFrederick QS, Rehan SM (2019) Wild bee pollen usage and microbial communities co-vary across landscapes. Microb Ecol 77:513–522. https://doi.org/10.1007/s00248-018-1232-y

51. McFrederick QS, Rehan SM (2016) Characterization of pollen and bacterial community composition in brood provisions of a small carpenter bee. Mol Ecol 25:2302–2311. https://doi.org/10.1111/mec.13608

52. McFrederick QS, Thomas JM, Neff JL et al (2017) Flowers and wild megachilid bees share microbes. Microb Ecol 73:188–200. https://doi.org/10.1007/s00248-016-0838-1

53. Wang J, Jia H (2016) Metagenome-wide association studies: fine-mining the microbiome. Nat Rev Microbiol 14:508–522. https://doi.org/10.1038/nrmicro.2016.83

54. Regan T, Barnett MW, Laetsch DR et al (2018) Characterisation of the British honey bee metagenome. Nat Commun 9:1–13. https://doi.org/10.1038/s41467-018-07426-0

55. Bovo S, Ribani A, Utzeri VJ et al (2018) Shotgun metagenomics of honey DNA: evaluation of a methodological approach to describe a multi-kingdom honey bee derived environmental DNA signature. PLoS One 13:e0205575. https://doi.org/10.1371/JOURNAL.PONE.0205575

56. Galbraith DA, Fuller ZL, Ray AM et al (2018) Investigating the viral ecology of global bee communities with high-throughput metagenomics. Sci Rep 8:8879. https://doi.org/10.1038/s41598-018-27164-z

57. Shell WA, Rehan SM (2022) Comparative metagenomics reveals expanded insights into intra- and interspecific variation among wild bee microbiomes. Commun Biol 5:603. https://doi.org/10.1038/s42003-022-03535-1

58. McFrederick QS, Wcislo WT, Taylor DR et al (2012) Environment or kin: whence do bees obtain acidophilic bacteria? Mol Ecol 21:1754–1768. https://doi.org/10.1111/J.1365-294X.2012.05496.X

59. Voulgari-Kokota A, Steffan-Dewenter I, Keller A (2020) Susceptibility of red mason bee larvae to bacterial threats due to microbiome exchange with imported pollen provisions. Insects 11:373. https://doi.org/10.3390/insects11060373

60. Figueroa LL, Maccaro JJ, Krichilsky E et al (2021) Why did the bee eat the chicken? Symbiont gain, loss, and retention in the vulture bee microbiome. MBio 12:e02317-e2321. https://doi.org/10.1128/MBIO.02317-21

61. Manirajan BA, Ratering S, Rusch V et al (2016) Bacterial microbiota associated with flower pollen is influenced by pollination type, and shows a high degree of diversity and species-specificity. Environ Microbiol 18:5161–5174. https://doi.org/10.1111/1462-2920.13524

62. Voulgari-Kokota A, Grimmer G, Steffan-Dewenter I, Keller A (2019) Bacterial community structure and succession in nests of two megachilid bee genera. FEMS Microbiol Ecol 95:218. https://doi.org/10.1093/FEMSEC/FIY218

63. Martinson VG, Moy J, Moran NA (2012) Establishment of characteristic gut bacteria during development of the honeybee worker. Appl Environ Microbiol 78:2830–2840. https://doi.org/10.1128/AEM.07810-11

64. Powell JE, Eiri D, Moran NA, Rangel J (2018) Modulation of the honey bee queen microbiota: effects of early social contact. PLoS One 13:e0200527. https://doi.org/10.1371/JOURNAL.PONE.0200527

65. Su Q, Wang Q, Mu X et al (2021) Strain-level analysis reveals the vertical microbial transmission during the life cycle of bumblebee. Microbiome 9:1–14. https://doi.org/10.1186/S40168-021-01163-1

