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Unveiling symbiotic bacterial communities in insects feeding on the latex-rich plant *Ficus microcarpa*

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Research Paper

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Abstract

The diversity and health of insects that feed on plants are closely related to their mutualistic symbionts and host plants. These symbiotic partners significantly influence various metabolic activities in these insects. However, the symbiotic bacterial community of toxic plant feeders still needs further characterisation. This study aims to unravel bacterial communities associated with the different species of insect representing three insect orders: Thysanoptera, Hemiptera, and Lepidoptera, along with their predicted functional role, which exclusively feeds on latex-rich plant species *Ficus microcarpa*. By using 16S rRNA gene high-throughput sequencing, the analysis was able to define the major alignment of the bacterial population, primarily comprising *Proteobacteria*, *Firmicutes*, *Bacteroidota*, *Actinobacteriota*, and *Acidobacteriota*. Significant differences in symbiotic organisms between three insect groups were discovered by the study: hemipterans had *Burkholderia* and *Buchnera*, and lepidopterans had *Acinetobacter*. At the same time, *Pseudomonas* was detected in high abundance in both lepidopteran and thysanopteran insects. Furthermore, these symbionts exhibit consistent core functions, potentially explaining how different insects can consume the same host plant. The identified core functions of symbionts open avenues for innovative approaches in utilising these relationships to develop environment-friendly solutions for pest control, with broader implications for agriculture and environmental conservation.

Introduction

Insects, with their inclusive variety of species, ability to acclimate to different environments, and significant biomass, play a vital role in global ecosystems. The complex link that exists between insects and the bacteria, fungi, viruses, protozoa, and archaea that live in their symbiotic relationship affects the diversity and adaptability of these organisms (Naveed *et al.*, 2020). Microbial symbionts associated with insects significantly contribute to their diversity and evolutionary pathways (Ali *et al.*, 2018; Id *et al.*, 2021). These symbionts perform numerous vital roles, such as influencing the use of host plants (Liu *et al.*, 2021; Naveed *et al.*, 2024), providing resistance to parasites or fungal pathogens (Tsuchida *et al.*, 2010), enhancing tolerance to thermal stress (Liu *et al.*, 2022), modifying body colouration (Tsuchida *et al.*, 2010), and influencing reproduction dynamics (Ali *et al.*, 2019; Liu *et al.*, 2021, 2022). There is ample evidence regarding the evolutionary dynamics of the symbiotic relationships that occur among individual microbial symbionts and their animal hosts (Bennett and Moran, 2015). However, the mechanisms governing community structure in long-term host–microbiome symbiosis still need to be understood (Groussin *et al.*, 2017). While a number of studies have planned diet as the primary driver influencing bacterial symbiotic composition (Ley *et al.*, 2008; Yatsunenko *et al.*, 2012; Carmody *et al.*, 2015), debates persist regarding the relative contributions of host genetics vs. environmental factors. Some authors suggest a minor impact of host genetics compared to ecological factors (Degnan *et al.*, 2012; Yatsunenko *et al.*, 2012), whereas others offer evidence supporting a more significant role of host genetics (Blekhman *et al.*, 2015). Furthermore, the configuration of bacterial populations within insects can be inclined by various factors, including food resources, exposure to pathogenic microbes, environmental conditions, and host plants (Wang *et al.*, 2020; Naveed *et al.*, 2024).

Many different poisonous and indigestible substances can be found in plant tissues, which forces insects that are herbivorous to develop adaptive strategies for different plant types. These strategies often involve symbiotic relationships that allow insects to exploit various host plants effectively (Liu *et al.*, 2020). Latex, an antifeedant primarily found in plants, is a potent deterrent among the many defences against herbivores that plants produce (Dillon and Dillon, 2004). The latex of various *Ficus* species contains several proteins, such as oxidases, protease inhibitors, chitinases, and cysteine protease, which are crucial in defending against herbivores (Konno, 2011). Cysteine proteases such as ficin and bromelain, abundant in the latex of *Ficus*,

exhibit toxicity against herbivores, contributing to the plant's defence mechanism against insects (Konno *et al.*, 2004). *Ficus microcarpa*, a widely cultivated ornamental tree, thrives in tropical regions worldwide and is commonly used in landscaping. It is frequently observed in cultivated and naturalised specimens near human activities, such as houses, buildings, and gardens (Starr *et al.*, 2003). Various pests, mainly insects, pose a significant threat to the development of this plant species. However, this plant species faces substantial threats from multiple pests, primarily insects. For example, *Mycopsylla* sp. is known to nourish exclusively on *Ficus*, and its nymphs yield a gummy substance at the inferior exterior of the leaves (Newman, 2004). A species of *Ormyrus Westwood* (*Ormyridae*) was also reported on *F. microcarpa* in Europe (Koutsoukos *et al.*, 2024).

Despite clear evidence of latex's deterrent effect on herbivory and the prevalence of latex-producing plants, the question of how herbivores navigate the inevitable inclusion of latex in their diets still needs to be explored. We hypothesise that bacterial symbionts enable insects to overcome plant defences by detoxifying plant toxins, such as latex, and facilitating nutrient acquisition. We predict that different insect species will harbour distinct bacterial symbionts specialised for these adaptations, reflecting their specific phylogenetic and dietary adaptations. Exploring these associations, with a keen consideration of taxonomy and feeding habits, could unlock crucial insights into this intriguing facet of herbivory. Some insects have evolved specific bacterial symbiotic relationships that are pivotal in combating phytotoxins (Naveed *et al.*, 2020). These symbionts are adept at breaking down chemical compounds found in latex, potentially serving as a mechanism for herbivores to adapt to latex-producing plants. Moreover, certain insects harbour bacterial symbionts that offer vital nutrients (Zhang *et al.*, 2023). This partnership not only aids in the breakdown of plant compounds but also enhances the adaptability of the insect's hosts to challenging environmental conditions. The complicated web of connections between the insects and their bacterial symbionts could provide a comprehensive understanding of how herbivores cope with latex-induced challenges and shed light on the broader dynamics of plant–insect interactions.

The expansion of high-throughput sequencing makes it possible to discover complete bacterial populations in insects with great ease. This work used 16S rRNA gene amplicon sequencing in order to examine the bacterial communities living within insects that are feeding on *F. microcarpa*. Furthermore, insights gained from studying these symbiotic relationships may also offer promising avenues for controlling and managing these insects' pests, contributing to more sustainable and effective pest management strategies.

