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INVERTEBRATE MICROBIOLOGY

Reduced Environmental Microbial Diversity on the Cuticle and in the Galleries of a Subterranean Termite Compared to Surrounding Soil

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Abstract
Termites are intimately tied to the microbial world, as they utilize their gut microbiome for the conversion of plant cellulose into necessary nutrients. Subterranean termites must also protect themselves from the vast diversity of harmful microbes found in soil. However, not all soil microbes are harmful, such as *Streptomyces* and methanotrophic bacteria that some species of termites harbor in complex nest structures made of fecal material. The eastern subterranean termite, *Reticulitermes flavipes*, has a simple nest structure consisting of fecal lined galleries. We tested the hypothesis that *R. flavipes* maintains a select microbial community in its nests to limit the penetration of harmful soilborne pathogens and favor the growth of beneficial microbes. Using Illumina sequencing, we characterized the bacterial and fungal communities in the surrounding soil, in the nest galleries, and on the cuticle of workers. We found that the galleries provide a more beneficial microbial community than the surrounding soil. Bacterial and fungal diversity was highest in the soil, lower in the galleries, and least on the cuticle. Bacterial communities clustered together according to the substrate from which they were sampled, but this clustering was less clear in fungal communities. Most of the identified bacterial and fungal taxa were unique to one substrate, but the soil and gallery communities had very similar phylum-level taxonomic profiles. Notably, the galleries of *R. flavipes* also harbored both the potentially beneficial *Streptomyces* and the methanotrophic *Methylacidiphilales*, indicating that these microbial associations in fecal material pre-date the emergence of complex fecal nest structures. Surprisingly, several pathogenic groups were relatively abundant in the galleries and on the cuticle, suggesting that pathogens may accumulate within termite nests over time while putatively remaining at enzootic level during the lifetime of the colony.

Keywords Actinobacteria · Methanotroph · Social insect · Blattodea · Rhinotermitidae

Introduction

Within insects, the evolution of eusociality has inevitably lead to remarkable ecological success, as evidenced by the rapid diversification of social insect species and their adaptation to a variety of different habitats [1]. However, eusociality inherently brings several costs to colony life. The dense aggregation of closely related individuals could promote the spread of disease epidemics. This is especially true for social insects that live in subterranean environments, as soil is teeming with microbial life. Many general pathogens that can infect a broad range of insect hosts are found in the soil, and their impact on subterranean social insects, such as ants and termites, has been heavily studied [2–6]. There are also host-specific pathogens that have coevolved with social insects. Fungi in the *Ophiocordyceps unilateralis* species complex are able to manipulate their host ant's behavior to promote their spread throughout the colony, with each species in this group having adapted to a different species of ant [7, 8]. The nest of social insect colonies, often constructed from organic material, can also be colonized by soil-dwelling microbes [9–11]. Although many of these saprophytes are not insect pathogens, colonies must still protect themselves from microbes that can infest and consume the nest. In collapsed

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laboratory colonies of the Formosan subterranean termite, *Coptotermes formosanus*, the colony is no longer able to inhibit the growth of microbes in the nest, and, as a result, the remaining resources of the nest are consumed by a succession of saprophytic organisms [12].

Social insects have evolved numerous defenses to contend with such microbial adversity, making them remarkably resilient to disease. These defenses are known as social immunity, the collective organization of individual behaviors and physiological defenses that protect the colony from harmful pathogens [13–15]. Ants have become dominant in soil environments, in part, due to their metapleural glands, a structure that produces antimicrobial secretions [16–18]. The compounds produced by this gland allow ants to continually disinfect themselves, their nestmates, and their nest structure. Although subterranean termites do not have a metapleural gland, they may be able to similarly reinforce their nests with antimicrobial activity. Termite fecal material, which is used to construct nests, exhibits inhibitory activity on fungal growth [19]. In *C. formosanus*, the carton nest built from accumulated fecal material contains potentially beneficial *Streptomyces* inoculated from surrounding soil [20]. Additionally, termites produce antifungal peptides in their salivary glands that may also be incorporated into the nest walls [21–24].

The termite nest primarily serves the vital function of housing the colony and providing fortification from external threats. Termite nesting strategies can be classified into one of three categories based on the nest's location relative to the colony's food source [25, 26]. In a one-piece nesting strategy, termites nest and feed in the same substrate. A separate-piece nesting strategy entails a central nest located in a substrate separate from the food source, best characterized by mound-building termites that must leave their nest to forage. Subterranean termites (Subfamily: Rhinotermitidae) utilize an intermediate-nesting strategy where, like one-piece nesting species, the colony is housed in a food source, but will then forage through the soil for additional food.

