Received: 5 March 2021 | Revised: 7 May 2021 | Accepted: 13 May 2021

DOI: 10.1002/mbo3.1201

ORIGINAL ARTICLE

MicrobiologyOpen Open Access WILEY

The bacterial and fungal community composition in time and space in the nest mounds of the ant *Formica exsecta* (Hymenoptera: Formicidae)

Stafva Lindström[1,2,3] | **Sari Timonen**[3] | **Liselotte Sundström**[1,2]

[1]Organismal and Evolutionary Biology Research Programme, Faculty of Biological and Environmental Sciences, University of Helsinki, Helsinki, Finland

[2]Tvärminne Zoological Station, Hanko, Finland

[3]Department of Microbiology, University of Helsinki, Helsinki, Finland

Correspondence
Stafva Lindström, Department of Microbiology, University of Helsinki, P O Box 56, 00014 Helsinki, Finland.
Email: stafva.lindstrom@helsinki.fi

Funding information
Academy of Finland, Grant/Award Number: 252411 and 284666

Abstract

In a subarctic climate, the seasonal shifts in temperature, precipitation, and plant cover drive the temporal changes in the microbial communities in the topsoil, forcing soil microbes to adapt or decline. Many organisms, such as mound-building ants, survive the cold winter owing to the favorable microclimate in their nest mounds. We have previously shown that the microbial communities in the nest of the ant *Formica exsecta* are significantly different from those in the surrounding bulk soil. In the current study, we identified taxa, which were consistently present in the nests over a study period of three years. Some taxa were also significantly enriched in the nest samples compared with spatially corresponding reference soils. We show that the bacterial communities in ant nests are temporally stable across years, whereas the fungal communities show greater variation. It seems that the activities of the ants contribute to unique biochemical processes in the secluded nest environment, and create opportunities for symbiotic interactions between the ants and the microbes. Over time, the microbial communities may come to diverge, due to drift and selection, especially given the long lifespan (up to 30 years) of the ant colonies.

KEYWORDS
bacteria, fungi, microbial communities, microbial ecology

1 | INTRODUCTION

The metabolic activities of microbes constantly modify the environment they live in (Ratzke et al., 2018; Schimel & Schaeffer, 2012). Reciprocally, the selection impelled by the biotic and abiotic environment largely explains the diversity of bacteria and microfungi (Koskella & Bergelson, 2020; Sorensen et al., 2013). Animals shape the microbial composition of their environment, by altering the physicochemical characteristics of soil, for example by burrowing, constructing dams, excavating tunnels, or building nests (Duff et al., 2016; Hastings et al., 2007; Jílková et al., 2012, 2017; Vander Meer, 2012). Especially in areas of cold climate, many species are compelled to modify their immediate environment and construct nests in or above the soil to protect themselves and their offspring (Jurgensen et al., 2008). Nest building (or equivalently, construction of niche) influences the evolution of other species sharing the same niche (Hastings et al., 2007).

A nest is the product of the material and the host's building skills, but it is also a physicochemical framework, in which the host creates and shapes a characteristic microbiome. The microbiota of a nest or a

dwelling can decisively influence the fitness of its inhabitants, be the birds, insects, or humans (Brandl et al., 2014; Broussard & Devkota, 2016; Voulgari-Kokota et al., 2019). Similar to the characteristic human gut microbiota (Coyte et al., 2015), or the plant root microbiome (Beckers et al., 2017), nest microbiota often differs from the one in the surrounding environment, regarding the taxonomic composition and patterns of abundance (Lindström et al., 2019). For example, bird nests, like those of reed warblers, contain bacteria originating from environmental sources, soil, food remains, or from the host plumage (Brandl et al., 2014). The incubation period before hatching, however, significantly changes the bacterial composition in bird nests. During this period, several harmful bacterial groups disappear, either due to antibiotic properties of the eggs, or the sanitation activities of the parents (Brandl et al., 2014). The inside of a termite nest, the termitosphere (Moreira et al., 2018), also illustrates the effects of the nest environment on the microbial communities. When building the nest walls, the workers mix soil and feces into micro-aggregates which promote antibiotic microorganisms within the nest environment (Moreira et al., 2018). The nest mounds of wood ants are less conspicuous than the massive termite domes, but they too provide a unique environment for bacterial and fungal communities, compared to the background forest soil (Lindström et al., 2019).