Materials and methods

Sample collection

From 2022 to 2023, we collected extensive insects from the latex-producing plant *F. microcarpa* in Fuzhou, Fujian, China. In this study, samples comprised of a diverse range of species, comprising *Perina nuda*, *Ocinara albicollis*, *Teleiodes saltuum*, *Bambusiphila vulgaris*, *Homoeocerus angulatus*, *Harmostes reflexulus*, *Aphis aurantii*, *Gynaikothrips uzeli*, and *Gynaikothrips ficorum*. These specimens were considerately selected to comprehensively represent insects that feed on *F. microcarpa*. Table 1 describes the comprehensive sample data used in a recent study.

DNA extraction

Larger insect samples only needed one individual, but we used two to three individuals for DNA extraction from small-bodied insects. All of the collected samples experienced a detailed washing process that involved three washes by using ultra-pure water. Afterwards, the total genomic DNA were extracted by using a DNeasy Blood and Tissue Kit from Qiagen. To maintain the integrity of our data, a sample of deionised water was used for negative control. The extraction of DNA was performed at an ultra-clean worktable to evade DNA contagion through environmental factors. The bacterial universal primers 8F and 1492R (Ledbetter *et al.*, 2007) were used to ensure that the DNA extraction was successful, a polymerase chain reaction (PCR) amplification method was employed for this purpose. Deionised water served as an additional negative control to further guarantee data accuracy. A 25 µl reaction mixture of 1 µl of DNA, 2.5 µl of 10× LA PCR Buffer-II (Mg^{2+}), 0.5 µl of dNTP, 0.5 µl of each primer (10 µM), 0.5 µl of TaKaRa LA-Taq (5 µM μl^{-1}) by TaKaRa Bio-Inc., Japan, and 19.5 µl of water was used for PCR amplification by ProFlex-TM Base (Applied Biosystems, Inc., Waltham, USA). PCR cycling conditions were used as follows: preliminary denaturation for 4 min at 94°C; also includes 30 rounds of denaturation for 30 s at 94°C; annealing at 65°C for 40 s; 90 s for the process of extension at 72°C; and 10 min of eventual extension at 72°C. PCR results were observed on a 1% agarose gel, and positive insect samples at about 1500 bp were stored at −20°C in a refrigerator for further 16S library synthesis. Particularly, the samples that were used as a negative control yielded no bands, demonstrating that DNA extraction has a lack of impurity during the process.

16S rRNA gene sequencing and amplification

Our focus was on amplifying the V3–V4 hypervariable area of the 16S rRNA gene using specific primers 338F and 806R (Liu *et al.*, 2022). The amplification process involved two PCR steps, first targeting the gene regions of interest and the second incorporating indices and adapter sequences. Positive PCR underwent purification and homogenisation to create a sequencing library, facilitating high-throughput sequencing.

Analysis of sequencing data

Sequencing data underwent merging of paired-end readings using FLASH and subsequent refinement and filtering by Trimmomatic (Bolger *et al.*, 2014). Chimaera sequences were eliminated, and remaining sequences were listed into operational taxonomic units (OTUs) using USEARCH. To interpret the differences in sequence depth between samples, rarefaction normalisation was used. The enduring sequences were categorised into OTUs with 97% similarity by USEARCH v10.0. OTUs that were counted for less than 0.005% of all sequences were removed (Bokulich *et al.*, 2013). Taxonomic classifications were applied using the RDP classifier, supplemented by BLAST searches against GenBank sequences for validation.

Diversity and statistical analysis

Various diversity indices were calculated using Mothur (Schloss *et al.*, 2009), including abundance-based coverage estimator (ACE), Chao1, Shannon, and Simpson indices. These indices included the ACE and Chao1 for estimating richness of species

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Table 1. Detailed sample information used in this study

Sl. no.	Sample ID	Collection date	Sample name	Insect species	Insect order	Feeding	Host plant
1	FWC1	16.11.2022	GfW.S.T	*G. ficorum* (winter)	Thysanoptera	Sap	*F. microcarpa*
2	FWC2						
3	FWC3						
4	FWA1	02.06.2023	GfS.S.T	*G. ficorum* (summer)		Sap	*F. microcarpa*
5	FWA2						
6	FWA3						
7	FWB1	11.06.2023	Gu.S.T	*G. uzeli*		Sap	*F. microcarpa*
8	FWB2						
9	FWB3						
10	FWD1	03.05.2023	Aa.S.H	*A. aurantii*	Hemiptera	Sap	*F. microcarpa*
11	FWD2						
12	FWD3						
13	FWJ1	11.06.2023	Ha.S.H	*H. angulatus*		Sap	*F. microcarpa*
14	FWJ2						
15	FWJ3						
16	FWO1	01.06.2023	Hr.S.H	*H. reflexulus*		Sap	*F. microcarpa*
17	FWO2						
18	FWO3						
19	FWM1	11.11.2022	PnW.L.L	*P. nuda* (winter)	Lepidoptera	Leaf	*F. microcarpa*
20	FWM2						
21	FWM3						
22	FWH1	07.06.2023	PnS.L.L	*P. nuda* (summer)		Leaf	*F. microcarpa*
23	FWH2						
24	FWH3						
25	FWE1	12.05.2023	Oa.L.L	*O. albicollis*		Leaf	*F. microcarpa*
26	FWE2						
27	FWE3						
28	FWG1	17.05.2023	Ts.L.L	*T. saltuum*		Leaf	*F. microcarpa*
29	FWG2						
30	FWG3						
31	FWF1	17.05.2023	Bv.L.L	*B. vulgaris*		Leaf	*F. microcarpa*
32	FWF2						
33	FWF3						

and the Simpson and Shannon directories for calculating community diversity. Higher values of Chao1, ACE, and Shannon, as well as lower Simpson values, imply greater diversity. Given the diverse symbiotic compositions, insect samples were compared using the Student's *t*-test.

We used beta diversity analysis to compare symbiotic bacterial compositions across different sample groups. For this goal, we used weighted UniFrac distances, which take into account phylogenetic information on the OTUs. These metrics help out to evaluate community variation and evaluation of different indices of beta diversity, taking into account both the existence and abundance of bacteria (Lozupone *et al.*, 2011).

