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# The microbiome of the ant-built home: the microbial communities of a tropical arboreal ant and its nest

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**Abstract.** Microbial life is ubiquitous, yet we are just beginning to understand how microbial communities are assembled. We test whether relationships between ant microbiomes and their environments resemble patterns identified in the human home microbiome. We examine the microbial communities and chemical composition of ants, their waste, their nest, and the surrounding soil. We predicted that the microbiome of the canopy ant, *Azteca trigona*, like that of humans, represents a distinct, relatively invariant, community compared to the soil community. Because *Azteca* build aboveground nests constructed from ant exudates mixed with chewed plant fibers, we predicted that nest-associated microorganisms should reflect their ants, not the surrounding environment. The ant microbiome was distinct from the soil, but contrary to initial predictions, ant microbiomes varied dramatically across colonies. This variation was largely driven by the relative abundance of *Lactobacillus*, a genus frequently associated with hymenopteran diets. Despite the origin of nests and their means of construction, nest-associated microorganisms were most similar to the surrounding soil. The microbiota of *Azteca* ants is thus distinct, but dimorphic across colonies, for reasons likely due to inter-colony differences in diet; microbiotas of the nests however mirror the surrounding soil community, similar to patterns of human home microbiota.

**Key words:** *Azteca trigona;* bacteria; built environment; *Lactobacillus;* microbial ecology; microbiome.

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## INTRODUCTION

Microbes are present in nearly every location on earth. Numerous studies are beginning to identify some of the rules by which microbial communities are assembled and vary geographically (such as the role of pH in microbial distribution [Fierer and Jackson 2006] or the high geographic endemism in fungal communities [Grantham et al. 2015, Barberán et al. 2015*b*]). Many of these studies have focused on the interactions humans and their microbiomes have with their "built environments" (hospitals, office buildings, and homes; Kembel et al. 2012, Hewitt et al. 2012, Barberán et al. 2015*a*). These have provided insight into how the geography of abiotic factors, like climate and physical structure, dictates which microbes colonize the home's exterior (Kembel et al. 2012, Barberán et al. 2015*b*, Matulich et al. 2015). Likewise, features of the home's occupants—their number, gender, and species, along with their associated microbiomes, can influence the home's internal microbial community (Täubel et al. 2009, Lax et al. 2014,

Barberán et al. 2015*b*). Our study highlights another organism known for constructing elaborate dwellings: the ant. Like humans, ant colonies build structures to live in, produce waste, and interact in ways that produce distinct microbiomes (Wheeler 1910, Hölldobler and Wilson 1990, 2009). We propose that like studies of the microbiome of the human home, ants and their built structures are intimately connected and capable of influencing one another's microbial assemblage.

The microbiota associated with social organisms are of particular interest as their colonial lifestyle provides a high risk of disease spread (Wilson 1975). To maintain colony health, many social organisms rely on associations with mutualistic microbes (Currie et al. 1999, 2006, Koch and Schmid-Hempel 2011, Kellner et al. 2015). Microbiota can aid in nest mate recognition (Richard et al. 2007, Theis et al. 2013, Dosmann et al. 2016) or provide protection through production of antimicrobial compounds (Promnuan et al. 2009, Sen et al. 2009, Barke et al. 2010, Visser et al. 2012, Madden et al. 2013). Because of these relationships, the microbiota of social organisms and their built structures are being explored as potential sources for novel antibiotic compounds (Pelaez 2006, Bode 2009, Poulsen et al. 2011), though detailed investigations of these environments are lacking (Madden et al. 2013, Kellner et al. 2015).

The Neotropical ant, *Azteca trigona*, forms high-density populations in Panama's seasonal forests (1–5 nests every 40 m) with colonies inhabited by >200,000 ants (Adams 1994, Clay et al. 2013). *Azteca trigona* societies build and maintain large papery carton nests (0.5–4 m in length) by chewing, regurgitating, and gluing together plant fibers (Fig. 1). This process creates ample opportunity for the ant microbiome to inoculate the building material. These colonies may live up to 30 years (M. Kaspari, *personal observation*), providing generous time for nests to develop distinctive microbiomes. Fueled on a diet of sugary honeydew and insects (Longino 2007), *A. trigona* are aggressive ants, with territories spanning multiple tree crowns and a consistent work force inhabiting, patrolling, and defending the nest's exterior. Each colony produces up to 10 g of organic refuse a day, depositing it on the ground directly below the nest. This refuse mainly consists of ant waste, as well as occasional parts of carrion and nest material. The constant refuse input generates a long-term interaction between canopy and forest floor microbial communities (Clay et al. 2013).

Our study uses *Azteca trigona* societies to pose similar questions pursued by studies of the microbiome of human societies: How do the microbiomes of individual colonies differ from the waste they produce, and to what extent do the bacterial communities shape the microbial communities of the nests they inhabit? We ask do the gut-origins of the exudates used in nest construction and maintenance make nest microbiotas an extension of the ant colony, or do they maintain microbiomes more similar to the surrounding environment? We further test the prediction, driven by assumption that core microbiota are maintained by ants (Hu et al. 2014), that inter-colony variation in the composition of the ant microbiome and refuse community will be smaller than, yet correlated with, the variation found in the nest and soil. Finally, because microbes are often metabolic and biogeochemical specialists, we explore how the chemical composition varies among the ants, their refuse, nest, and soil. Through these questions, we aim to shed light on how the microbiome of a species interacts with and is shaped by the surrounding environment.