The eastern subterranean termite, *Reticulitermes flavipes*, implements an intermediate-nesting strategy, and the nest walls of this species are known to harbor a rich microbial community, although the taxonomic identity of this community remains unknown [11]. If *R. flavipes* can selectively maintain the microbial communities in their nests, they may also be able to limit the penetration of harmful soilborne pathogens. We tested the hypothesis that the fecal material lining the galleries can serve as a selective medium that buffers pathogens while favoring the growth of beneficial microbes. To do this, we characterized the bacterial and fungal communities of the soil surrounding colonies, the substrate lining foraging galleries, and the cuticle of the termites themselves. We examined diversity patterns in these three substrates (i.e., soil, gallery, and cuticle) and taxonomically identified the bacteria and fungi in the communities we sampled. Then, we compared the relative abundance of species within targeted genera of interest among soil, gallery, and termite cuticle samples.

Methods

Soil, Gallery, and Termite Collections

Sampling was performed at the Sam Houston State University Center for Biological Field Studies, a heavily forested site within the Sam Houston National Forest (Huntsville, TX, USA). Samples were taken from 10 collection points that were separated from each other by at least 15 m. Based on previous studies, this was a sufficient distance to ensure that termites collected at each point represented distinct colonies [27–29]. At each collection point, wood debris was opened and inspected for termite activity. In order to sample microbial communities on the cuticle of the termites, 20 workers and one soldier were collected into vials using forceps. Gallery substrate (a mixture of soil, feces, and wood particles lining the foraging galleries) was then scraped into sampling bags. Finally, a 10-cm-deep soil sample was collected from directly beneath the wood debris that contained termites. All samples were collected in triplicate using sterilized tools, stored on ice, and brought back to the lab for immediate processing. In the lab, each sampled group of termites was washed for 15 min by gentle rotation in 500 μL of 0.1% TWEEN®80 (Sigma-Aldrich Chemie N.V., the Netherlands) diluted in ddH2O. The solution was decanted out and used for DNA extraction.

DNA Extraction, Barcode Amplification, and Sequencing

All sampled colonies were confirmed as *R. flavipes* by extracting DNA of one worker from each collection point and sequencing its mitochondrial 16S rRNA gene (Supplementary Information S1). Then, DNA was extracted from cuticle washes using a modified phenol:chloroform DNA extraction protocol (Supplementary Information S1). Gallery and soil samples were each homogenized by shaking, and DNA was extracted from 0.25 g of each substrate using the DNeasy PowerSoil Kit (Qiagen). To identify bacterial communities present in each sample, extracted DNA was amplified at the v4 hypervariable region of the 16S rRNA gene using the primers 515f (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806r (5′-GGACTACHVGGGTWTCTAAT-3′) [30]. Fungal communities were identified by amplifying the ITS2 gene region using the primers ITS3 (5′-GCATCGATGAAGAACGCAGC-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) [31] (see Supplementary Information S1 for PCR protocols). Following PCR, amplified DNA was sent to the RTSF Genomics Core at Michigan

State University for sequencing. PCR products were batch normalized using SequalPrep (Thermo Scientific). Pooled amplicons were cleaned using a 0.8X Ampure XP beads/pool ratio (Beckman Coulter). Quality was quantified using a combination of Qubit dsDNA HS (Invitrogen), Advanced Analytical Fragment Analyzer High Sensitivity DNA (Agilent), and Illumina Library Quantification qPCR assays (KAPA). Each pool was loaded onto an Illumina MiSeq Standard v2 flow cell (Illumina, San Diego, CA, USA) and sequenced in a 2 × 250 bp paired-end format using a MiSeq v2 500 cycle reagent cartridge. Base calling was performed by Illumina Real Time Analysis v1.18.54, and the output was demultiplexed and converted to FastQ format with Illumina Bcl2fastq v2.19.1.

Data Analysis

All analyses were performed using the microbiome bioinformatics platform QIIME 2 [32]. Paired-end reads were filtered for quality control and merged using the DADA2 pipeline [33]. With a target median quality score of 30, 16S and ITS sequences were each joined at 250 bp and then identified as amplicon sequence variants (ASVs). Processing the 16S sequence data produced 32,804 ASVs from 5,592,120 reads across 90 samples. Based on a rarefaction curve of observed operational taxonomic units (OTUs) (Supplementary Information S2), a sampling depth of 19,000 reads was determined to provide sufficient coverage to estimate the diversity of the bacterial communities in soil, gallery, and cuticle samples. Ten samples with low coverage were removed from the analysis to achieve this sequencing depth ($N = 80$). For fungal community analysis, 5803 ASVs were identified from 4,329,649 across 90 samples with the ITS sequences. The rarefaction curve of fungal observed OTUs (Supplementary Information S2) indicated that a sampling depth of 10,000 reads provided sufficient coverage to estimate fungal diversity. Two samples with low coverage were removed from the analysis to achieve this sequencing depth ($N = 88$).