To investigate bacterial community changes across all samples, as well as three different insect orders, non-metric multidimensional scaling (NMDS) was employed to cluster the communities of bacteria in the package *vegan* v2.3.0 (Looft *et al.*, 2012) and generated two-dimensional plots using ggplot2 v3.1.1 (Wickham, 2016) by using R programming environment version 3.1.1 (Team RC, 2014). NMDS is trustworthy when the stress value is less than 0.2. The Circos sample and species relationship map was created using the Circos-0.67-7 (http://circos.ca/). We employed permutational multivariate analysis of variance (PERMANOVA) and analysis of similarities (ANOSIM) to identify statistically noteworthy differences in symbiotic bacterial

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composition among the above explained classification systems. The weighted UniFrac and Bray–Curtis distance matrices were utilised in these studies. ANOSIM and PERMANOVA were achieved by the anosim and adonis functions with the vegan v2.3.0 package (Oksanen *et al.*, 2015) in the R v3.1.1 (Team RC, 2014). The *R* value in ANOSIM nearer to one recommends greater variation across groups than within them (Anderson and Walsh, 2013). A higher value of R^2 in PERMANOVA showed that the grouping variable is very important in clarifying overall variation (Anderson, 2014). *P*-values of less than 0.05 for PERMANOVA and ANOSIM represent high test reliability.

PICRUSt2 v2.3.0b0 (Douglas, 2006) was used to forecast the composition of the functional genes in order to investigate prospective functional changes in symbiotic bacterial communities. This entailed comparing bacterial species configuration data achieved from 16S rRNA gene sequences. Data obtained through PICRUSt2 were analysed at Kyoto Encyclopedia of genes and genomes (KEGG) (Kanehisa *et al.*, 2012) orthology level 3. We used STAMP (Parks *et al.*, 2014) v2.1.3 to analyse relative abundance differences. Individual *t*-tests were done between groups, with a *P*-value of ≤0.05 representing statistical significance.

Results

Analysis of 16s rDNA sequencing results

We obtained 2,433,868 high-quality sequences through 16S rRNA gene amplicon sequencing from 33 distinct DNA samples of various insects. On average, each sample yielded approximately 73,753 reads. After sequence filtering, 2,294,886 reads were retained, per sample averaging around 69,542 reads (table S1). The gene assemblies paired sequences of the 16S rRNA represent a typical length of 421 base pairs.

Cluster analysis, using a 97% sequence match threshold, identified 1493 OTUs (table S2). These OTUs encompassed 24 phyla, 49 classes, 130 orders, 257 families, and 552 genera. Evaluation through the Shannon index rarefaction curve (fig. S1) signifies the adequacy of sequencing volume and saturation of sequencing depth. Further increasing the sample volume is not anticipated to yield additional OTUs.

Comparison of alpha and beta diversity in samples

Alpha diversity indices were engaged to assess species richness (ACE and Chao1) and bacterial diversity (Simpson and Shannon) across all the samples (table S2). The analysis exposed prominent differences in the microbial communities between the several samples (fig. 1, table S2). The four indices of alpha diversity were widely spread through all samples, extending from 177.61 to 1362.67 (927.12 ± 59.91) for the ACE index, from 169.2 to 1374.5 (938.34 ± 63.09) for the Chao1 index, from 0.72 to 8.68 (6.27 ± 0.42) for the Shannon index, and from 0.10 to 0.99 (0.85 ± 0.04) for the Simpson index (table S2). The ace and Chao1 indexes demonstrated the abundance of symbionts in *P. nuda* (summer) was maximum monitored by *T. saltuum*, *P. nuda* (winter), *G. ficorum* (winter), *O. albicollis*, *G. ficorum*

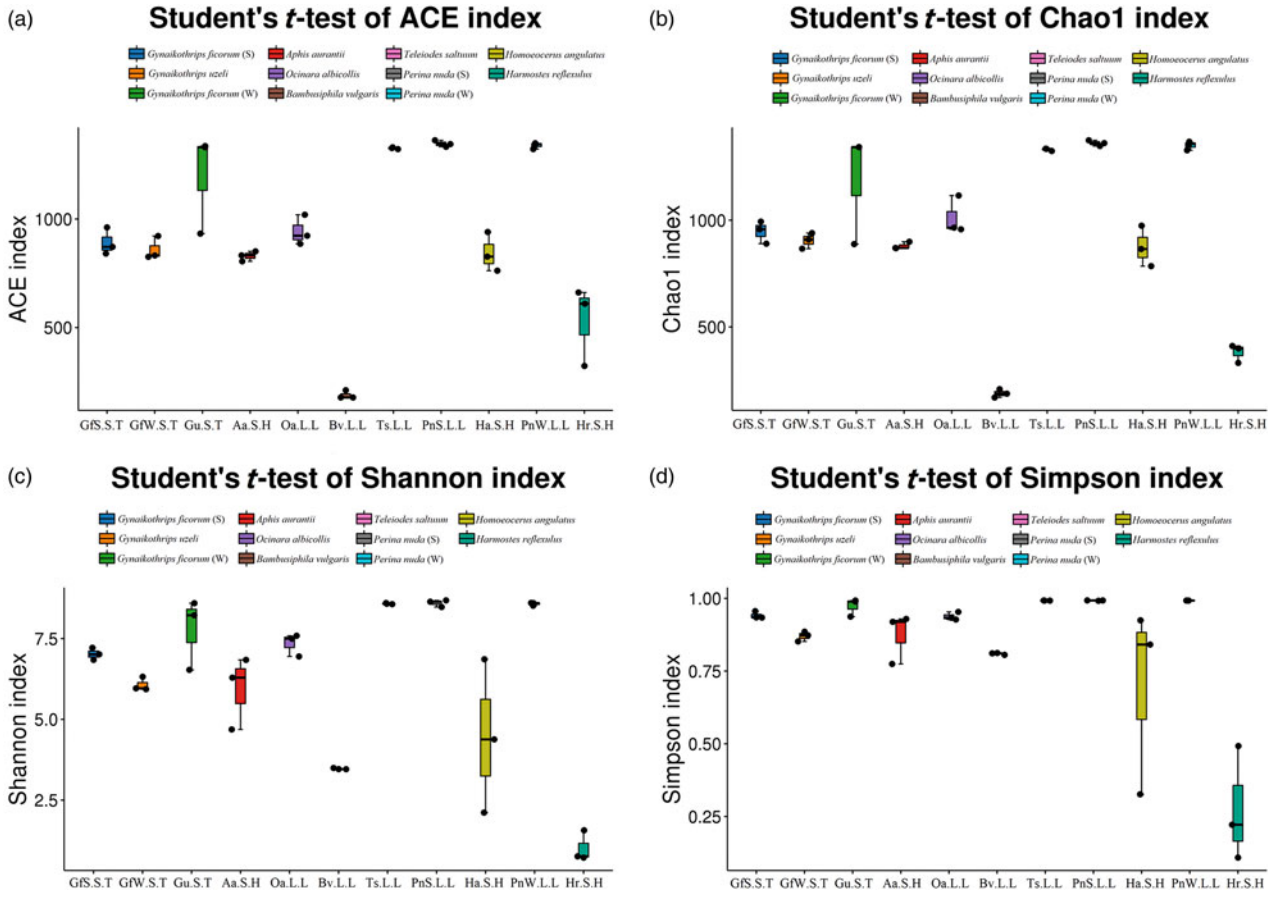

Figure 1. Alpha diversity indices across all groups: (A) ACE index, (B) Chao1 index, (C) Shannon index, and (D) Simpson index.

