



Ant colonies promote the diversity of soil microbial communities

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Abstract

Little is known about the role of ant colonies in regulating the distribution and diversity of soil microbial communities across large spatial scales. Here, we conducted a survey across >1000 km in eastern Australia and found that, compared with surrounding bare soils, ant colonies promoted the richness (number of phylotypes) and relative abundance of rare taxa of fungi and bacteria. Ant nests were also an important reservoir for plant pathogens. Our study also provides a portfolio of microbial phylotypes only found in ant nests, and which are associated with high nutrient availability. Together, our work highlights the fact that ant nests are an important refugia for microbial diversity.

Some ant species build massive colonies that modify soil texture, promote soil fertility, and provide refugia for plant species [1]. Recent studies have also demonstrated that ant colonies can increase the activity of soil microbial communities and modify their community composition at the scale of individual nests [2–5], but little is known about the microbiome associated with ant colonies and their nests across multiple locations and at large spatial scales. Identifying the role of these ubiquitous biotically engineered patches in driving the diversity and community composition of microbial communities is essential to better understand

the spatial distribution and history of soil microbial communities in terrestrial ecosystems. This knowledge also has important implications for the functioning of these ecosystems [6].

Because ants are central-place foragers, they accumulate a wide range of material in their nests including plant litter and invertebrates, creating unique soil environments in terms of increased soil nutrient availability and properties (e.g., pH) [7] with potential implications for the diversity and composition of soil microbial communities [8–10]. Here, we hypothesized that ant nests would increase the richness (number of phylotypes) of rare microbial species by creating nutrient hotspots and altering soil pH. Because of the expected increase in nutrient availability in ant nests than surrounding areas, we also predicted that ant nests would support unique microbial assemblies, including microbial species related to different fungal (e.g., larger fungal saprobe and plant pathogen) and bacterial (copiotrophic—e.g., Bacteroidetes—vs. oligotrophic—e.g., Acidobacteria—sensu [11]) functional groups. Ants are known to transport large amount of litter to their nests. Fungal saprobes and plant pathogens obtain resources from decayed plant litter, and therefore, would be expected to be more abundant in this microsites compared with bare soils.

To test these hypotheses, we conducted a large-scale (>1000 km) survey across 22 locations and 2 microsites (Fig. S1; ant nest and bare soil; see Supplementary Methods for details) from eastern Australia. We aimed to identify the role of nests of *Iridomyrmex purpureus*, which build large, long lived nest structures that support many hundreds of thousands of individuals (Fig. S1), in regulating the richness and identity of rare and common soil microbial communities. Our study is based on a blocked design. Put simply,