By constructing their nests, mound-building ants modify the physical and chemical characteristics of soil (Frouz & Jilková, 2008; Kilpeläinen et al., 2007), thereby influencing the main drivers causing seasonal changes in the structure and composition of microbial communities. Seasonal variations in humidity (Evans & Wallenstein, 2014; Sorensen et al., 2013), acidity, nutrient availability, and carbon sequestration (Kaiser et al., 2016; Lauber et al., 2008; Nacke et al., 2016; Rasche et al., 2011; Strickland et al., 2009; Tecon & Or, 2017) shape the composition of soil bacteria and fungi. Nest construction and maintenance affect porosity, aeration, water permeability (Dostál et al., 2005; Duff et al., 2016; Holec & Frouz, 2006), pH (Boots et al., 2012; Dean et al., 1997) (Frouz & Jilková, 2008) as well as carbon and nutrient concentrations in the nest environment (Domisch et al., 2009; Dostál et al., 2005).

Biotic processes, such as seasonal plant processes, also strongly influence the temporal patterns of microbial communities in forest topsoil (Kaiser et al., 2016; Prescott & Grayston, 2013; Zhou et al., 2017), for example, those of mycorrhizal fungi (Sietiö et al., 2018; Timonen et al., 2017) or fungal decomposers (Baldrian, 2017a). The occurrence rate of fungal decomposers varies, being frequent in early spring and late autumn, whereas mycorrhiza forming fungi are predominant during the photosynthetically active period before leaf decay (Santalahti et al., 2016; Žifčáková et al., 2016). Active, maintained ant nest mounds are mostly void of live plants (Frouz & Jilková, 2008; Laakso & Setälä, 1998), and hence less, or not at all, affected by the plant processes.

1.1 | Mound-building *Formica* ants

Mound-building wood ants, such as the *Formica exsecta*, are characteristic of the boreal forest (Jurgensen et al., 2008). They construct their nest mounds above ground (Collingwood, 1979; Seifert, 2011), by gathering their nest material (pine and spruce needles, pieces of moss, and soil) from the surrounding forest floor (Littlewood & Young, 2008). Like other Formicas, their nest mounds are void of plant cover, and due to continuous maintenance, the nests contain fewer or finer roots than the soils surrounding the nest (Frouz & Jilková, 2008; Laakso & Setälä, 1998). The *F. exsecta* ants select sunlit, open spots for their nest, to secure enough insolation during summer (Katzerke et al., 2010; Littlewood & Young, 2008). The decomposition of the organic nest material, together with abundant solar radiation, maintains a steady and relatively high temperature inside the nest mound. The protective characteristics of the nests allow for sufficient duration of suitable climatic conditions for brood development (Rosengren et al., 1987), but the nest also provides a favorable environment for microbes with low tolerance to sub-zero temperatures. This could have a basic influence on the composition of the microbial communities, in particular during sub-zero periods without an insulating snow cover (Margesin & Miteva, 2011; Rankinen et al., 2004). Furthermore, the social behavior of these ants, such as removing microbes from the cuticle of fellow ants, cleaning the nest, or bringing in pieces of antimicrobial, coniferous resin to the nest modifies the microbial communities inside the nests (Brütsch & Chapuisat, 2014; Brütsch et al., 2017; Reber et al., 2011; Ugelvig et al., 2010).

1.2 | Aims of study

In our previous work, we have shown that the nest environment, continuously maintained by its host, shelters bacterial and fungal communities, which are distinct from the surrounding reference soils (Lindström et al., 2019). The microbial communities in nest mounds are unique with respect to both the relative representation, and the presence of certain taxa, whereas the reference soils are significantly less divergent. The nest microbes also assemble into modular networks, which are largely separated from those in the reference soils. The patterns of both indicative taxa, and the modular networks, were consistently maintained across a three-month sampling period (Lindström et al., 2019). This suggests that the factors which drive seasonal changes in the microbial communities differ between the nest and the reference soil environments. This raises the question of whether these effects also persist across seasons. If so, other factors than the typical seasonal shifts in temperature, precipitation, plant cover, and plant species composition, which drive the microbial communities in the upper soil layers of a boreal forest, may drive the microbial communities of the nests.