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(summer), *G. uzeli*, *H. angulatus*, *A. aurantii*, *H. Reflexulus*, and *B. vulgaris*, which showed the lowermost richness. The values of samples in the Chao1 and ace indices were expressively diverse from each other (fig. 1A, B). All of the previously mentioned indices were proved that *P. nuda* (summer) had the maximum levels of the diversity and microbial community richness (fig. 1C, D). Significant variances were detected when relating alpha diversity indexes through distinct insect orders and seasons (table S2).

At the OTU level, the samples' beta diversity showed similarities and variations in the species composition and community structure. We considered NMDS to relate the bacterial community differences centred on Weighted UniFrac distance metrics across all the studied samples and three insect orders. The stress value was 0.0285 for both categories (fig. 2). The abscissa and ordinate axes in scatter plots represent two vital variables determining sample differences. We employed PERMANOVA and ANOSIM to statistically judge the differences in bacterial populations across various samples and insect orders (table 2). These studies revealed significant alterations in bacterial populations through all sample groups.

Table 2. Results of ANOSIM and PERMANOVA based on Bray–Curtis and weighted UniFrac distances

Beta diversity distance	Bacterial community	ANOSIM (R,p)	PERMANOVA (R^2,p)
Bray–Curtis	All samples	0.601, *0.001*	0.735, *0.001*
	Insect orders	0.259, *0.001*	0.215, *0.001*
Weighted UniFrac	All samples	0.685, *0.001*	0.854, *0.001*
	Insect orders	0.234, *0.003*	0.291, *0.001*

Statistically significant P values ($P < 0.01$) are highlighted in italics; see table 1 for detailed sample information.

Comparison of microbial community composition at different taxonomic levels

The composition of the community for each insect sample was analysed at the phylum and genus levels (fig. 4). The 'Others' category contains a limited number of phyla (0.01%) that are only existent in specific samples which have less abundance. At the phylum level, insect samples were primarily confined to *Proteobacteria* (54.31%), *Firmicutes* (23.60%), *Bacteroidota* (8.73%), *Actinobacteriota* (3.32%), and *Acidobacteriota* (2.58%). *Proteobacteria* were principally detected across all samples, mainly in the species of hemipteran with higher abundance and one lepidopteran species (*B. vulgaris*) (figs 3A and 4A).

At the genus level, 22 highly prevalent genera were identified in all samples (fig. 4B), with the top ten genera being *Burkholderia* and *Pseudomonas*, followed by *Acinetobacter*, *Muribaculum*, *Bacteroides*, *Buchnera*, *Dubosiella*, *Lactobacillus*, *Staphylococcus*, and *Oscillospiraceae*. Notably, *Burkholderia* was found in only two kinds of bugs (98% in *H. reflexulus* and 61% in *H. angulatus*) with higher abundance (fig. 5, table S3); on the other hand *Acinetobacter* was mainly detected in one species of Lepidoptera (79% in *B. vulgaris*) and also found in different species of insects with low abundance (fig. 5, table S3). *Pseudomonas* was detected in all insect orders, but its relative abundance varied in all samples except for *H. reflexulus*. *Muribaculum* was detected in two insect orders, Thysanoptera and Lepidoptera, with high abundance, but in hemipterans, these were detected in very low abundance. Intriguingly, *Buchnera* was exclusively detected in aphid species with high abundance (figs 4B and 5). In contrast, all the genera *Bacteroides*, *Dubosiella*, *Lactobacillus*, *Staphylococcus*, and *Oscillospiraceae* were detected in all samples but with less abundance (fig. 5, table S3).

Notably, *Proteobacteria* were prevalent among all samples, predominantly in species of hemipteran with the higher abundance rate (fig. 3A). From the 1493 OTUs, maximum OTUs were detected in *P. nuda* (summer and winter), followed by *T. saltuum*,

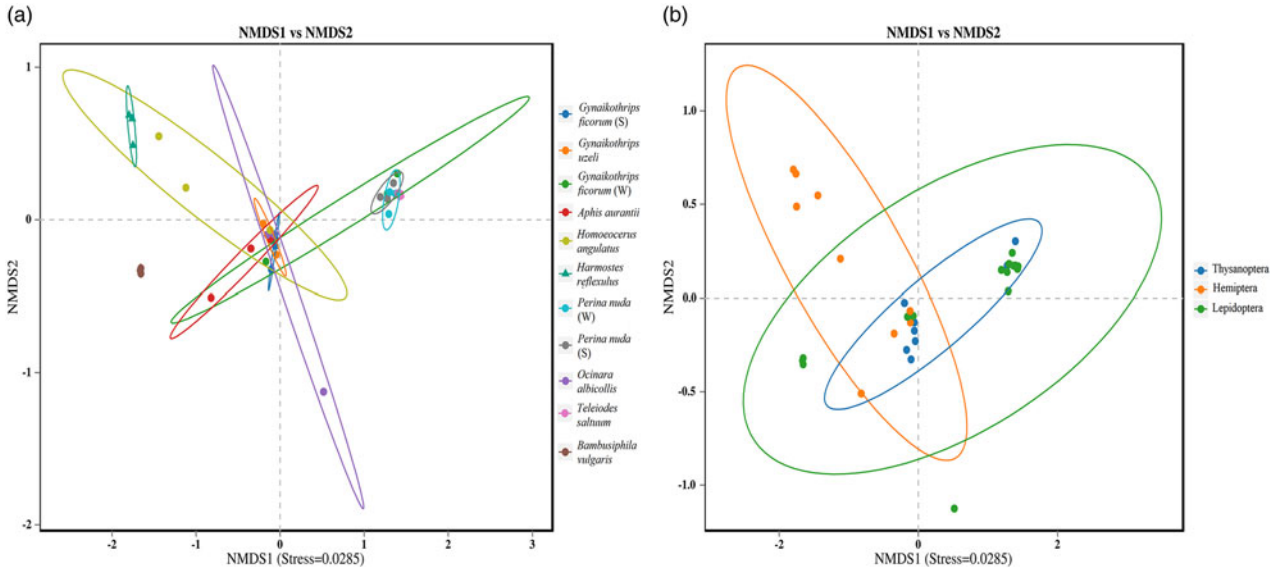

Figure 2. Beta diversity based on weighted UniFrac distance of bacterial population displayed in NMDS plots for (A) all insect groups and (B) three different insect orders.