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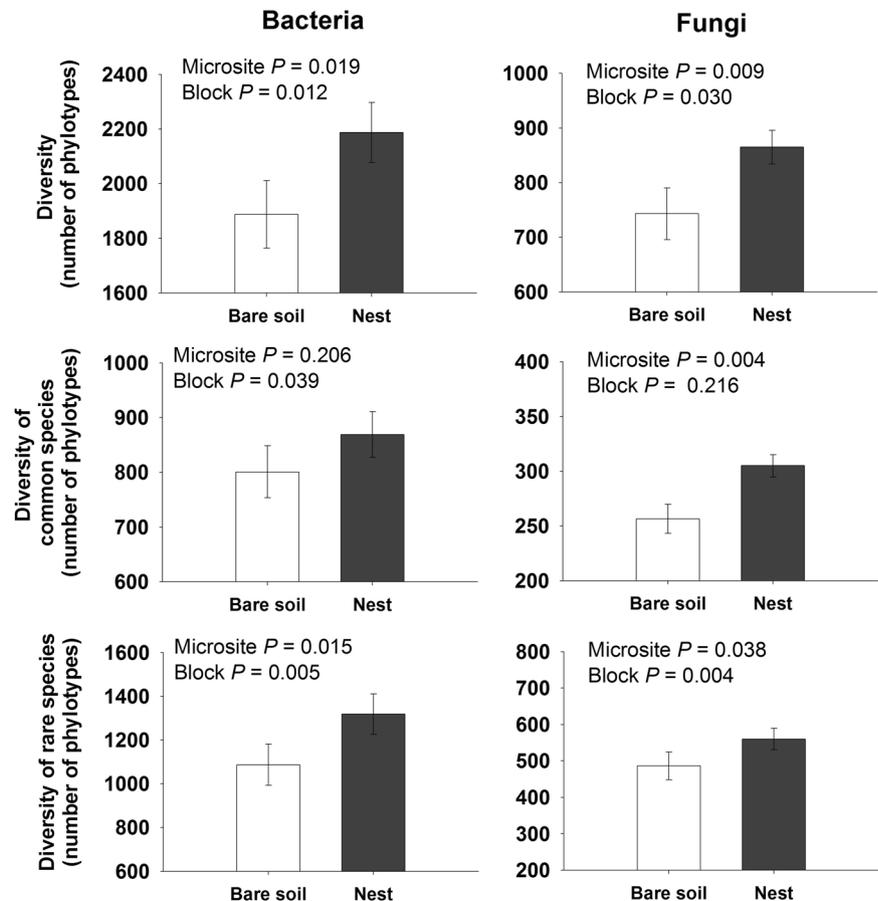
our study area was not a local single plot, but a whole region from eastern Australia (Fig. S1a) with 22 replication units. At each site (block), we established a 30 m × 30 m plot, which represented the replication unit. Composite soil samples (ten 0–5 cm deep cores) were collected from ant nest and bare soil areas within each plot. We characterized the microbial community of our soils via bacterial 16S rRNA and fungal ITS gene amplicon sequencing (Supplementary Methods). Soil nutrient availability and properties were determined as explained in the Supplementary Methods.

Ant colonies showed a larger richness (number of phylotypes; aka operational taxonomic units) of fungi and bacteria than bare soils, and were particularly important for the richness of rare bacteria and fungi, and common fungi (Fig. 1; Supplementary Methods). Some of these patterns were related to the larger nutrient availability (fungi) and pH (fungi and bacteria) found in ant nests as compared to bare soils (Fig. S2; Table 1). For example, samples with a higher pH and/or nutrient availability corresponded to a higher richness of rare bacteria and common fungi (Table 1). The importance of pH and nutrient availability as major drivers of the diversity of soil bacteria and fungi is

widely known [9, 10, 12], however, their importance in regulating the richness of rare and common species remains largely unexplored. Unlike the results for richness and community composition (Fig. S3), we did not find any significant differences in microbial biomass C and the abundance of bacteria and fungi between ant nests and bare soil (Fig. S4).

Our results further indicate that ant colonies can potentially regulate the functional groups of bacteria and fungi by increasing nutrient availability. For example, we found that the relative abundance of *Acidobacteria* (Fig. 2; Table S1), typically classified as an oligotroph and therefore preferring environments of low nutrient availability [11, 13], was negatively related to nutrient content, and had a lower relative abundance in the nests (Table 1). Conversely, the relative abundance of *Bacteroidetes* (Fig. 2; Table S1) (classified as a copiotroph), and therefore preferring high nutrient availability [11, 13], was positively related to nutrient content and showed a higher relative abundance in the nests (Table 1). Similarly, ant colonies promoted the relative abundance of fungal plant pathogens (Fig. 2; Fig. S5), typically considered as opportunistic fast-growers, which were also positively related to nutrient content

Fig. 1 Richness (mean ± SE) of bacteria and fungi in bare soil and ant nest microsites ($n = 44$). Block (location) is included in these statistical analyses to control for any effect of location on our results. The diversity of common and rare species is defined as the richness of the top 10%, and bottom 90% of phylotypes sorted by their relative abundance, respectively [22]. A more detailed description can be found in the Method section and Fig. S13



(Table 1; Table S1). Our 16S rRNA primer set was not specifically designed to evaluate changes in soil archaea (<0.09% of all 16S rRNA sequences were classified as

Table 1 Correlation (Spearman) between selected soil nutrients and pH, and selected microbial attributes.