## Materials and Methods

All samples for this study were collected during July 2014 in the Barro Colorado National Monument (BCNM), Panama. BCNM consists of Barro Colorado Island (BCI) and the surround mainland Gigante Peninsula. BCNM is a seasonally wet tropical forest that receives ~2600 mm of rain annually, with the majority of rain falling from mid-April to mid-December (Wieder and Wright 1995).

### *Field samples*

For this study, we located 10 nests along the Edwin Willis trail on the Gigante Peninsula and 10 nests along the Thomas Barbour trail on BCI. Studied nest had no host tree specificity and ranged in size from 0.5 to 3.5 m. We selected nests within 2 m from the ground to aid in sampling. Refuse collection buckets were placed below each nest to collect refuse before it could be inoculated with soil microbial communities,

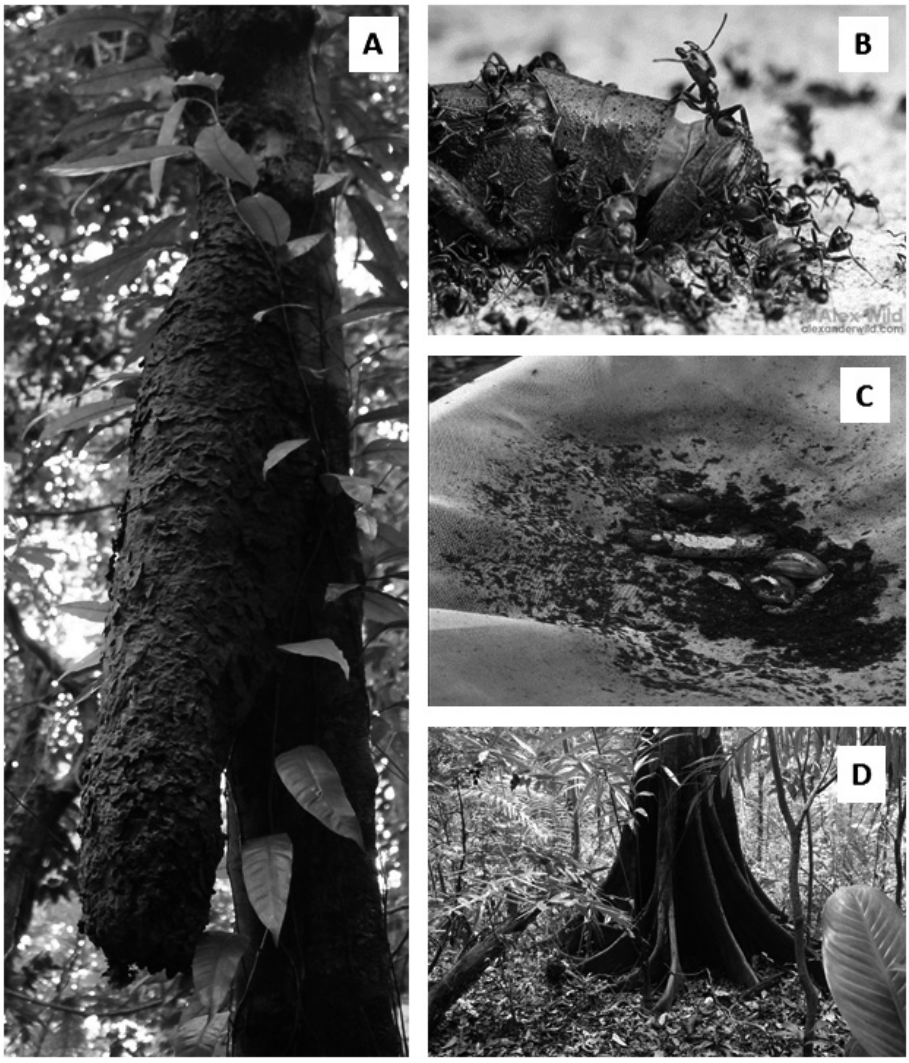


Fig. 1. Photos of (A) *Azteca trigona* nest, (B) ant, (C) refuse in collection bucket, and (D) environmental landscape. Photos A, C, and D were taken by Jane Lucas. Photo B was taken by Shannon Hartman (www.antweb.org).

as described in Clay et al. (2013). Due to the close proximity to the forest floor, collection buckets capture >90% of the refuse fall. Each nest was given 5 days to allow for adequate refuse accumulation before sampling.

Microbial reference samples were taken from each colony's ants, refuse, nest, and surrounding soil. Hydrogen peroxide- and ethanol-sterilized forceps were used to collect each sample. Roughly 20–30 ants (0.5 g total) were collected from the outside of the nest to ensure that workers from the same colony were being examined. Ants were surface-sterilized with a 95% ethanol wash but not dissected (Kautz et al. 2012). However, we acknowledge that a 95% ethanol wash may not be a fully sufficient way of eliminating surface bacteria (Moreau 2012), and therefore, microbial ant samples represent entire ant microbiomes. Nest samples consisted of a 0.5 g piece of nest material taken from the external portion of the nest. Nest portions sampled were located at least 50 cm away from the bottom of the nest to avoid potential contamination with refuse material. For refuse samples, we collected 0.5 g of refuse from collection buckets (Clay et al. 2013). Finally, we took 0.5 g soil samples from locations 0.5 m away from directly below the center of nests. Due to collection buckets collecting the majority of refuse,

and the distinct coloration difference between blackened refuse and red soils, we are confident that samples taken 0.5 m away from nests were not contaminated by falling refuse.