66. Wu J, Lang H, Mu X et al (2021) Honey bee genetics shape the strain-level structure of gut microbiota in social transmission. Microbiome 9:225. https://doi.org/10.1186/S40168-021-01174-Y

67. Holley JAC, Jackson MN, Pham AT et al (2022) Carpenter bees (*Xylocopa*) harbor a distinctive gut microbiome related to that of honey bees and bumble bees. Appl Environ Microbiol 88:e0020322. https://doi.org/10.1128/AEM.00203-22

68. Handy MY, Sbardellati DL, Yu M et al (2022) Incipiently social carpenter bees (*Xylocopa*) host distinctive gut bacterial communities and display geographical structure as revealed by full-length PacBio 16S rRNA sequencing. Mol Ecol. https://doi.org/10.1111/MEC.16736

69. Liberti J, Kay T, Quinn A et al (2022) The gut microbiota affects the social network of honeybees. Nat Ecol Evol 6:1471–1479. https://doi.org/10.1038/s41559-022-01840-w

70. Neu AT, Allen EE, Roy K (2021) Defining and quantifying the core microbiome: challenges and prospects. Proc Natl Acad Sci U S A 118:e2104429118. https://doi.org/10.1073/PNAS.2104429118

71. Custer GF, Gans M, van Diepen LTA et al (2023) Comparative analysis of core microbiome assignments: implications for ecological synthesis. mSystems 8:e01066-22. https://doi.org/10.1128/msystems.01066-22

72. Risely A (2020) Applying the core microbiome to understand host–microbe systems. J Anim Ecol 89:1549–1558. https://doi.org/10.1111/1365-2656.13229

73. Anderson KE, Maes P (2022) Social microbiota and social gland gene expression of worker honey bees by age and climate. Sci Rep 12:10690. https://doi.org/10.1038/s41598-022-14442-0

74. Copeland DC, Anderson KE, Mott BM (2022) Early queen development in honey bees: social context and queen breeder source affect gut microbiota and associated metabolism. Microbiol Spectr 10:e00383-e422. https://doi.org/10.1128/spectrum.00383-22

75. Jones JC, Fruciano C, Marchant J et al (2018) The gut microbiome is associated with behavioural task in honey bees. Insectes Soc 65:419–429. https://doi.org/10.1007/S00040-018-0624-9

76. Kowallik V, Mikheyev AS (2021) Honey bee larval and adult microbiome life stages are effectively decoupled with vertical transmission overcoming early life perturbations. MBio 12:e02966-e3021. https://doi.org/10.1128/mBio.02966-21

77. Almeida EL, Ribiere C, Frei W et al (2022) Geographical and seasonal analysis of the honeybee microbiome. Microb Ecol. https://doi.org/10.1007/S00248-022-01986-X

78. Sun H, Mu X, Zhang K et al (2022) Geographical resistome profiling in the honeybee microbiome reveals resistance gene transfer conferred by mobilizable plasmids. Microbiome 10:69. https://doi.org/10.1186/S40168-022-01268-1

79. Nguyen PN, Rehan SM (2022) The effects of urban land use gradients on wild bee microbiomes. Front Microbiol 13:992660. https://doi.org/10.3389/FMICB.2022.992660

80. Bleau N, Bouslama S, Giovenazzo P, Derome N (2020) Dynamics of the honeybee (*Apis mellifera*) gut microbiota throughout the overwintering period in Canada. Microorganisms 8:1146. https://doi.org/10.3390/MICROORGANISMS8081146

81. Manirajan BA, Maisinger C, Ratering S et al (2018) Diversity, specificity, co-occurrence and hub taxa of the bacterial–fungal pollen microbiome. FEMS Microbiol Ecol 94:112. https://doi.org/10.1093/femsec/fiy112

82. Sookhan N, Lorenzo A, Tatsumi S et al (2021) Linking bacterial diversity to floral identity in the bumble bee pollen basket. Environ DNA 3:669–680. https://doi.org/10.1002/EDN3.165