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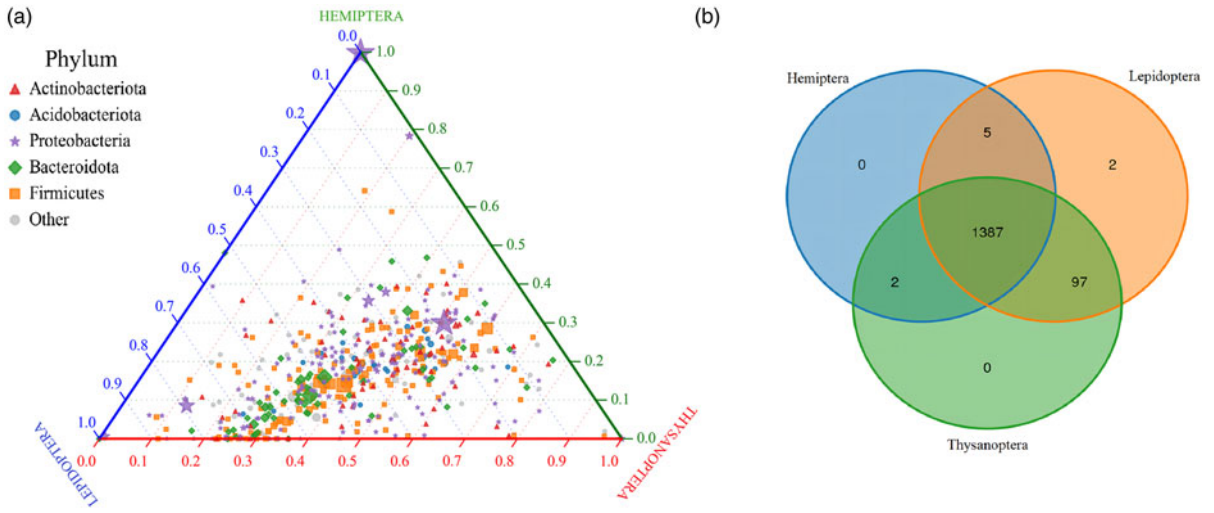

Figure 3. Plot shows the OTUs distribution in ternary form: (A) across three distinct insect orders and (B) Venn diagram at the OTU level for three distinct insect orders.

G. ficorum (winter), *O. albicollis*, *G. ficorum* (summer), *G. uzeli*, *A. aurantii*, *H. angulatus*, *H. reflexulus*, and *B. vulgaris* (table S2). In total, 1387 OTUs were common between the three orders, as represented in Venn diagram, while only two OTUs were specific to Lepidoptera. In contrast to the comparisons, Thysanoptera and Lepidoptera shared more OTUs (fig. 4B).

Deviation of dominant symbionts and their functional prediction

All collected samples showed differences in relative abundance and composition of dominating symbionts (table S3). Among the five samples, *G. ficorum* (summer), *G. uzeli*, *G. ficorum* (winter), *P. nuda* (summer), and *O. albicollis*, the dominant symbiont was *Pseudomonas*. *Buchnera* was detected in *A. aurantii*, *Burkholderia* was found in *H. reflexulus* and *H. angulatus*, and *Acinetobacter* was found in *T. saltuum* and *B. vulgaris*. In contrast, *Muribaculum* was found dominant in *P. nuda* (winter) (fig. 5, table S3). Seasonal variations were observed in the abundance of bacterial genera in two species of insects, *G. ficorum* and *P. nuda*. Specifically, *Bacteroides* and *Pseudomonas* showed higher levels in summer than in winter, whereas *Muribaculum* and *Lacticaseibacillus* were more abundant in winter than in summer (fig. S2).

Results of the PICRUSt2 examination are represented in fig. S3. The *t*-test conducted on prevalent functional gene pathways across the five comparison groups, categorised by dominant symbionts, discovered a major rise in the abundance of genes linked with 'metabolic pathways' (fig. S4). *Pseudomonas* displayed remarkably higher relative abundance in 'biosynthesis of secondary metabolites', 'metabolic pathways', and 'biosynthesis of antibiotics' compared to *Burkholderia*, *Buchnera*, and *Muribaculum* (figs S4D, E, G). *Acinetobacter* displayed particularly higher richness in 'metabolic pathways', 'biosynthesis of secondary metabolites', 'biosynthesis of antibiotics', and the biosynthesis of amino acids associated with *Burkholderia* (fig. S4B), but *Burkholderia* unveiled especially higher abundance in 'microbial metabolism in diverse environments', 'ABC transporters', and 'Quorum sensing' when equated with *Acinetobacter*, *Pseudomonas*, and *Muribaculum* (fig. S4B–D).

Discussion

This study symbolises the initial investigation of bacterial symbiotic communities in various insect species that feed on latex-rich toxic plants. The symbiotic relationships between insects and their bacterial communities are vital for survival. It is essential to recognise insects' morphology and physicochemical conditions, which can vary significantly (Zhao *et al.*, 2018). These deviations may influence the distinctive bacterial ecology particular to each host (Mariño *et al.*, 2018). Accepting these dynamics is important for an inclusive understanding of insect ecology. Recent advancements in microbiome investigation using high-throughput sequencing have made it easier to explore host–microbe interactions. Using 16S rRNA amplicon sequencing, this study provides the first in-depth look at the bacterial diversity and relationships among the insects that feed on the latex-producing plant *F. microcarpa*. However, a distinguished gap in the study differentiates variations in symbiotic bacteria among different species and their relationship with their host (Liu *et al.*, 2022). Our understanding of the interface among diverse insects and their associated symbionts is currently limited due to the exploration of only a few models. Compared to other insects and herbivores, our samples exhibit a vibrant and diverse bacterial population. This underscore the exclusive and intricate bacterial ecosystems connected with these insects.