### *Microbial community analysis*

All samples were placed in sterile 1.5-mL tubes containing 750 mL of Zymo's Xpedition Lysis/Stabilization solution and bashing beads. Within 2 h of sampling, all samples were ground and homogenized by bead-beating tubes at 10,000 g for 10 min using the Vortex-Genie tube adaptor (Scientific Industries, Inc., Bohemia, New York, USA), after which DNA was stabilized. Preserved field samples were stored at −40°C. Immediately prior to DNA extraction, samples were re-homogenized using a BioSpec Mini-Beadbeater (BioSpec Products, Inc., Bartlesville, Oklahoma, USA) for 60 s. Total DNA was extracted according to the manufacturer's protocol (Zymo Soil/Fecal Xpedition mini kit protocol, Zymo Research Corp., Irvine, California, USA).

Libraries of small-subunit (16S) rRNA gene fragments representative of bacterial phylotypes were generated from each DNA sample using the primers S-D-Arch-0519-a-S-15/S-D-Bact-0785-b-A-18 (Klindworth et al. 2012). The S-D-Arch-0519-a-S-15 primer was modified to include a 16-bp M13 sequence (GTAAAACGACGGCCAG) at the 5′ end to allow for the attachment of a unique 12-bp "barcode" in a subsequent PCR. The 50-μL PCR containing 2 μL of 1:10 diluted template DNA, 0.2 μmol/L each of forward and reverse primers, and 1 μmol/L of 5 Prime Master Mix (5 PRIME) was carried out in a Techne TC-512 Gradient Thermal Cycler (Techne Inc., Burlington, New Jersey, USA). Initial denaturation was held at 96°C for 3 min, followed by 30 cycles, each consisting of 96°C for 30 s, 52°C for 45 s, and 72°C for 45 s. The final extension was held for 10 min at 75°C. Appropriate PCR products were verified on 1% agarose gel. PCR products were purified using SPRIselect beads following the manufacturer's protocol (Beckman Coulter, Brea, California, USA).

A unique 12-bp "barcode" was attached to each library using a subsequent six-cycle PCR. Unique barcode sequences are presented in Appendix S1: Table S1. The attached forward primers consisted of a unique barcode, two spacer nucleotides, and the 16-bp adapter sequence (GTAAAACGACGGCCAG); the reverse primer was S-D-Bact-0785-b-A-18. This unique "barcode" labeling reaction was a total of 50 μL and contained 4 μL of the purified PCR product, 0.2 μmol/L each of forward and reverse primers, and 1 μmol/L of 5 PRIME. Six cycles of PCR thermal cycling were carried out in a Techne TC-512 Gradient Thermal Cycler (Techne Inc., Burlington, New Jersey, USA), as described above. The resulting products were cleaned using SPRIselect beads and quantified using the Qubit fluorometer and dsDNA HS assay kit (Life Technologies, Grand Island, New York, USA). Equimolar amounts of each uniquely barcoded PCR product were pooled and submitted for Illumina MiSeq (San Diego, California, USA) using TruSeq 250 bp PE V2 chemistry.

### *Sequence data analysis*

All 16S sequencing reads were analyzed and demultiplexed using QIIME (Caporaso et al. 2010). We removed sequencing reads that contained errors in the barcoded region, ambiguities, homopolymers (greater than six nucleotides in length), or an average quality score <25. Primer sequences were trimmed, and chimeric sequences were eliminated using USEARCH (version 6.1) and the "gold" reference database (Edgar 2010). Then sequences were clustered into de novo operational taxonomic units (OTUs) at 97% similarity. Microbial taxonomic classification was assigned via the SILVA reference database (Quast et al. 2013) using the pyNAST aligner. All raw data are available in the NCBI BioSamples databank (accession nos. SAMN04576300–SAMN04576371).

### *Chemistry analysis*

We analyzed how chemistry changes across environments by collecting additional samples (~5 g) from ants, refuse, nest, and soil. Due to the partially destructive nature of nutrient sampling, all chemistry samples were taken after microbial samples were taken; however, we were only able to obtain large enough refuse samples from 11 of the 20 nests. Ant, nest, refuse, and soil samples were air-dried and then weighed to 2 g. Samples analyzed for cations and P were extracted in Mehlich-3 solution (Mehlich 1984) with detection by ICP-OES on an Optima 2100 (PerkinElmer, Waltham, Massachusetts, USA). Total C and N were measured in 0.5 mol/L $K_2SO_4$ extracts and determined by automated colorimetry on a Lachat Quikchem 8500 (Hach Ltd, Loveland, Colorado, USA). All samples

were analyzed by the Soil Analysis Laboratory at the Smithsonian Tropical Research Institute (Panama City, Panama); detailed methods can be found in Turner and Romero (2009).

### *Statistical analysis*

Rarefaction curves were constructed from the estimated number of OTUs in each sample using observed species richness in QIIME (Hu et al. 2014). Libraries were rarefied to 3000 reads (the size of the smallest sequence library; Appendix S1: Fig. S1). Observed species richness and Chao richness were calculated in QIIME. Alpha diversity was compared among samples for each environment (i.e., ants, nest, refuse, and soil) using a one-way ANOVA.