83. Morris MM, Frixione NJ, Burkert AC et al (2020) Microbial abundance, composition, and function in nectar are shaped by flower visitor identity. FEMS Microbiol Ecol 96:fiaa003. https://doi.org/10.1093/FEMSEC/FIAA003

84. Keller A, McFrederick QS, Dharampal P et al (2021) (More than) Hitchhikers through the network: the shared microbiome of bees and flowers. Curr Opin Insect Sci 44:8–15. https://doi.org/10.1016/J.COIS.2020.09.007

85. Warren ML, Kram KE, Theiss KE (2020) Characterizing the nectar microbiome of the non-native tropical milkweed, *Asclepias curassavica*, in an urban environment. PLoS One 15:w0237561. https://doi.org/10.1371/JOURNAL.PONE.0237561

86. Christensen SM, Munkres I, Vannette RL (2021) Nectar bacteria stimulate pollen germination and bursting to enhance microbial fitness. Curr Biol 31:4380. https://doi.org/10.1016/J.CUB.2021.07.016

87. Westreich LR, Westreich ST, Tobin PC (2022) Bacterial and fungal symbionts in pollen provisions of a native solitary bee in urban and rural environments. Microb Ecol 1:1–12. https://doi.org/10.1007/S00248-022-02164-9

88. Botías C, David A, Hill EM, Goulson D (2017) Quantifying exposure of wild bumblebees to mixtures of agrochemicals in agricultural and urban landscapes. Environ Pollut 222:73–82. https://doi.org/10.1016/J.ENVPOL.2017.01.001

89. Botías C, David A, Horwood J et al (2015) Neonicotinoid residues in wildflowers, a potential route of chronic exposure for bees. Environ Sci Technol 49:12731–12740. https://doi.org/10.1021/ACS.EST.5B03459

90. Pozo MI, van Kemenade G, van Oystaeyen A et al (2020) The impact of yeast presence in nectar on bumble bee behavior and fitness. Ecol Monogr 90:e01393. https://doi.org/10.1002/ECM.1393

91. Yordanova M, Evison SEF, Gill RJ, Graystock P (2022) The threat of pesticide and disease co-exposure to managed and wild bee larvae. Int J Parasitol Parasites Wildl 17:319–326. https://doi.org/10.1016/J.IJPPAW.2022.03.001

92. Hotchkiss MZ, Poulain AJ, Forrest JRK (2022) Pesticide-induced disturbances of bee gut microbiotas. FEMS Microbiol Rev 46:1–22. https://doi.org/10.1093/FEMSRE/FUAB056

93. Kakumanu ML, Reeves AM, Anderson TD et al (2016) Honey bee gut microbiome is altered by in-hive pesticide exposures. Front Microbiol 7:1255. https://doi.org/10.3389/fmicb.2016.01255

94. Vidau C, Diogon M, Aufauvre J et al (2011) Exposure to sublethal doses of fipronil and thiacloprid highly increases mortality of honeybees previously infected by *Nosema ceranae*. PLoS One 6:e21550. https://doi.org/10.1371/JOURNAL.PONE.0021550

95. Motta EVS, Raymann K, Moran NA (2018) Glyphosate perturbs the gut microbiota of honey bees. Proc Natl Acad Sci U S A 115:10305–10310. https://doi.org/10.1073/PNAS.1803880115

96. Raymann K, Motta EVS, Girard C et al (2018) Imidacloprid decreases honey bee survival rates but does not affect the gut microbiome. Appl Environ Microbiol 84:e00545-e618. https://doi.org/10.1128/AEM.00545-18

97. Daisley BA, Trinder M, McDowell TW et al (2017) Neonicotinoid-induced pathogen susceptibility is mitigated by *Lactobacillus plantarum* immune stimulation in a *Drosophila melanogaster* model. Sci Rep 7:2703. https://doi.org/10.1038/s41598-017-02806-w