Phylogenetic trees were internally constructed for ASVs using MAFFT FastTree implemented in QIIME 2 [34] in order to estimate diversity within samples. Different metrics using different approaches were calculated to provide a comprehensive picture of the diversity within samples (i.e., alpha diversity): observed OTUs, ENS/PIE, Faith's phylogenetic diversity, Simpson diversity, and Shannon diversity. In order to estimate diversity between samples (i.e., beta diversity), weighted and unweighted UniFrac distances were calculated between each sample and plotted using a PCoA [35].

ASVs from all samples ($N = 90$) were taxonomically identified using classifiers trained on either the 16S rRNA gene or ITS region. The 16S ASVs were classified using the SILVA v132 database with 99% sequence identity from the 515F/806R sequence region [36]. The ITS ASVs were classified using the UNITE fungal database classifier trained on ITS sequences [37]. A Venn diagram was constructed showing the number of classified ASVs that were unique or shared among substrates. For each sample, barplots representing the taxonomic profile of bacteria and fungi classified at the phylum-level were created, as this taxonomic level was the most informative for identifying differences between the three substrates. Heatmaps were created to show the relative abundance of species of interest.

Results

Diversity

Bacterial communities assessed from 16S rRNA gene sequences showed significant differences in all alpha diversity metrics among substrates (Fig. 1a). Diversity was highest in the soil, lower in the galleries, and least on the cuticle. Fungal communities estimated from ITS gene sequences followed a similar pattern only when measuring observed OTUs and Faith's phylogenetic diversity (Fig. 1b). No significant differences in effective number of species/probability of intra- or interspecific encounter (ENS/PIE), Shannon diversity, or Simpson diversity were found for fungal communities separated by substrate. To compare community similarity across samples, a principal coordinates analysis (PCoA) was used to plot unweighted and weighted UniFrac distances. Bacterial communities were largely separated according to the substrate from which they were sampled (Fig. 2a); in most cases, the samples from the gallery clustered between those from the soil and the cuticle. However, this pattern was less clear in fungal communities (Fig. 2b). When plotting unweighted UniFrac values, soil and cuticle samples separated, but gallery samples did not. In the plot of weighted UniFrac scores, which accounts for abundance, there was no separation by substrate.

Taxonomic Analysis

ASVs were taxonomically identified using classifiers trained on bacterial and fungal databases. We found a majority of these identified taxa (bacteria, 83.19%; fungi, 85.47%) to be exclusive to a single substrate (Fig. 3, Supplementary Information S3). Of the taxa shared between two substrates, the highest numbers were those shared between soil and gallery (bacteria, 9.39%; fungi, 5.82%), and the lowest were those shared between soil and cuticle (bacteria, 0.53%; fungi, 1.36%). Barplots of bacterial phyla diversity show that the communities sampled from the cuticle are distinct from soil and gallery samples, largely due to the ~ 50% increase in relative abundance of *Spirochaetes* (Fig. 4a). However, while the phylum-level taxonomic profiles of soil and gallery samples are similar, one notable difference is the greater