We compared microbial communities across environmental sites using PERMANOVA in QIIME (1000 permutations). We also ran pairwise PERMANOVAs to identify differences among individual sample types and corrected for multiplicity using a Bonferroni correction. Community similarity was calculated using weighted UniFrac distance (Lozupone and Knight 2005). We used a non-metric multidimensional scaling (NMDS) ordination to visualize relationships among microbial communities within ant workers, refuse, nest walls, and soil. We used QIIME to generate NMDS coordinates and then fit environmental vectors on this ordination using the Vegan package in R v3.2.1 (Oksanen et al. 2011). Microbial community data were arcsine transformed to improve normality, and we confirmed normality both visually and with the Shapiro–Wilk test.

To examine which particular phyla were driving compositional differences, we determined differences among sample types using a Wilcoxon rank sum test and then effect size using soil as the control environment. The Wilcoxon test was performed in R (v3.2.1), and the effect size was calculated (Cohen's $d$ [1988]) on all significant microbial phyla. Effect sizes allow a standardized comparison of strong differences in the units of SDs, and we treat effect sizes of $>|1|$ as large.

## Results

A total of 1,204,544 bacterial/archaeal 16S rRNA gene sequences were retained and analyzed. Nest and soil samples averaged 58% more microbial OTUs than samples coming from ants and their refuse ($P < 0.001$; Fig. 2). Nest and refuse samples contained the highest percentage of unclassified at 5.8%, followed by soil at 4.0% and ants at 3.6%. Our rarefaction analyses (at 97% identity threshold) indicated that the majority of our samples were adequately sampled.

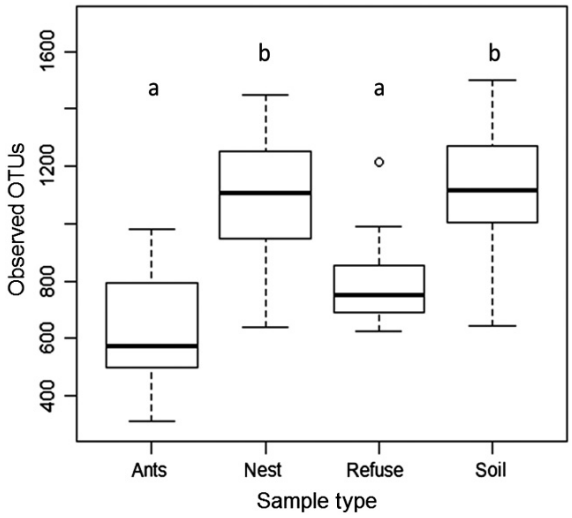


Fig. 2. Alpha diversity for each sample type calculated from observed OTUs. Letters denote significant differences between sample types identified using ANOVA. OTU, operational taxonomic units.

### *Comparing microbial composition across the four sample types*

The microbial community composition differed across all four sample types (full model: pseudo-$F = 22$, $P = 0.001$; Figs. 3 and 4; pairwise comparisons: pseudo-$F > 8$, $P < 0.001$). Contrary to predictions, the microbiome of ants varied dramatically across colonies and were more variable than refuse and nest samples ($F_{3,65} = 2.63$, $P = 0.049$; Fig. 3).

Ant microbiomes were unique in the dominance of one common order, Lactobacillales (33% ± 23%), that was bimodally distributed with >40% relative abundance in 13 of 18 colonies sampled, and <5% in the rest (Table 1). The four next most common orders were Oceanospirillales, Micrococcales, Corynebacteriales, and Rhodospirillales, which made up 5–34% of the ant worker microbiome. These orders averaged >5% relative abundance in the other sample types.

The other three sample types were distinct from each other, but lacked a dominant order such as

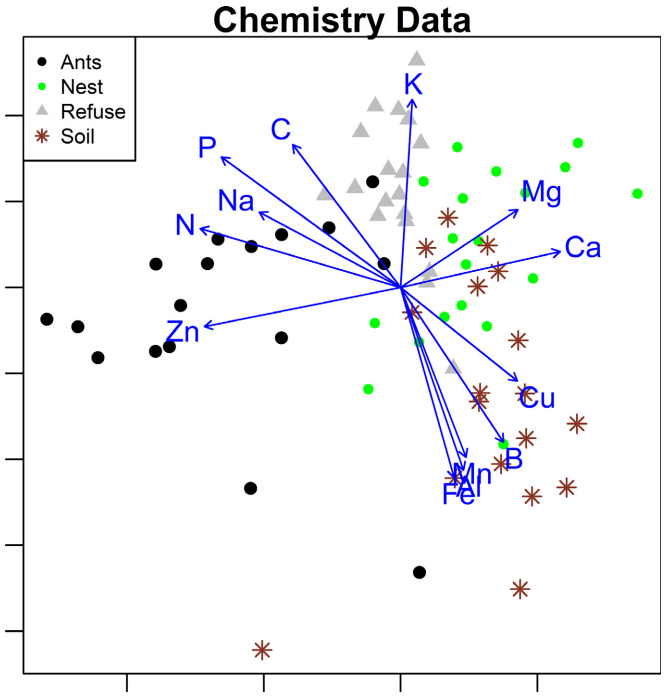


Fig. 3. NMDS representation of bacterial communities of *Azteca trigona* ants, their refuse, nest wall, and surrounding soil. Distances are based on dissimilarity matrices of sequence-based weighted UniFrac distances. Sample types differ significantly from each other (PERMANOVA: $P = 0.001$, pseudo-$F = 22.27$). Chemical composition of all nutrients was correlated with compositional trends in ordination. The strength of each correlation is proportional to the vector length ($P$ is the strongest; $r^2 = 0.60$). NMDS, non-metric multidimensional scaling.