Thus, we hypothesize that the nest environment alleviates some of the seasonal effects, which drive the microbial communities in the surrounding boreal topsoil in nests mounds of the ant *F. exsecta*. To test this, we assessed the level of within-season and between-season variation of bacterial and fungal taxa in the nests over three years. We identify the most consistent taxa, evaluate their potential functions, and compare their frequency and abundance to that in the surrounding soils during one season.

To achieve this, we used community fingerprinting (T-RFLP) to inform NGS (Illumina-MiSeq) read abundance and taxonomy data. We analyzed the similarity of the bacterial and fungal communities in the nests, based on their Bray–Curtis dissimilarities, with ordination methods and permutational ANOVAs (PERMANOVA). We produced diversity indices for the nest communities and tested them for variation within and between seasons with mixed-model ANOVAs. Finally, we assessed the turnover rates, and the consistency of the bacterial and fungal taxa, and discussed some potential functional reasons for their consistent presence in the nests.

2 | MATERIALS AND METHODS

2.1 | Study organism, sampling, and extraction of DNA

The ant *Formica exsecta* is common in Finland (Czechowski et al., 2002; Douwes et al., 2012), where it inhabits meadows and open woodlands (Sundstrom et al., 1996). The perennial nests have an average lifespan of 6.5 years (Haag-Liautard et al., 2009), but healthy nests can stay active for up to 30 years (Pamilo, 1991). At our study sites on the SW coast of Finland, on the two islands of Furuskär and Joskär close to the Tvärminne zoological station (59°84′196″N, 23°20′182″E), the *F. exsecta* populations have been monitored since 1994 (Sundstrom et al., 1996; Vitikainen et al., 2011, 2015). The biotopes of the study sites consist of pine and spruce thickets intermixed with granite cliffs, dry meadows, and some lusher patches of the grove. In addition to pine and spruce, the vegetation consists mainly of junipers and ericoid shrubs. The immediate surroundings of the nests encompass plant communities that vary both spatially and temporally. The soil type consists mainly of thin layers of leptosol intermixed with stratified podzol (Lindström et al., 2018). The uppermost litter layer consists mostly of small twigs and needles of coniferous trees. The characteristic shared by all the sampled nest locations is that they are all built on rather open and dry spots.

We sampled six nests during May, June, and August in 2013–2015, three nests from the island Furuskär (ca 1.5 km^2), and three from the island of Joskär (ca 2 km^2). The nests included in the study were distributed across the islands, usually at more than a 50 m distance from each other. In addition, we collected reference soil samples from the surroundings of three of these nests in May, June, and August 2015, in total nine reference soil samples, in addition to the 54 nest samples. The samples, (~0.2 L) of the nest or reference soil material, were collected by hand at a depth of 10–15 cm, using sterile gloves. The samples were placed in sterile zip-lock bags and stored at −80°C until further processing. DNA was extracted from a ~0.25 g subsample of the nest material, using the MoBio PowerSoil® (Qiagen) DNA Isolation Kit, according to manufacturer's instructions, except using TissueLyser II (Qiagen) for 3 min at 20Hz, instead of vortexing (Lindström et al., 2018) instead of vortexing, during the cell lysis.

2.2 | Molecular and bioinformatic procedures

All samples were subjected to T-RFLP (Liu et al., 1997). The PCR and purification of the soil DNA for the T-RFLP analysis were conducted as in Lindström et al. (2018). For bacteria, FAM-tagged forward primer 27F (AGAGTTTGATC(A/C)TGGCTCAG, Chung et al., 2004, Weisburg et al., 1991) and the reverse primer 1387R (GGGCGG(A/T) GTGTACAAGGC, Wade et al., 1998) were used. For fungi, the protocol was modified to encompass the whole ITS area (both ITS1 and ITS2), instead of ITS2 only, using the TAMRA-tagged primer ITS1F (CTTGGTCATTTAGAGGAAGTAA, Gardes, & Bruns, 1993) and ITS4 (TCCTCCGCTTATTGATATGC, White et al., 1990). The enzymes HaeIII and MspI were used for the digestion of the bacterial and fungal sequences, as in Lindström et al. (2018).