Studies have highlighted the significant impact of host diet and taxonomy on shaping symbiotic communities (Ley *et al.*, 2008). While some research emphasises the predominant role of diet in structuring microbiomes, others suggest that common ancestry holds sway in determining microbiome community (Karasov *et al.*, 2011; Zhao *et al.*, 2016). However, there is still a need for more studies on this subject. In this investigation, we examined symbiotic bacterial communities across different species of insects that share the same plant species. Notably, while observing the same plant species, variations in bacterial community structures were evident between different insect orders and within the same order. Previous studies found that *Proteobacteria*, *Firmicutes*, and *Bacteroidota* are normally establish inside numerous insects and mammals (Chen *et al.*, 2018), as we reported *Proteobacteria* (54.3%), *Firmicutes* (23.6%), *Bacteroidota* (8.7%), and *Actinobacteriota* (3.32%) in all insect samples but their

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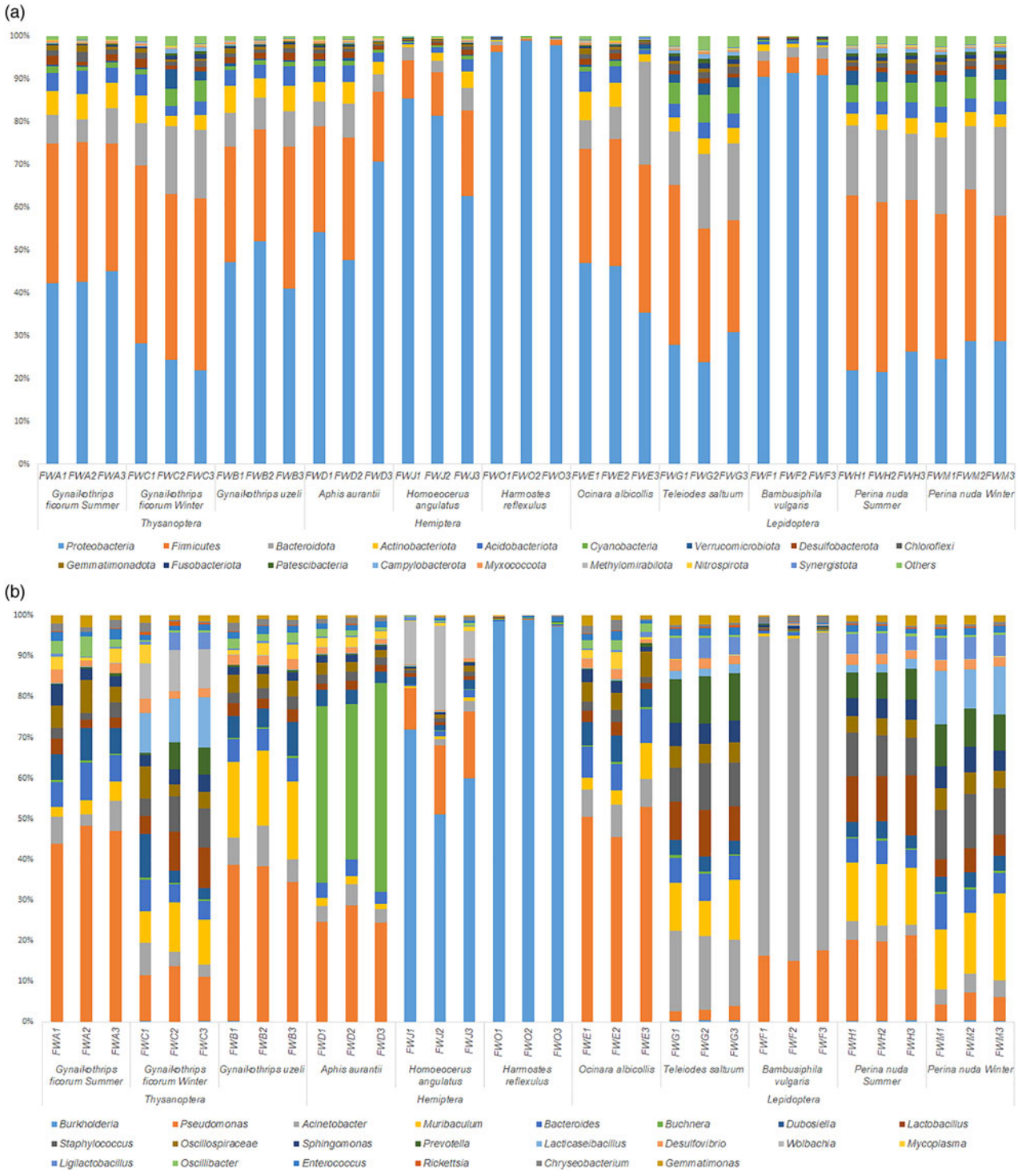

Figure 4. Taxonomic diversity and number of symbiotic bacteria associated with each sample: (A) at the phylum level and (B) on the genus level.

abundance was varied in all samples (fig. 4A). *Proteobacteria* often dominate the microbiota of wide range of invertebrates (Zhang *et al.*, 2022) including *Harpalus pensylvanicus*, *Aspidimorpha sanctaecrucis* (Schmid *et al.*, 2014), *Hypothenemus hampei* (Mariño *et al.*, 2018), and *Bactrocera dorsalis* (Andongma *et al.*, 2015). *Proteobacteria* are pivotal in carbohydrate degradation in the Wood Borer, *Saperda vestita* (Delalibera *et al.*, 2005), synthesis of vitamins (McCutcheon and Moran, 2007), and pesticide detoxification in the fruit fly *B. dorsalis* (Itoh *et al.*, 2018). The second most prevalent phylum, *Firmicutes*, abundant in termites and honeybees (Martinson *et al.*, 2011; Auer *et al.*, 2017), enhances food resource metabolism, aiding energy conversion from the diet and assisting in cellulose and hemicellulose consumption (Brown *et al.*, 2012). *Bacteroidota*, the third predominant phylum in our 16S rRNA dataset, is recognised for producing crucial enzymes like xylanase, glucanase, and mannanase, contributing to intricate