Lactobacillales (Table 1). The five most common orders in refuse (Burkholderiales, Flavobacteriales, Sphingobacteriales, Xanthomonadales, Chromatiales) were entirely distinct from those of ants. In nests, the top five dominant orders were Sphingobacteriales, Sphingomonadales, Xanthomonadales, Rhizobiales, and Micrococcales; in the soil they were Xanthomonadales, Planctomycetales, Myxococcales, Rhizobiales, and Burkholderiales.

### *Variation in the* Azteca *microbiome and its products compared to the soil*

The 20 ant colonies we sampled were at least ~50 m apart, with the furthest distance among any pair of colonies ~5 km. This likely represented a wide variety of soil microbial communities (Barberán et al. 2015*a*). We used the soil community near each colonies as baseline against which to compare variation in the microbiomes of the *Azteca* ants, their nests, and refuse (Fig. 5). The abundance of some bacterial orders is highly correlated with a specific environment. The microbiota of ants consisted of >1 SD more OTUs of SR1 and BD1-5 (Firmicutes yielded a Cohen's $d = 0.72$, but with *Lactobacillus*, Cohen's $d = 1.7$, driving the majority of separation). Compared to soil, ant microbiomes had fewer Armatimonadetes, Planctomycetes, Gemmatimonadetes, and Verrucomicrobia. As with ants workers, ant refuse had >1 SD more SR1, as well as Deinococcus-Thermus. Refuse had fewer members of the Armatimonadetes and Planctomycetes as well as Spirochaetae, and Acidobacteria. The microbiome of ant nests was most similar to the soil but contained higher levels of Actinobacteria (Cohen's $d$: 1.27) while hosting fewer Verrucomicrobia (Cohen's $d$: −1.69), Gemmatimonadetes (Cohen's $d$: −1.36), and Planctomycetes (Cohen's $d$: −1.07).

### *Chemistry composition correlates with microbial community structure*

The biogeochemistry of the soil, ant workers, refuse, and nests was distinct, but the magnitude of these differences varied among nutrients

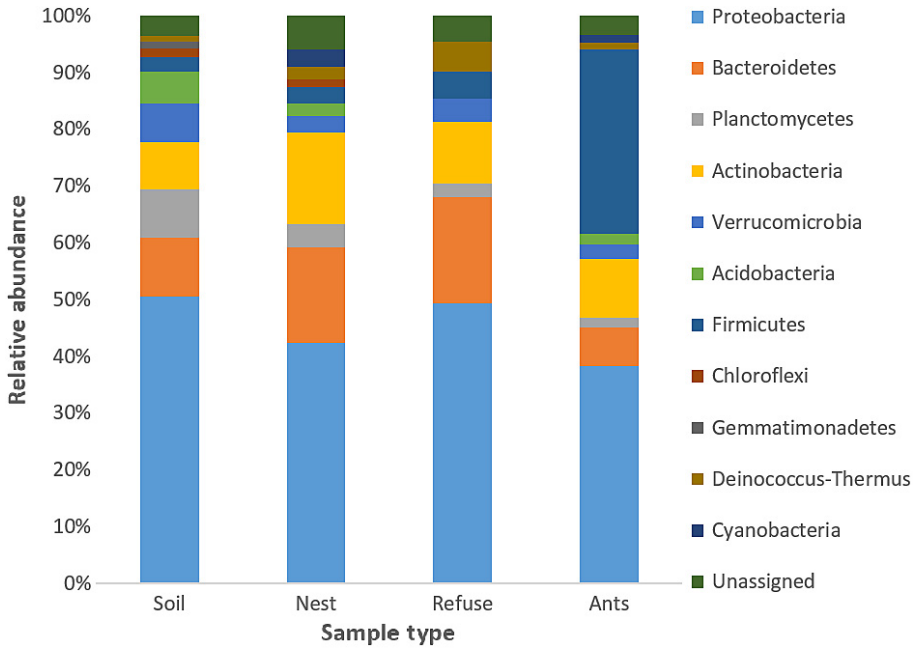


Fig. 4. Mean relative abundance of the bacterial phyla across sample types. Bacterial phyla present in >0.01% relative abundance across samples are shown.

(Table 2, Fig. 3). Nutrients that are correlated with microbial composition are displayed as vectors on the NMDS (Oksanen et al. 2013). Phosphorus had the strongest correlation with microbial community composition, while Mg had the weakest correlation (Appendix S1: Table S2). Ant works were associated with the largest concentrations of P, N, Zn and Na. Refuse concentrated K, while both ants and refuse were high in C. Soil was characterized by high Fe, Mn, B and Cu. Finally, the nests were relatively enriched in Mg, K and Ca.