Microbial group	Variable	pH	DIN	aP
Bacteria	Richness bacteria	.551**		.490**
	Richness rare bacteria	.623**		.523**
	Bacterial NMDS 1	-.660**	-.324**	-.555**
	Bacterial NMDS 2			
	Acidobacteria		-.378*	-.447**
	AD3			-.418**
	Bacteroidetes	.370*	.479**	.467**
	Gemmatimonadetes	.547**	.357*	.546**
	Nitrospirae	.664**		.613**
	MVP21	.366*		
	OP3			
	TM6		.312*	
	TM7		.523**	
	WPS2	-.559**		-.471**
Fungi	Richness fungi			
	Richness rare fungi			
	Richness common fungi		.373*	.304*
	Chytridiomycota	.519**	.361*	.668**
	Plant pathogens		.337*	.373*

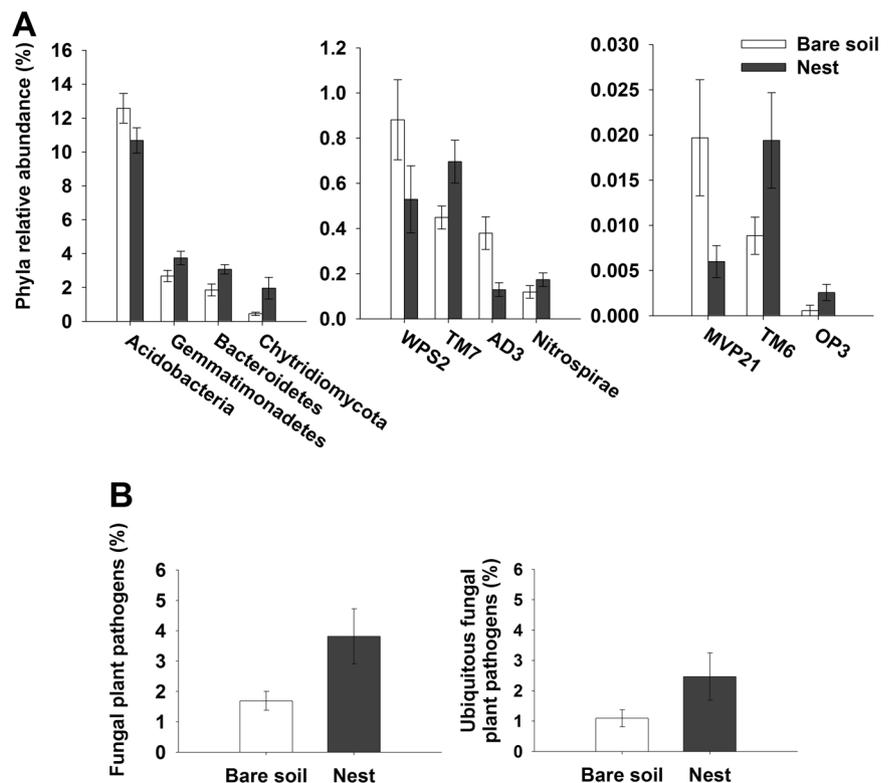
Red and blue colors indicate positive and negative correlations, respectively. *DIN* dissolved inorganic nitrogen, *aP* dissolved inorganic phosphorus.

archaea), and therefore, changes in archaeal richness were not determined. However, we found that the relative abundance of Thaumarchaeota (all phylotypes belonging to Candidatus Nitrososphaera, an ammonia oxidizing archaeon) was marginally larger ($P = 0.08$) in ant nests compared with bare soils (Fig S6).