The processing (separation, peak scoring, noise filtering, alignment, and binning) of the T-RFs (terminal restriction fragments) was conducted as in Lindström et al. (2018), except the minimum height of fungal T-RFs. Instead of applying 70 fluorescent units (fu) for fungi, we set the height at 100 fu for both bacteria and fungi (the T-RFLP data are available at https://doi.org/10.6084/m9.figshare.14547558). Prior to further analysis, the T-RF data were normalized with the function decostand in R, package vegan (Oksanen et al., 2017). The preliminary analysis showed comparable results for data generated by both of the enzymes, so the data generated by MspI were chosen for further analysis, for both bacteria and fungi. The number of T-RFs was counted, and the height of the normalized T-RFs was used as a proxy for abundance.

A subset of nest samples from three nests (years 2013–2015), and corresponding reference soil samples (the year 2015), a total of 36 samples, with an unbroken series of observations during the whole timeline, were submitted to Illumina MiSeq sequencing. The nests that were selected for sequencing had the most complete set of samples throughout the timeline. Preparation of libraries, sequencing, and the bioinformatics pipeline were performed as in Lindström et al. (2018). In brief, sequences from the bacterial 16S rRNA region were amplified with the FAM-tagged forward primer 27F (AGAGTTTGATC(A/C)TGGCTCAG, Chung et al., 2004; Weisburg et al., 1991) and the reverse primer 1387R (GGGCGG(A/T) GTGTACAAGGC, Wade et al., 1998). Sequences from the fungal ribosomal ITS2 region were amplified with the same TAMRA-tagged forward primer as in the T-RFLP analysis. Library sequencing (pair-end mode) was carried out by the DNA Sequencing and Genomics Laboratory, Institute of Biotechnology, University of Helsinki. Read filtering and the clustering of OTUs at the identity of 97% were conducted using UPARSE (Edgar, 2013). A higher taxonomic resolution could have been reached by the use of ASV (with a 99%–100% identity, Callahan et al., 2017; Edgar, 2018), instead of OTU. For this report we, however, decided to use the same methodology which we used in (Lindström et al., 2018, 2019), to retain full congruence with earlier results on the same colonies. The SILVA database v128 (Quast et al., 2013) was used as a reference for the alignment of bacterial sequences and the UNITE v7 database (Kõljalg et al., 2013) for the fungal sequences. The negative controls of the sequencing

showed very low numbers and abundance of contaminants, suggesting the risk of inflated read abundancies due to contamination was negligible.

2.3 | Data analysis

The diversity and number of taxa were assessed based on the OTU data as the number of reads and taxa per year, month, and nest. When possible, taxa were identified to the level of genus, when genus-level information was unavailable a higher taxonomic level was used. All identified taxa are referred to as "GOH (Genus Or Higher) taxa", and the taxonomic level is indicated separately. Prior to further analysis, the OTU data were rarefied to the lowest number of reads in the nest samples (to 24354 reads in bacteria and 14693 reads in fungi) in R, package vegan, function rrarefy (Oksanen et al., 2017), and the proportion of reads identified to the level of phylum and genus was calculated. The number of rarefied reads was used as a proxy for abundance (hereafter referred to as TA, total abundance).

Variation across the bacterial and fungal communities between the years, months, and nests was compared by generating Bray–Curtis inter-sample dissimilarity matrices of both the OTU and the T-RF data, which were then plotted in a principal coordinates analysis (PCoA). In a PCoA, the data are visualized by placing the communities in an n-dimensional space according to their dissimilarity, such that similar communities cluster close together, whereas dissimilar communities are placed further away from each other. The matrices were further subjected to a permutational ANOVA (PERMANOVA, Legendre & Anderson, 1999) with 999 permutations to test the effects of "year," "month," and "nest," with "nest" as stratum to account for repeated measures. The matrices were performed with the function vegdist, the PCoAs with the function capscale, and the PERMANOVAs with adonis2, all in R, package vegan (Oksanen et al., 2017).