## Discussion

Distinct microbial communities exist across *A. trigona* and their refuse, and these communities are separate from the surrounding nest and soil communities (Fig. 3). The distinct community present within the ant samples compared to its surrounding environment is consistent with previous studies (Ishak et al. 2011, Kellner et al. 2015) and suggests that *A. trigona* microbial communities are not a result of accidental contamination (Kellner et al. 2015). This finding supports the hypothesis that ants are capable of shaping and maintaining their microbial symbionts (Fernandez-Marin et al. 2009, Kellner et al. 2015). Refuse, a product thought to mainly consist of ant frass, has a rapid and significant shift in its microbial composition upon introduction to the environment outside the nest. This is a pattern consistent with previous analysis of the refuse piles of leaf-cutter ant (Scott et al. 2010, Ishak et al. 2011), and this distinct shift from the ant microbiome suggests that refuse may be made up of a greater variety of materials than previous thought.

### *Microbiomes of ant nests*

Despite the intimate nature in which ants build and inhabit their nests, the two are no more similar than the relationship seen between humans and the external microbiome of their homes (Barberán et al. 2015*a*). The strong correlation between nest and soil samples suggests that the surrounding environment, rather than the occupants of the nest, is the main source for microbial colonization for external structures (Barberán et al. 2015*a*). Furthermore, external portions of the nest are recycled frequently, allowing for constant resampling of the surrounding environmental community. Our results also support the hypothesis that microbial communities are specialized to their environments and can experience rapid shifts once introduced to

Table 1. Core microbiota of ant, refuse, nest wall, and surrounding soil.

| Sample type | Bacteria genera | % | Sample type | Bacteria genera | % |
|---|---|---|---|---|---|
| Ant | Lactobacillus | 30 | Refuse | Rheinheimera | 6.2 |
| | Marinobacterium | 5.1 | | Truepera | 5.1 |
| | Acinetobacter | 2.7 | | Weeksella | 3.5 |
| | Saccharibacter | 2.3 | | Acinetobacter | 3.1 |
| | Gordonia | 2.3 | | Lampropedia | 2.5 |
| | Weeksella | 2.2 | | Lactobacillus | 2.3 |
| | Sulfurimonas | 2.1 | | Gordonia | 2.2 |
| | Sandaracinaeceae uncultured | 1.4 | | Azoarcus | 2.1 |
| | Marinobacter | 1.3 | | Saprospiraceae uncultured | 1.9 |
| | Corynebacterium | 1.3 | | Comamonadaceae other | 1.8 |
| | Chthoniobacteriales uncultured | 1.3 | | Leucobacter | 1.8 |
| | Truepera | 1.2 | | Olivibacter | 1.6 |
| | Acidobacteria uncultured | 1.1 | | Pseudofulvimonas | 1.5 |
| | Proteobacteria uncultured | 1.1 | | Muricauda | 1.4 |
| | Myceligenerans | 1 | | Myceligenerans | 1.4 |
| | Unassigned | 3.3 | | Achromobacter | 1.3 |
| | | | | Olivibacter | 1.3 |
| | | | | Pseudomonas | 1.3 |
| | | | | Myceligenerans | 1.2 |
| | | | | Rhodobacteraceae other | 1 |
| | | | | Luteimonas | 1 |
| | | | | Unassigned | 5.8 |
| Nest | Sphingomonas | 3.4 | Soil | Planctomycetaceae uncultured | 2.5 |
| | Nocardioides | 3.1 | | Opitutus | 2.1 |
| | Pseudoxanthomonas | 2.5 | | Marinobacterium | 2.1 |
| | Truepera | 2.3 | | Xanthomonadales uncultured | 1.9 |
| | Olivibacter | 2 | | Planctomycetes | 1.9 |
| | Chryseobacterium | 2 | | Comamonadaceae other | 1.7 |
| | Pedobacter | 1.8 | | Lactobacillus | 1.6 |
| | Weeksella | 1.7 | | Blastocatella | 1.6 |
| | Fructobacillus | 1.6 | | Dongia | 1.4 |
| | Rhizobium | 1.4 | | Haliangium | 1.2 |
| | Sphingomonadaceae other | 1.3 | | Sorangium | 1.1 |
| | Luteimonas | 1.2 | | Xanthomonadaceae other | 1.1 |
| | Planctomycetes | 1.1 | | Diaphorobacter | 1.1 |
| | Brachybacterium | 1.1 | | Myxococcales uncultured | 1.1 |
| | Cytophagia other | 1 | | Cytophagaceae uncultured | 1 |
| | Flavobacterium | 1 | | Chitinophagaceae uncultured | 1 |
| | Unassigned | 5.8 | | Sphingomonas | 1 |
| | | | | Unassigned | 3.5 |

*Notes:* Values displayed are the percent relative abundance of bacterial genera in each sample type. Only genera present with more than 1% relative abundance are shown. (For a complete list of bacterial genera, see Data S1.)

new environmental conditions. While additional sampling of internal portions of the nests is required to confirm whether colonization patterns are similar to those of the interiors of human homes, our results suggest that microbial assembly in ant-built dwellings is comparable to those seen in human dwellings.