We did not find overall significant differences between ant nest and bare soil for other dominant phyla (Figs. S7 and S8; Table S1) or fungal functional groups such as mycorrhizal fungi and soil saprobes (Fig. S9; Table S1). Moreover, ant nests had similar relative abundance of animal pathogens than surrounding bare soil, and also of important ant pathogens such as *Beauveria* sp. and *Metarhizium* sp. (Table S2). However, they had a higher proportion of ant-microbial symbionts than surrounding bare soils including *Aspergillus pseudonominus*, *Aspergillus NRRL-32683*, *Mucor circinelloides*, and *Cryptococcus laurentii*, which are involved in the production of antibiotics [14]. These findings are in agreement with recent research [15], and suggest that ant nests maintain a hygienic environment in their nests.

Ant nest had a lower relative abundance of less common phyla such as *AD3*, *WPS2*, and *MVP21* (Fig. 2; Table S1). Some phyla were lower in relative abundance in ant nests (*AD3*, *WPS2*, and *MVP21*), while others were increased (Chytridiomycota (fungi) and Gemmatimonadetes, *TM7*, Nitrospirae, *TM6*, and *OP3*—*Caldiserica*) (Fig. 2; Figs. S7 and S8; Table S1). The higher litter accumulation and greater porosity of these large nests [16] might explain the

Fig. 2 a Relative abundance (mean ± SE) of bacterial and fungal phyla for which we found significant differences between bare soil and ant nest microsites ($n = 44$). See Table S1 for results of statistical analyses. **b** Relative abundance (mean ± SE) of fungal plant pathogens and ubiquitous fungal plant pathogens (total) for the bare soil and ant nest microsites ($n = 44$)



higher relative abundance of *Nitrospirae*, a phylum that contains taxa involved in the nitrification process. The relative abundance of *TM7* was positively correlated with the concentration of dissolved inorganic N. In support of this notion, ammonium and urea fertilization have been reported to increase the relative abundance of *TM7* in terrestrial environments [17]. Importantly, further analyses suggested that the relative abundance of taxa positively associated with ant nests (Fig. 2) were also positively correlated with the activities of multiple extracellular enzymes related to decomposition rates and C (starch and chitin degradation), N (protein degradation), and P (P mineralization) cycling (Table S3) [18]. This result suggests that microbial taxa associated with ant nests could play an important role in maintaining the functioning of terrestrial ecosystems across large spatial scales.

Finally, following the criterion explained in the Supplementary Methods, we identified a subset of 71 exclusive and commonly distributed microbial phylotypes for ant nests, which were classified in two major ecological clusters (Figs. S11 and S12; Table S4). The first cluster includes taxa related to high N content such as *Variovorax paradoxus* and *Methylotenera mobilis* associated with N cycling (e.g., denitrification [19, 20]). A second major ecological cluster was related to P availability and included *Azospirillum* sp, a genus that fixes N, which requires high levels of energy, and therefore P (e.g., ATP), and *Algoriphagus* sp., a genus that includes species that could potentially contain alkaline phosphatases [21]. The exclusive ant microbiome also contained phylotypes associated with saprobic, pathogenic, and ectomycorrhizal functional groups (Table S4).

Together, our work provides novel evidence that ant colonies increase the richness and include unique assemblages of microbial taxa across a large region, suggesting that they are an important *refugia* for microbial diversity in terrestrial environments. Many of these taxa could follow copiotrophic (e.g., Bacteroidetes) and opportunistic (e.g., fungal plant pathogens) strategies and benefit from the larger amount of nutrients within ant nests compared to bare ground areas, with implications for the ecosystem functioning of terrestrial ecosystems.

Data accessibility

The primary data have been deposited in figshare: <https://figshare.com/s/ced10679d675e27dfb00> (<https://doi.org/10.6084/m9.figshare.7092920>). The raw sequence data have been deposited in figshare: <https://figshare.com/s/55813554972fd4a51195> (<https://doi.org/10.6084/m9.figshare.7092950>).

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Author contributions M.D-B. conceived the idea of this study. M.D-B. and D.J.E. conducted soil samplings. B.K.S. provided Illumina data. K.H. conducted bioinformatics analyses. M.D-B. conducted statistical modeling. The manuscript was written by M.D-B, edited by D.J.E., and all co-authors significantly contributed to improve it.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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