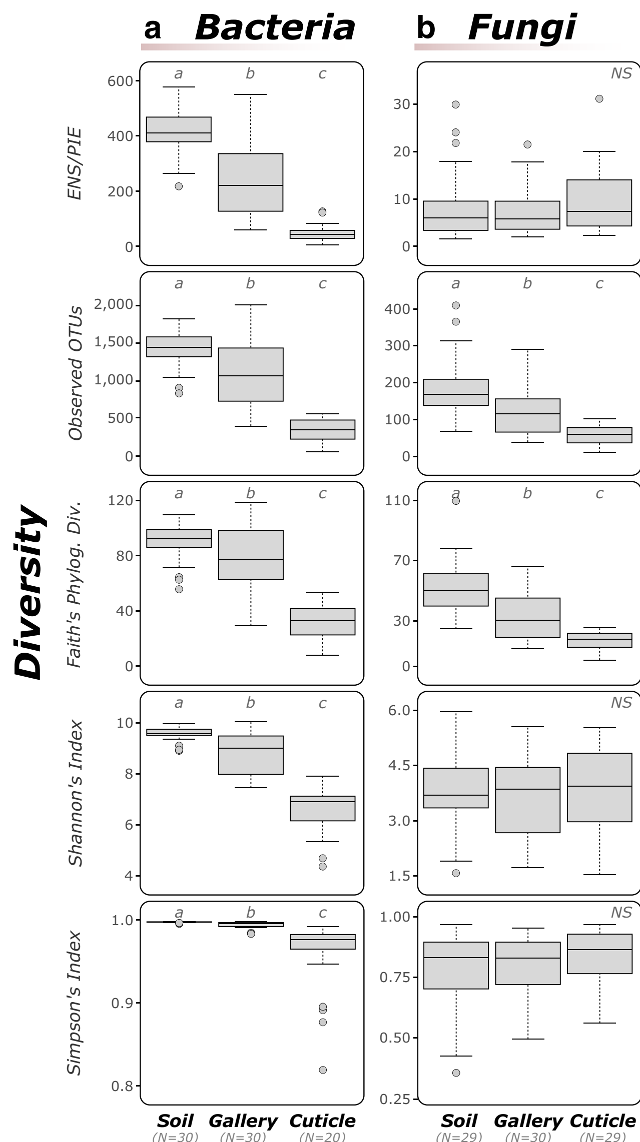

Fig. 1 Alpha diversity metrics for bacterial and fungal communities in *R. flavipes* colonies. Boxplots show values for effective number of species/probability of intra- or interspecific encounter (ENS/PIE), observed OTUs, Faith's phylogenetic diversity, Shannon diversity, and Simpson diversity in bacterial (**a**) and fungal (**b**) communities, separated by substrate. Letters denote significance ($p < 0.001$)

proportion of *Spirochaetes* in the galleries. In fungal communities, phyla diversity is largely dominated by high proportions (> 60%) of *Basidiomycota* in each sample (Fig. 4b). However, cuticle samples are distinct from the soil and galleries in that most cuticle communities have higher proportions of other phyla, including more unassigned or unidentified fungi. Patterns in phyla diversity may not reflect the changes that occur in microbes that are in low abundance, but are of ecological importance. Heatmaps were generated to examine the relative abundance of bacterial and fungal species identified in genera of interest (*Streptomyces*, methanotrophic bacteria, and pathogens). Of the three *Streptomyces* species identified, the most diversity and highest relative abundance was found in the gallery communities (Fig. 5a). The two methanotrophic groups identified were *Methylomonas* and an uncultured strain of *Methylacidiphilales* (Fig. 5b). *Methylacidiphilales* was the only reported methanotrophic microbe found in the gallery substrate, and it was found in relatively high abundance. *Methylomonas* was present in low abundance in soil and cuticle samples but was absent from the gallery samples.

A high degree of variation among the relative abundance patterns of potentially harmful microbes was identified (Fig. 5c). Some of these genera have been directly investigated as termite pathogens (e.g., *Bacillus*, *Pseudomonas*, *Serratia*, *Aspergillus*, and *Metarhizium* [6]), while others are known entomopathogens (e.g., *Metacordyceps chlamydosporia* and *Lecanicillium antillanum* [5, 38]). Interestingly, only five pathogenic fungi were found on the cuticle, whereas nine were found in the gallery and 12 in the soil (Fig. 5c). The fungal genus *Trichoderma* was found in all three substrates [12]. The unclassified *Bacillus* sp. and *Trichoderma hamatum* had high relative abundance in the soil that decreased in gallery and cuticle samples. *Lecanicillium antillanum* and the unclassified *Serratia* sp. were present in highest proportions on termite cuticle, while unclassified *Pseudomonas* sp. was relatively abundant in all three substrates. There were many differences in relative abundance among the classified species of *Aspergillus*. However, the cuticle communities harbored high proportions of the unclassified *Aspergillus* sp. All *Metarhizium* taxa identified were found in the soil, but varied in relative abundance in the galleries and on the cuticles. Interestingly, *Metarhizium robertsii* was present in all three substrates, but showed a lowest relative abundance in the galleries.

Discussion

We characterized bacterial and fungal communities associated with *R. flavipes* in the surrounding soil, the gallery material and the surface of the insect cuticle. We found a significant reduction in bacterial and fungal diversity in the galleries and on the cuticle of workers, with the communities in the galleries being intermediate between those in the soil and the cuticle. The reduction in microbial diversity on termite cuticle probably results from allogrooming, an immune behavior by which nestmate workers remove microbes from each other [15, 39, 40]. The reduction observed in gallery material is likely due to nest hygiene behaviors through the deposition of antimicrobial substances in the fecal material lining nest chambers and galleries, a phenomenon that has also been suggested in *C. formosanus* [19, 20]. We found that beneficial microbes associated with complex nest structures in other termite species were also harbored in *R. flavipes* foraging galleries. These