Nest communities had high levels (15% relative abundance) of the antimicrobial-producing group Actinomycetes. Actinomycetes are commonly found in the nests of social organisms (e.g., paper wasps, Madden et al. 2013; termites, Visser et al. 2012; bees, Promnuan et al. 2009; and ants, Sen et al. 2009, Barke et al. 2010). Social living brings an increased risk of disease spread, and many social organisms have developed relationships with antimicrobial-producing organisms to help deter infections. Previous studies have emphasized the value in examining arthropod nest structures as a source of novel antibiotic-producing

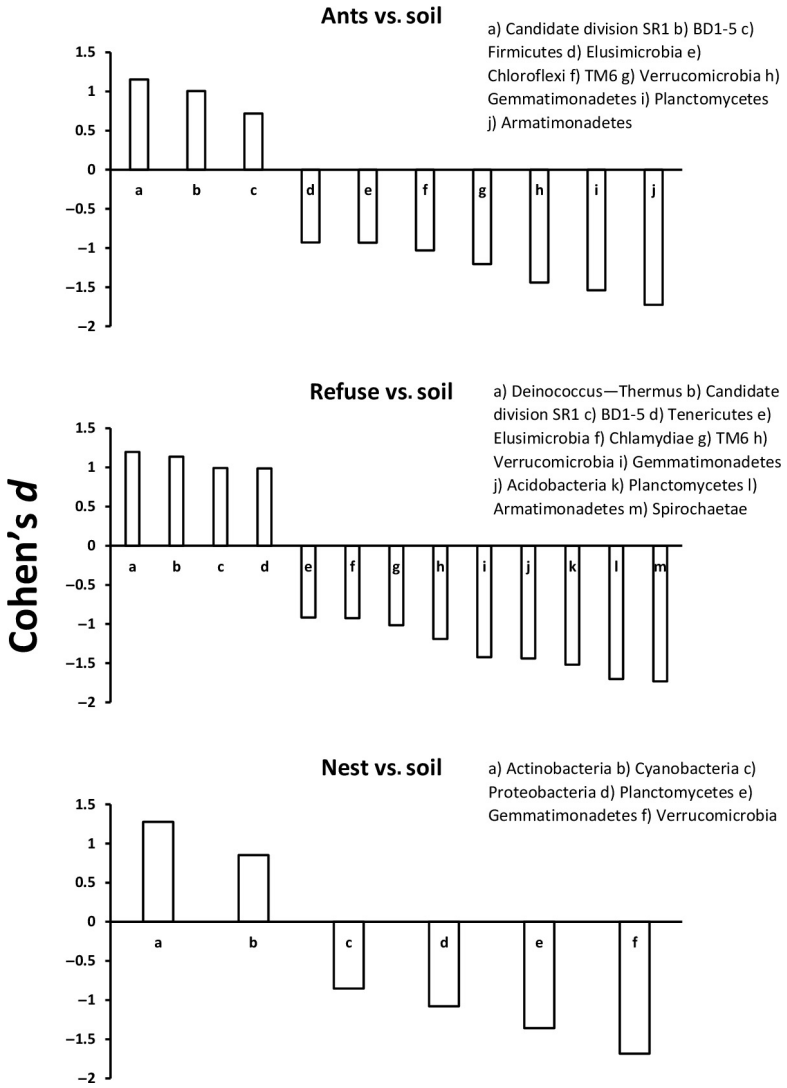


Fig. 5. Bacterial phyla that differ significantly on each sample type compared to soil samples. Only phyla with large effect sizes (Cohen's $d > \pm 0.7$) are shown. Positive values represent an increase in sample type over soil; negative values represent higher abundance on soil samples.

bacteria (Bode 2009, Poulsen et al. 2011, Madden et al. 2013). Further examination and isolation of the Actinomycete community occurring on *A. trigona* nests is required to assess its level of antimicrobial properties and potential role in nest hygiene.

### *Natural ant microbial community variability*

The *A. trigona* microbiome was not highly conserved across individual colonies. This pattern is almost entirely driven by the relative abundance of the Firmicute *Lactobacillus*. The variability of *Lactobacillus* abundance is a pattern demonstrated in multiple ant species (Hu et al. 2014, Kellner et al. 2015), with diet likely driving the variability. *Lactobacillus* facilitates the breakdown of sugars into lactic acid and is known to increase dramatically in the presence of high sugar substrates (Shamala et al. 2000). Likewise, human microbiome studies found higher ratios of Firmicutes to Bacteroidetes in obese individuals compared with lean individuals, a ratio that was adjustable through the restriction of carbohydrate intake (Ley et al. 2006).

Table 2. Average chemical concentration and the SE in ants (17), nest (18), refuse (10), and soil (18).

| Elements | Ants | Nest | Refuse | Soil |
|---|---|---|---|---|
| Al | 0.48 ± 0.1 | 4.22 ± 0.84 | 3.04 ± 1.02 | 26.72 ± 4.47 |
| B | 0 ± 0 | 0.05 ± 0 | 0.05 ± 0 | 0.11 ± 0.01 |
| % C | 49.61 ± 0.75 | 40.19 ± 0.56 | 43.28 ± 0.39 | 30.76 ± 2.86 |
| Ca | 2.4 ± 0.29 | 10.3 ± 0.68 | 9.05 ± 1.14 | 9.07 ± 1.15 |
| Cu | 0.02 ± 0 | 0.04 ± 0 | 0.06 ± 0 | 0.08 ± 0 |
| Fe | 0.53 ± 0.09 | 4.14 ± 0.95 | 3.1 ± 0.92 | 34.19 ± 6.5 |
| K | 16.84 ± 1.46 | 35.42 ± 2.76 | 35.67 ± 3.8 | 7.34 ± 1.72 |
| Mg | 1.45 ± 0.05 | 3.21 ± 0.22 | 3.48 ± 0.16 | 2.98 ± 0.4 |
| Mn | 0.15 ± 0.02 | 0.29 ± 0.07 | 0.29 ± 0.08 | 1.16 ± 0.23 |
| % N | 8.22 ± 0.22 | 2.64 ± 0.13 | 4.9 ± 0.2 | 3.19 ± 0.32 |
| Na | 1.93 ± 0.13 | 0.91 ± 0.23 | 0.59 ± 0.07 | 0.27 ± 0.04 |
| P | 7.71 ± 0.21 | 2.98 ± 0.2 | 5.24 ± 0.4 | 1.9 ± 0.37 |
| Zn | 0.19 ± 0.01 | 0.06 ± 0.01 | 0.09 ± 0.01 | 0.08 ± 0.01 |