To assess community dynamics, we counted richness of species and calculated Shannon–Wiener indices of diversity (H') on GOH taxa and T-RFs, and tested the effect of "year" and "month" on the diversity in a mixed-model ANOVA (JMP v.13, SAS Institute Inc., Cary, NC, U.S), with "month" nested within "year" as the main factor, and "nest" as a random factor. The rate of yearly turnover (appearance and disappearance of the bacterial and fungal taxa) in nest samples was calculated with the package codyn (function turnover, Hallett et al., 2016) in R. In the nest samples, also the abundance and frequency of GOH taxa across years and months were measured in a consistency analysis, and a subset of consistent GOH taxa was derived, sensu Shade and Handelsman (2012). A GOH taxon was characterized as consistent if it was detected in all sampled nests, months, and years. The proportional distribution of the 20 most abundant bacterial and all the fungal GOH taxa that were consistently detected in all nest samples or reference soil samples was plotted in a stacked bar graph.

Finally, we calculated the ratio of total abundance in nests and reference soils as an estimate of enrichment in the nest environment. To ensure comparability, we only included data from 2015. We then used repeated measures ANOVA on ln-transformed values to test for statistical significance for taxa that showed a 1.5-fold or higher, or 0.5-fold or lower prevalence in nests, compared to reference soils. To account for multiple tests, we adjusted the *p*-values for the false discovery rate (Benjamini-Hochberg, 1995).

3 | RESULTS

The Illumina MiSeq DNA sequence data on the 27 nest samples produced in total 1,371,127 bacterial and 1,765,128 fungal sequence reads, which clustered into 33,580 bacterial and 8489 fungal OTUs, respectively (Table A1). The corresponding data for the nine reference soil samples produced 1,061,232 bacterial and 199,875 fungal reads, which clustered into 3776 bacterial and 3764 fungal OTUs, respectively. In total, 383 unique bacterial and 294 unique fungal taxa were detected in the nest samples, and 340 and 216, respectively, in the reference soil samples (Table A1). When possible, taxa were identified to the level of genus, when genus-level information was unavailable a higher taxonomic level was used. In the bacterial data, 97.7% of the taxa were identified at the phylum and 57.4% at the genus level; in the fungal data, these numbers were 98.2% and 53.4%, respectively. Identified taxa are referred to as "GOH (Genus Or Higher) taxa" in the text, and the taxonomic levels are indicated separately. The T-RFLP fingerprinting and restriction with enzyme MspI yielded 160 bacterial and 158 fungal terminal restriction fragments (T-RFs).

Both the bacterial and the fungal communities were clustered according to the nest (Figure 1). The PERMANOVA further confirmed nest location as the main source of community variation in the bacterial nest data, both for OTU and T-RF data (Table 1). In addition, the fungal T-RF nest data showed significant effects of year and month (Table 1). We found no significant variation in either diversity or number of GOH taxa across years or months in the OTU nest data, in neither bacteria nor fungi. However, the bacterial T-RF data showed significant yearly variation in both the diversity and the number of T-RFs. The corresponding fungal data showed no such effects of year and month (Tables 2 and A2). The yearly turnover of bacterial GOH was on average lower than the one of fungi, as 10% of the bacterial taxa appeared or disappeared during 2013–2014, and 31% during 2014–2015. The corresponding figures for fungi were 27% and 40%, respectively (Table 3), indicating that the fungal communities were in general temporally less stable than the bacterial ones.

Forty-five bacterial GOH taxa were present in all 26 nest samples. These encompassed 12% of all bacterial GOH taxa and 75% of the bacterial TA (Table A3). The three most abundant and consistent phyla (Acidobacteria, Actinobacteria, and Proteobacteria) encompassed 68% of the TA. The ten most abundant of these taxa, each of which encompassed over 2% of the TA, were the Acetobacteraceae (Family), Acidobacteria Gp1, Actinomycetales (Order), Bradyrhizobiaceae (Family), *Burkholderia*, *Granulicella*, *Massilia*, *Mycobacterium*, Rhizobiales (Order),