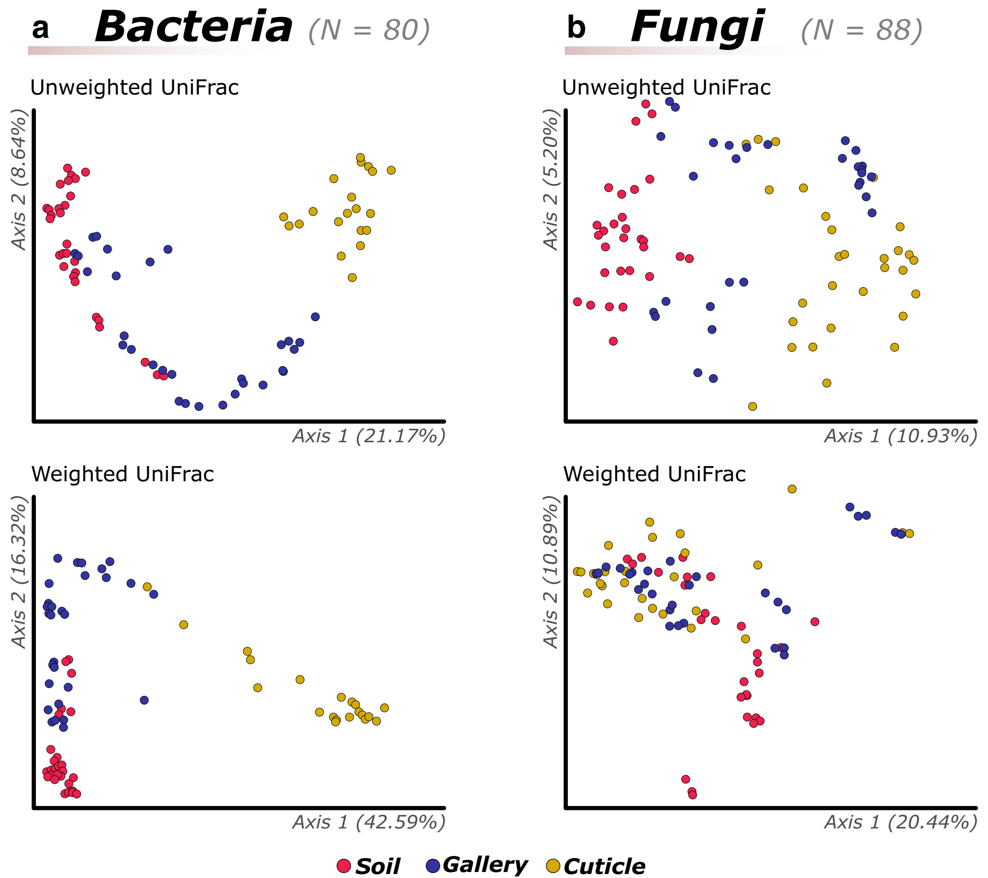

Fig. 2 Bacterial and fungal community similarity in *R. flavipes* colonies. Principal coordinates analysis (PCoA) of UniFrac distances for bacterial (**a**) and fungal (**b**) communities. Each community is colored according to whether it was sampled from the soil, galleries, or cuticle

results support the hypothesis that the fecal material lining termite galleries helps protect the colony by selectively limiting pathogens while favoring the growth of beneficial microbes. These findings in the simple nest structure of *R. flavipes* suggest that microbial associations with termite fecal material predate the origin of complex nest structures.

Our results show that the bacterial communities differ according to the substrate from which they were collected. However, this pattern was less clear for fungal communities, which suggests that *R. flavipes* colonies are less likely to harbor specific fungal associations in their nest and may have less control over the exclusion of harmful fungi. This absence of association may also suggest that some fungi remain dormant and undetectable, potentially allowing them to accumulate over time. Notably, known entomopathogenic microbes were found in various abundances in the galleries and on the cuticle, where they were potentially maintained at enzootic levels, only reaching pathogenic levels when the colony weakens.

Termites are characterized by their many intimate interactions with microbes. This is best studied in the termite gut, where obligate symbioses allow them to feed on difficult to digest food sources [41, 42]. In some species of termites, these

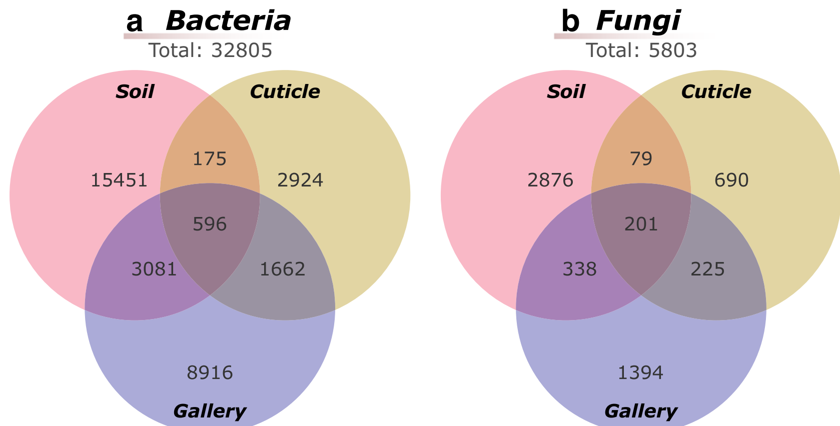

Fig. 3 Venn diagram of classified bacterial and fungal ASVs in *R. flavipes* colonies. Venn diagram depicts the number of bacterial and fungal ASVs that were unique to a substrate (soil, gallery, or cuticle) or were shared between substrates