98. Motta EVS, Powell JE, Leonard SP, Moran NA (2022) Prospects for probiotics in social bees. Philos Trans R Soc B 377:202110156. https://doi.org/10.1098/RSTB.2021.0156

99. Cohen H, Ponisio LC, Russell KA et al (2022) Floral resources shape parasite and pathogen dynamics in bees facing urbanization. Mol Ecol 31:2157–2171. https://doi.org/10.1111/MEC.16374

100. Dillard BA, Chung AK, Gunderson AR et al (2022) Humanization of wildlife gut microbiota in urban environments. Elife 11:76381. https://doi.org/10.7554/ELIFE.76381

101. Chua KO, Song SL, Sen YH et al (2018) Microbial community composition reveals spatial variation and distinctive core microbiome of the weaver ant *Oecophylla smaragdina* in Malaysia. Sci Rep 8:10777. https://doi.org/10.1038/s41598-018-29159-2

102. Arroyo-Correa B, Jordano P, Bartomeus I (2023) Intraspecific variation in species interactions promotes the feasibility of mutualistic assemblages. Ecol Lett 26:448–459. https://doi.org/10.1111/ELE.14163

103. Perfectti F, Gómez JM, Bosch J (2009) The functional consequences of diversity in plant–pollinator interactions. Oikos 118:1430–1440. https://doi.org/10.1111/J.1600-0706.2009.17491.X

104. Davis MF, Rankin SC, Schurer JM et al (2017) Checklist for One Health Epidemiological Reporting of Evidence (COHERE). One Heal 4:14–21. https://doi.org/10.1016/J.ONEHLT.2017.07.001

105. Mackenzie JS, Jeggo M (2019) The One Health approach—why is it so important? Trop Med Infect Dis 4:88. https://doi.org/10.3390/TROPICALMED4020088

106. Trinh P, Zaneveld JR, Safranek S, Rabinowitz PM (2018) One Health relationships between human, animal, and environmental microbiomes: a mini-review. Front Public Heal 6:235. https://doi.org/10.3389/FPUBH.2018.00235

107. Mays Z, Hunter A, Campbell LG, Carlos-Shanley C (2021) The effects of captivity on the microbiome of the endangered Comal Springs riffle beetle (*Heterelmis comalensis*). FEMS Microbiol Lett 368:121. https://doi.org/10.1093/FEMSLE/FNAB121

108. Chiang E, Deblois CL, Carey HV, Suen G (2022) Characterization of captive and wild 13-lined ground squirrel cecal microbiotas using Illumina-based sequencing. Anim Microbiome 4:1. https://doi.org/10.1186/S42523-021-00154-9

109. Chua PYS, Bourlat SJ, Ferguson C et al (2023) Future of DNA-based insect monitoring. Trends Genet. https://doi.org/10.1016/J.TIG.2023.02.012

110. Tonietto RK, Larkin DJ (2018) Habitat restoration benefits wild bees: a meta-analysis. J Appl Ecol 55:582–590. https://doi.org/10.1111/1365-2664.13012

111. Daisley BA, Pitek AP, Chmiel JA et al (2019) Novel probiotic approach to counter *Paenibacillus* larvae infection in honey bees. ISME J 14:476–491. https://doi.org/10.1038/s41396-019-0541-6

112. Corby-Harris V, Snyder LA, Schwan MR et al (2014) Origin and effect of Alpha 2.2 *Acetobacteraceae* in honey bee larvae and description of *Parasaccharibacter apium* gen. nov., sp. nov. Appl Environ Microbiol 80:7460–7472. https://doi.org/10.1128/AEM.02043-14

113. Floyd AS, Mott BM, Maes P et al (2020) Microbial ecology of European Foul Brood disease in the honey bee (*Apis mellifera*): towards a microbiome understanding of disease susceptibility. Insects 11:555. https://doi.org/10.3390/INSECTS11090555