We suggest three working hypotheses for the bimodality in the relative abundance of Lactobacillus in *Azteca* microbiomes. First, *Azteca*, like most ants, are omnivorous, harvesting both sugars directly from plants and homopteran honeydew, as well as protein from both live and dead prey (Kaspari 2000). It is possible that this bimodality in microbiomes represents bimodality among colonies in feeding habits. We are currently manipulating food sources for colonies, and extracting microbial communities from the ant gut and hind gut to determine whether diet is the main cause of variation across ant colonies. Secondly, Firmicutes, like *Lactobacillus*, are strongly associated with xylophagous insects. Because the nest-building behavior of *A. trigona* includes consumption of woody material, this behavior is another possible source of *Lactobacillus* colonization (Colman et al. 2012). Finally, high and low Lactobacillus abundance may represent cryptic species differences in this currently poorly resolved genus (Longino 2007). We are currently exploring this possibility via DNA barcoding. We do not predict host tree identity to have a strong influence over ant microbiome, due to the large territory these ants inhabit and the variety of extra-floral nectaries they feed at.

Another feature of the *Azteca* microbiome is worth noting. The exclusive presence of the genus *Saccharibacter* (a bacterium isolated from pollen [Jojima 2004]) in ant samples suggests that *A. trigona* are feeding on arboreal pollen. Ants from the arboreal genus *Cephalotes* often rely on pollen as an important source of protein and may contain special internal structures for digesting pollen (Roche and Wheeler 1977). The presence of *Saccharibacter* in *A. trigona* suggests that pollen consumption by canopy ants may be more widespread than previously predicted and that this genus may be a useful bacterial indicator for pollenophagy.

### *Ecological impacts of refuse deposition*

Nutrient-rich refuse below *A. trigona* nests can accelerate decomposition and alter the composition of the invertebrate community in the soil (Clay et al. 2013). While previous studies of refuse dumps have emphasized an enrichment in nutrients and higher fine root density (Farji-Brener and Werenkraut 2015), our results suggest that the microbial community structure of refuse can also contribute to accelerated decomposition rates and provide a favorable environment for root growth. *A. trigona* refuse contains the bacterial fertilizer Bacillus spp. (Suslow et al. 1979) and plant-growth-promoting rhizobacteria such as Pseudomonas spp., Rhizobiales spp., and Enterobacter spp. (Vessey 2003). Because refuse deposition is frequently on or close to the host tree's root system, this suggests a working hypothesis that trees hosting *A. trigona* benefit from the twin input of nutrients and beneficial bacteria. *A. trigona*, with stable, nutrient- and microbe-rich refuse piles, can provide long-term "hot spots" for diversity and productivity, and may be an important driver of habitat heterogeneity.

### *Chemical composition and microbial community correlates*

Each sample type in our study had a distinctive chemistry. Unsurprisingly, ant samples contained

the highest levels of carbon and nitrogen, essential nutrients for animal life, but also high levels of metabolically active Zn. Nest samples were high in Ca and Mg, critical elements for cell wall structure and photosynthesis, respectively, in plants (Shaul 2002, White and Broadley 2003). Refuse samples had elevated levels of K. Ants must regulate the amount of K consumed in order to maintain appropriate $Na^+/K$ levels, a task made more difficult given the abundance of K, but not Na, in plant tissue (Kaspari et al. 2009). The twofold increase of K in refuse samples compared to ants emphasizes the constant effort ants must exert to maintain proper chemical balances. While the results of our chemical and microbial analysis are strictly correlative, they provide a foundation for future work to address the relationship between chemical availability and microbial community composition.

To conclude, the composition of local soils is a good predictor of the composition of the exterior of both *Azteca* nests and human homes. Similarly, we found that ants, like humans, show a distinct but variable microbiome. Whereas in humans, some of this variation can be due to diet, location, and genetics (Shamala et al. 2000, Spor et al. 2011, Yatsunenko et al. 2012), the origins of Azteca's biomodal microbiome are still unresolved. It is intriguing, however, that the amount of sugar available to an ant colony, like a human, may be dramatically reflected in its microbiome. Quantification of diet preference and its relationship to internal microbial assemblage is thus important to discerning how microbial communities interact with and influence the surrounding environment.

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## Data Accessibility

Supporting Information accompanies this paper on the Ecosphere website. All microbial data have been uploaded and are available at NCBI's BioSamples databank (accession nos. SAMN04576300–SAMN04576371).

## Supporting Information

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.1639/full