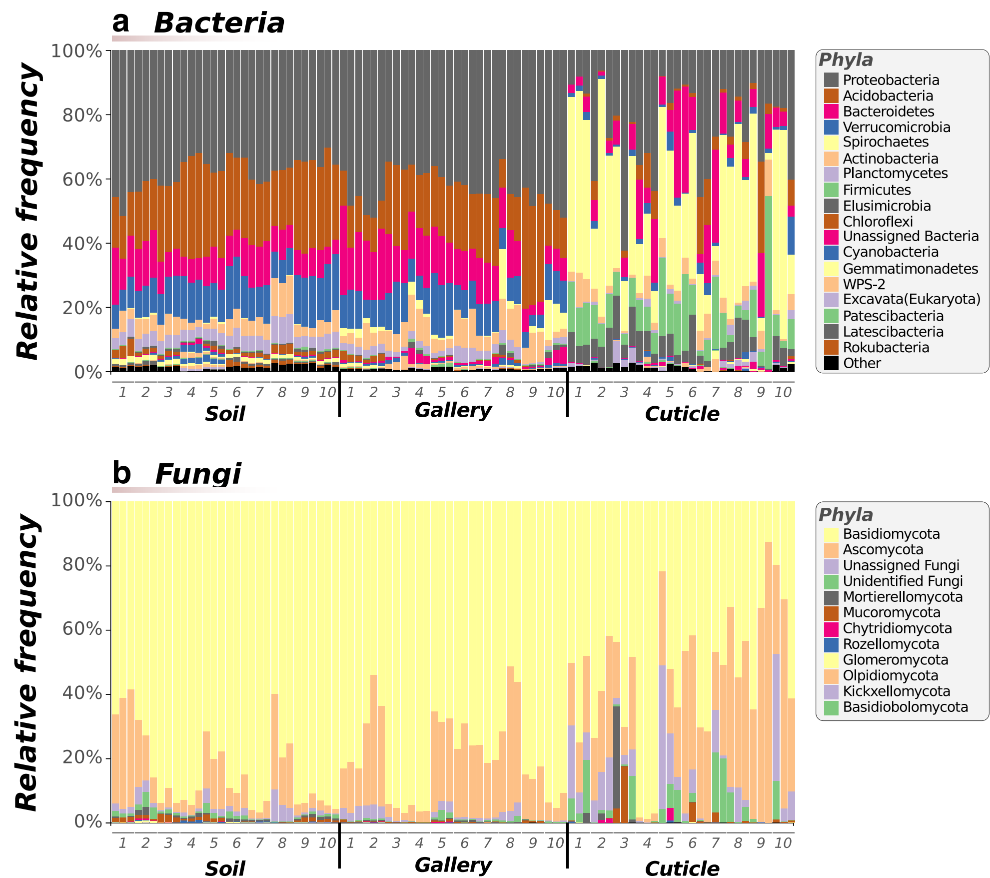

Fig. 4 Relative frequency of bacterial and fungal phyla in *R. flavipes* colonies. Phylum-level taxonomic profile of bacterial (**a**) and fungal (**b**) communities in each sample. Phyla identified from kingdoms other than bacteria and fungi are noted as such

symbiotic digestive processes have been externalized such that colonies obtain their food from cultivated fungal gardens. Fungal cultivation in termites requires them not only to obtain their cultivar from the environment but also to maintain their garden combs by weeding out unwanted microbes [43, 44]. The beneficial associations with *Actinobacteria,* a bacterial phylum known for its antimicrobial properties [45, 46], has been shown to prevent the spread of harmful microbes in the combs of these fungus-growing termites [47–49]. Interestingly, similar associations with beneficial microbes for the inhibition of garden parasites have been reported in fungus-growing ant species [50, 51]. In fungus-growing termites, the fungal comb is constructed from termite feces and likely evolved from a structure similar to the carton nest in *C. formosanus*, which is not used to cultivate fungi [9, 26, 52]. The carton of *C. formosanus* has also been suggested to enhance colony homeostasis through the presence of beneficial *Actinobacteria* [20, 53]. Interestingly, *Actinobacteria* were reported in the gut of *R. flavipes,* suggesting that they could ultimately end up on nest galleries [54]. In this study, we identified the presence of *Streptomyces* bacteria in *R. flavipes.* This bacterial genus is also harbored in carton material of *C. formosanus* and provides additional disease resistance against *Metarhizium* fungi [20, 55]. In this species, *Streptomyces* is inoculated from surrounding soil into the fecal material used to build the carton nest, rather than from the termites themselves [55]. Our results suggest a similar finding in *R. flavipes*, as the soil harbored a higher relative abundance of *Streptomyces* than the cuticle.