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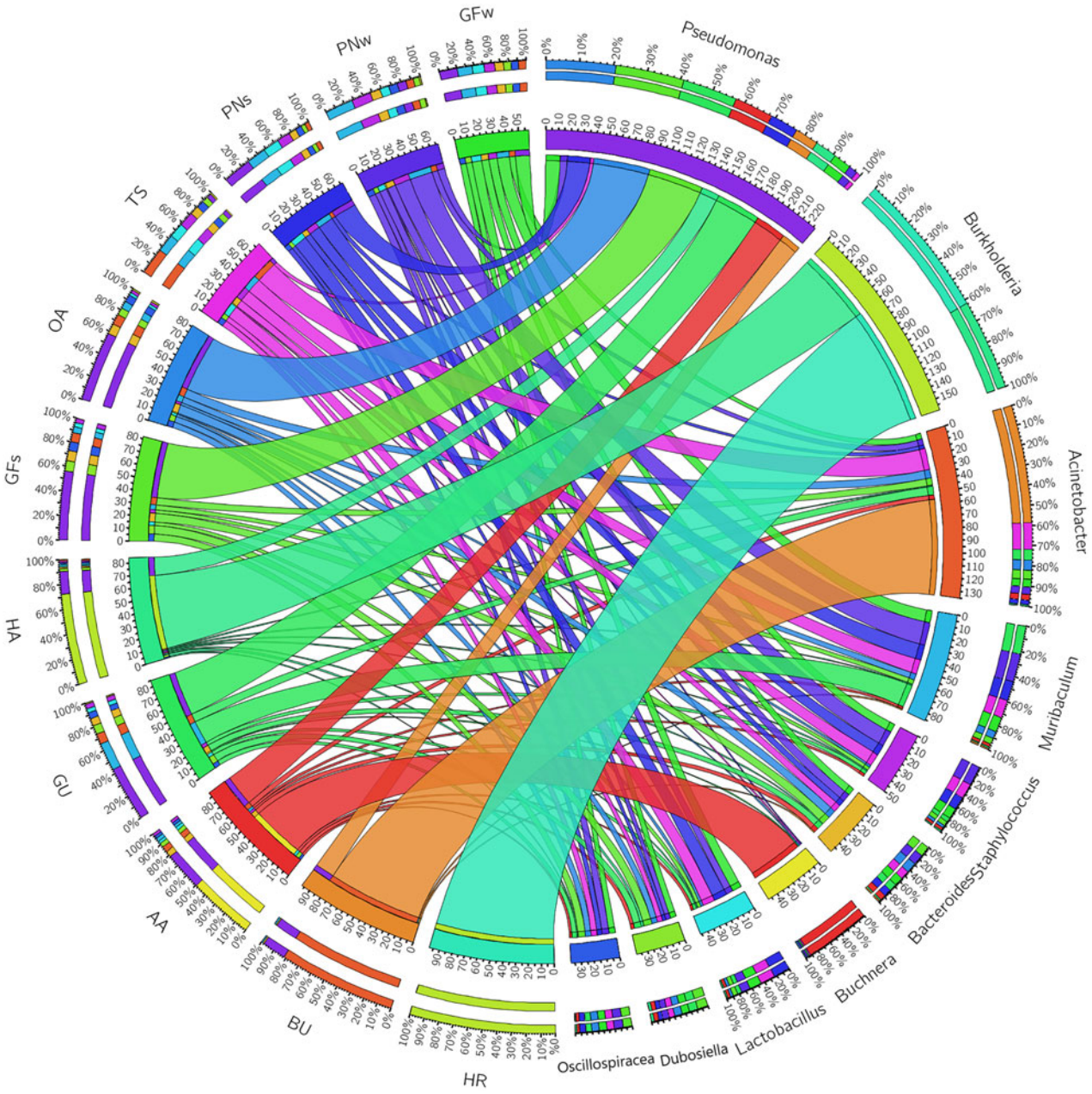

Figure 5. Circos displays the distribution percentage of the top ten genera in each sample and the distribution percentage across samples. GFw, *G. ficorum* (winter); PNw, *P. nuda* (winter); PNs, *P. nuda* (summer); TS, *T. saltuum*; OA, *O. albicollis*; GFs, *G. ficorum* (summer); HA, *H. angulatus*; GU, *G. uzeli*; AA, *A. aurantii*; BU, *B. vulgaris*; HR, *H. reflexulus*.

carbohydrate metabolism (Dai *et al.*, 2012). *Actinobacteria*, also abundant, augment metabolic versatility, enabling the exploitation of diverse nutritional resources such as cellulose and hemicelluloses in termites (Schäfer *et al.*, 1996), and they produce secondary metabolites with antibiotic properties (Gawande *et al.*, 2019). These fluctuations in phyla abundance might stem from varying species taxonomies and their respective feeding habitats.

At the genus level, we found that thysanopteran insects harbour *Pseudomonas* in high abundance except for *G. ficorum* (winter) (fig. 4B, table S3). This genus was also previously reported in the symbiotic bacteria of *Thrips tabaci*, accounting for 4.5% abundance (Gawande *et al.*, 2019). *Pseudomonas*, known for its ability to degrade latex, dominates in thrips species and other insect species (fig. S2B, table S3). This bacterial genus plays a vital role in helping its host detoxify harmful compounds, enabling the insect to feed on the host plant and obtain essential nutrients. On the other hand, *Pseudomonas* emerged as the most prevalent genus in *Nesothrips brevicollis* and *Elaphrothrips curvipes*, consistent with earlier studies on thrips (Dickey *et al.*, 2014; Gawande *et al.*, 2019). The dietary differences among these species are notable: *G. ficorum* and *G. uzeli* primarily feed on *F. microcarpa* leaves, extracting phloem, while *Ayyaria chaetophora*, *Dendrothripoides innoxious*, and *Nepenthes gracilips* prefer flowers, and *T. tabaci* forages on onion leaves. These distinct dietary preferences may significantly impact the abundance of specific genera within their associated bacterial communities.

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In hemipteran insects, we found that two species of bugs, *H. reflexulus* and *H. angulatus*, were highly abundant with *Burkholderia* (fig. 5, table S3). Largidae species were explicitly associated with *Burkholderia* strains. *Riptortus pedestris* consistently showed 93.1% *Burkholderia* presence over a 2-year survey, aligning with findings from a field study in Japan (Kikuchi *et al*., 2005). Additionally, *Burkholderia* presence was reported in populations of the leafhopper *Macrosteles striifrons* (Ishii *et al*., 2013). The evolutionary origin of *Burkholderia* is believed to be traced back to the last common ancestor of Lygaeoidea, Coreoidea, and Pyrrhocoroidea (Gordon *et al*., 2016). However, exceptions were observed; for instance, *Stenomacra tungurahuana* displayed a substantial number of reads from *Rickettsia*, and a *Dysdercus* specimen exhibited an unusual microbiome with a high concentration of *Bartonella* (Gordon *et al*., 2016). *Burkholderia* strains are recognised for conferring various advantages to host fitness, comprising increased fecundity, provision of nutrients, enhanced insecticide resistance, and larger body size (Takeshita and Kikuchi, 2017). Aphid species in our study were abundant with *Buchnera* (fig. 5, table S3), which is crucial in providing nutrition (fig. S2A, table S3). Earlier studies stated that *Pseudoxenodon bambusicola* has lost its capability to get feed from *Bambusa* independently, relying on its symbiotic bacteria for nutritional support (Zhang *et al*., 2023), enhanced insecticide resistance (Naveed *et al*., 2020), the diversity of bacterial communities in hemipterans varied, potentially influenced by their feeding habitats, thus affecting the abundance of specific genera within their hosts and these symbionts help them to get nutrition from the indigestible host (Naveed *et al*., 2024).