Termites can also passively alter the microbial communities in the soil around them, as a result of the organic material produced by a colony [10]. Many termite species produce methane through the digestive processes in their gut [56, 57], and the high numbers of individuals in a colony once led researchers to believe that termites could be significant contributors to the global methane budget [58, 59]. However, this assumption has been disproved due to the presence of methanotrophic archaea and bacteria oxidizing the methane produced by the colony [59]. These methanotrophs are harbored in the large mound structure that houses the colonies of many termite species, but their taxonomic identity remains unknown [59]. In this study, we identified the methanotrophic bacterial groups *Methylacidiphilales* and *Methylomonas* present in *R. flavipes* colonies. *Methylacidiphilales* was found in all substrates, with a highest concentration in the galleries. In contrast, *Methylomonas* was found only in the soil and on the cuticle of the termites. Similar to *Streptomyces,* associations with methanotrophic bacteria have previously been reported in the carton nest of *C. formosanus* [20]. *Coptotermes* and *Reticulitermes* are

closely related genera within the termite phylogeny [60, 61]. The fecal material lining the galleries of *R. flavipes* is homologous to the fecal material used to build the carton nests of *Coptotermes*. The presence of similar microbial associations found in the amorphous galleries of *R. flavipes* supports the idea that the fecal material of both species have a similar function, regardless of its diffuse use within galleries or its accumulation into a complex structure. It also suggests that such microbial associations present in fecal material are ancient in termites [60, 61]. Overall, considering the examples of *Streptomyces* in carton material and methanotrophs in termite mounds, it is clear that the termite nest serves a pivotal role in microbial interactions.

We compared the relative abundance of known termite pathogens across the three substrates and found considerable variation between different groups of pathogens. Notably, although these genera have been associated with termite health, they can actually be quite versatile in ecological function and may not be obligate entomopathogens [5]. *Metarhizium* has received the most attention as a termite pathogen and has been repeatedly associated with termites where it has likely been an important selective agent, although its actual ecological relevance to termites has been questioned [6, 62]. All *Metarhizium* species we identified had lower relative abundance in the galleries, compared to the soil, suggesting that growth may be inhibited in the galleries. Similar reductions in relative abundance were also observed in the genera *Bacillus*