Our findings revealed that lepidopteran insects were predominantly associated with three bacterial phyla: *Proteobacteria*, *Firmicutes*, and *Bacteroidota* (fig. 4A, table S3). However, prior examination has also identified *Proteobacteria* and *Firmicutes* as prevailing phyla in certain lepidopteran insects such as, *Helicoverpa armigera*, *Lymantria dispar*, *Bombyx mori*, and *Manduca sexta* (Gao *et al*., 2019). Furthermore, *Firmicutes* symbolise a significant part of *Solenopsis invicta* and many other insects (Ishak *et al*., 2011), the same as some lepidopterous insect species harbouring *Firmicutes*. At the genus level, *Pseudomonas* substantially contributes to the symbiotic community of some lepidopterous samples (fig. 5, table S3). This genus is known for harbouring species capable of degrading natural latex, rubber, and alkaloids (Vilanova *et al*., 2016). *Staphylococcus* is predominant in Lepidoptera of the families Noctuidae and Sphingidae and was detected in some lepidopterous species such as *P. nuda* and *T. saltuum* (fig. 5, table S3) (Visôtto *et al*., 2009). We also detected *Acinetobacter* in lepidopterous species but highly abundant in *B. vulgaris* and *T. saltuum* (fig. S2B, table S3). This genus has been reported in *H. armigera*, demonstrating robust esterase activity promoting the metabolism of the insecticide cypermethrin, thus increasing insect resistance (Malhotra *et al*., 2012). Additionally, *Acinetobacter*, isolated from *Plutella xylostella* larvae, may enhance the nitrogen-fixation capacity (Indiragandhi *et al*., 2008), and has similar functions observed in ants and termites, aiding carbon–nitrogen metabolism (Moreira-Soto *et al*., 2017). Both *Pseudomonas* and *Acinetobacter* genera collectively play a crucial role in detoxifying toxic chemicals for their hosts and facilitating nutrient absorption.

Various insects have been shown to exhibit seasonal changes in their bacterial communities (Wei *et al*., 2014; Sandeu *et al*., 2022), highlighting the significant influence of seasonal dynamics on insect-associated microbiota. Our findings illustrate seasonal changes in the bacterial communities linked with different insect species, *G. ficorum* and *P. nuda*, collected in both summer and winter. Seasonal deviations in symbiotic bacterial communities have been detected in different insects (Wei *et al*., 2014; Chen *et al*., 2019; Sandeu *et al*., 2022), this underscores the importance of seasonal dynamics in influencing bacterial communities associated with insects.

To predict metabolic capabilities of bacterial communities, we conducted KEGG ontology analysis, enlightening that many OTUs demonstrated substantial metabolic potential regardless of their relationship with specific insect samples (fig. S3). PICRUSt2 study shows no significant dissimilarities in the number of anticipated gene pathways in symbiotic communities of bacteria. This proposes that, based on the functional assessment generated using the 16S rRNA gene data, that may not be significant differences in the genetic pathways used by different bacterial communities within the examined environment. *Proteobacteria* was the dominant phylum across all species, mainly in hemipteran species with greater abundance (fig. 4A). Furthermore, phyla such as *Proteobacteria* and *Firmicutes* may perform pivotal roles in providing vital nutrition for various biological procedures in hosts, including reproduction, growth, and detoxification of toxic chemicals (Gurung *et al*., 2019; Chamankar *et al*., 2023). These findings provide further support for the notion that these insects possess the capability to metabolise toxic substances like latex. The presence of these bacteria within insects highlights their need on symbiotic relationships with communities of bacteria to successfully break down and use toxic chemicals in the diet. This ability is critical for such insects, allowing them to extract nutritional compounds derived from plants that would otherwise be indigestible. It emphasises the importance of the bacterial community in regulating host physiology. Simultaneously, PICRUSt2 analysis showed no significant dissimilarities in the proportion of projected gene pathways in the bacterial communities among diverse insect species.

A recent study observed a diverse bacterial composition in all samples. Some insects primarily have detoxifying symbionts (symbiotic organisms alter a toxic substance's chemical composition or structure in a way that benefits the host organism). In contrast, others host nutritional symbionts (symbiotic organisms function to sustain host metabolism by supplying vital nutrients, such as amino acids or vitamin B, enabling hosts to subsist on diets lacking in essential nutrients) (fig. S3). Interestingly, both types of symbionts play a vital role in facilitating their hosts to extract nutrients from toxic plants. Despite this diversity, their core functions seem consistent, potentially explaining why these insects can consume the same host plant. These results enhance our understanding of symbiotic interactions in ecological dynamics and provide practical insights for sustainable pest management strategies.

Conclusions

Our investigation unveils the intricate relationship between diverse insects feeding on the latex-rich plant *F. microcarpa* and their symbiotic bacterial communities. By comparing bacterial communities through insects from three distinctive orders (Thysanoptera, Hemiptera, and Lepidoptera), all feeding on *F. microcarpa*, we uncovered substantial deviations in symbiotic bacterial presence, abundance, and composition influenced by multifaceted factors such as seasonal changes and host phylogeny. Remarkably, despite different insect species nurturing on the same plant, they harbour distinct bacterial communities yet

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exhibit consistent functional capabilities among these symbionts. These crucial symbiotic partnerships are pivotal in aiding hosts in extracting nutrients from toxic plants. Our findings deepen our understanding of the dynamic interactions among insects and bacteria, providing inferences for insect biology, ecology, and beyond. Moreover, these insights pave the way for further exploration into the practical applications of manipulating symbiotic relationships for pest management. By leveraging the functional abilities identified in these symbiotic partnerships, we may uncover innovative approaches for developing environmentally friendly strategies to control pests, contributing to more sustainable agricultural practices and ecosystem conservation efforts. Our study isn't just about insects and microbes; it's a journey into the unseen world beneath our feet, where tiny allies pave the way for a greener tomorrow.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0007485324000439.

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