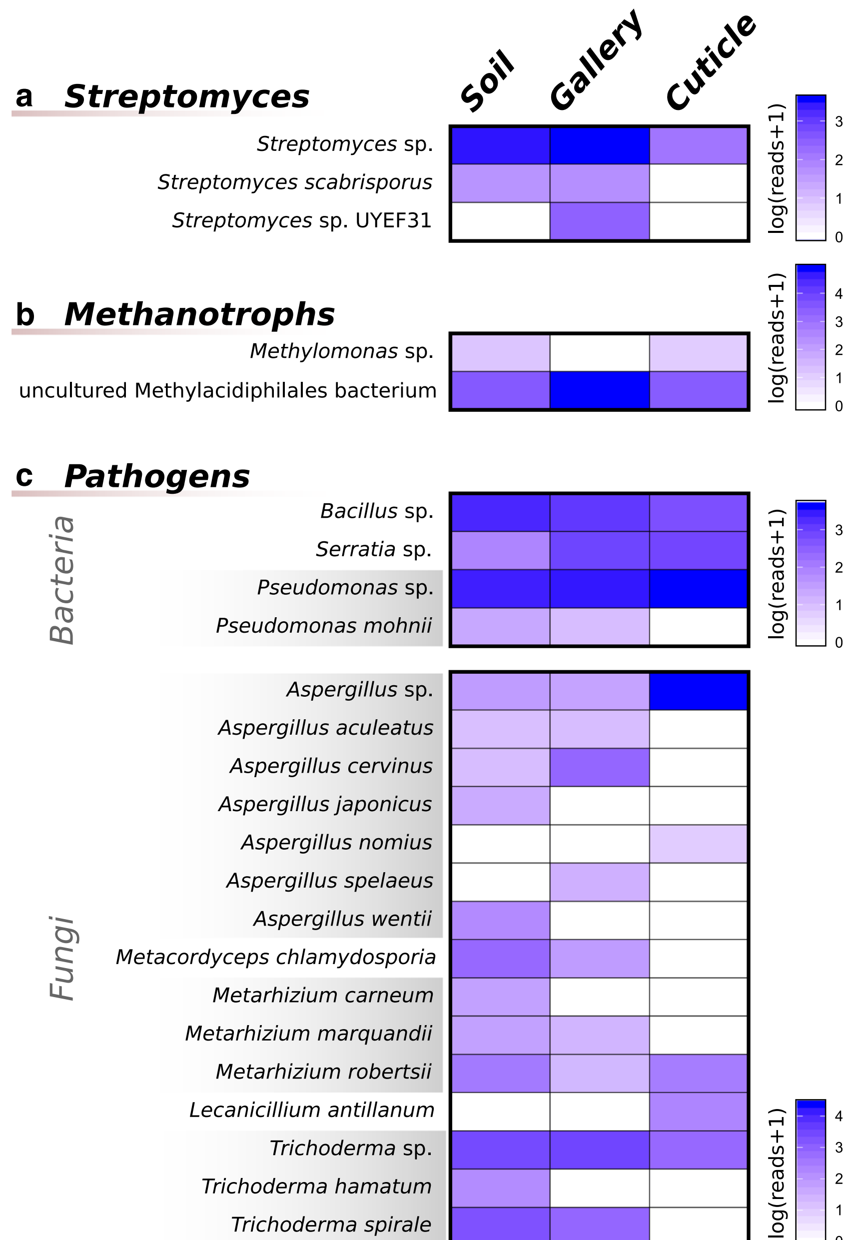

Fig. 5 Relative abundance of bacterial and fungal species of interest. Heatmaps depict the relative abundance across substrates for *Streptomyces* species (**a**), methanotrophic bacteria (**b**), and pathogenic microbes (**c**). Heatmap scales are based on the log value of the number of reads attributed to each taxon. Pathogenic microbes from the same genus are grouped together in shaded areas. Taxa that have only been identified to genus level could not be classified further using the SILVA or UNITE databases

and *Trichoderma*. The fungal genus *Trichoderma* does not directly infect termites, but it can colonize their nests [12]. This reduction could be due to the antimicrobial effects of termite feces and salivary gland secretions that are incorporated into the galleries, or the presence of *Streptomyces* in the galleries mentioned above [19, 20, 23]. Interestingly, *Metarhizium robertsii*, *Lecanicillium antillanum*, and the unclassified *Pseudomonas* sp., *Serratia* sp., and *Aspergillus* sp. had the highest relative abundance on the cuticle, indicating that some pathogens are able to persist in the nest. Unfortunately, as the samples used in this study were collected from mature colonies in the field, we are unable to determine whether factors such as colony age affect the presence of pathogens in the nest, notably those present in higher relative abundance on the cuticle compared to the galleries. One possible explanation is that although some pathogens are able to penetrate the nest, the routine maintenance of a healthy colony prevents them from developing to a propagative stage [12]. Termites have been shown to maintain strong immune defenses due to their social behavior [63], but as termite colonies decline in health from stress or age, epidemics are more likely to occur [12, 64]. Phoretic mites are also commonly found in termite colonies, but do not appear to be detrimental until the entire colony begins to decline [65, 66]. In this study, we did not attempt to identify other soilborne threats to termites, such as mites or nematodes, which may influence the overall health of the colony and therefore the relative abundance of additional harmful microbes. Thus, the high diversity of bacteria and fungi identified do not represent all of the potentially harmful organisms in the soil that subterranean termite colonies actually face [4, 66]. A similar example is seen in *R. speratus* where a “termite-ball” fungus is able to mimic termite eggs and trick workers into bringing it into the nest [67–69]. However, this fungus cannot germinate until the termites’ nest has been vacated [70]. Termite colonies are resistant to disease, but pathogens may accumulate in the nest over time.

Surprisingly, little is known about the microbial pressure that termites naturally face. By using metagenomics, we are beginning to understand the complex interactions between subterranean termites and soil microbiota. Subterranean termite colonies can live several years, experiencing the seasonality of their environment while experiencing large differences in colony size as the colony ages. This study supports the notion that the general microbiome within the fecal nest material may buffer environmental changes, maintaining homeostatic conditions within their nests [71]. While most of the focus on termite-microbe interaction has historically been on their gut, there is still much to learn about how termite colonies interact with microbes in their immediate nest environment. Understanding how microbiota-termite interactions change throughout the lifespan of a colony may elucidate the factors that contribute to natural colony collapse and provide novel targets for future termite management strategies.

Conclusions

We examined the bacterial and fungal communities in colonies of *R. flavipes* and found that within a highly diverse soil environment, colonies protect themselves through hygienic behavior that significantly reduces the diversity of microbial communities in the nest and on the cuticle. Although associations with *Streptomyces* and methanotrophic bacteria have previously been known only from specialized nest structures in other termite species, we found that these groups were harbored in *R. flavipes* foraging galleries. Several pathogens were relatively abundant on the termite cuticle, suggesting that colonies accumulate pathogen loads as they age. However, future work in this area is needed to fully understand the threshold of when a colony finally succumbs to disease.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00248-020-01664-w.

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Authors’ Contributions CMA, PAE, TLC, and ELV designed the study. CMA collected and analyzed the data. CMA, PAE, TLC, and ELV wrote the manuscript.

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Data Availability Metadata and raw reads of 16S rRNA gene and ITS gene amplicons have been deposited in the Open Science Framework database, https://osf.io (https://doi.org/10.17605/OSF.IO/4CSN2).

Compliance with Ethical Standards

Competing Interests The authors declare that they have no competing interests.

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