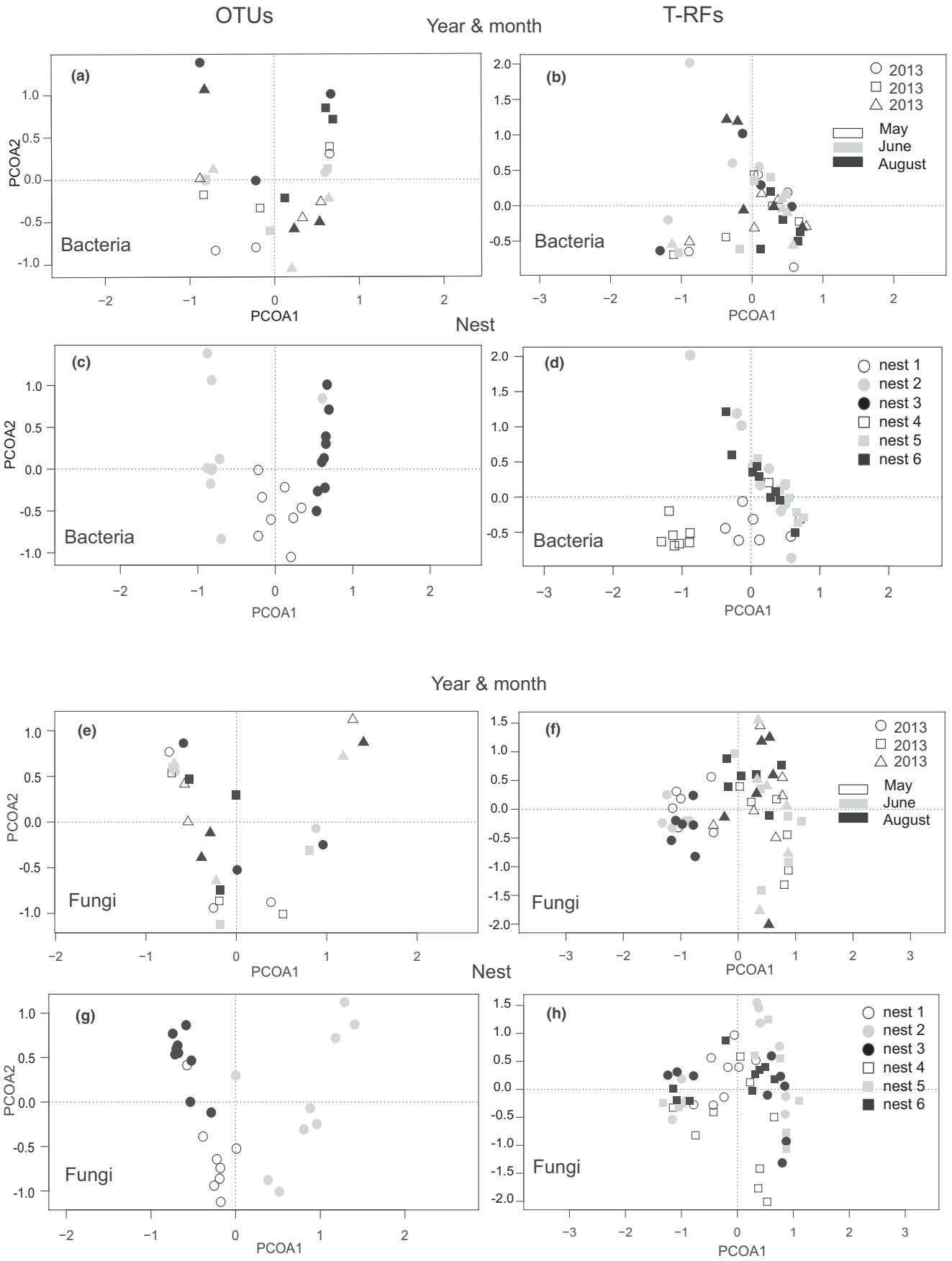

FIGURE 1 Principal coordinates analyses on bacterial OTU data (a and c) and T-RF data (b and d), fungal OTU data (e and g), and T-RF data (f and h). PCoA:s indicated by letters a, b, e, and f show clustering according to Bray–Curtis dissimilarities by year and month, and c, d, g, and h show clustering by nests

TABLE 1 PERMANOVA tests of the effects of year, month, and nest on the bacterial and fungal Bray–Curtis dissimilarities

	OTU-data				T-RF data			
Effect	***F***	R^2	***p***	***n***	***F***	R^2	***p***	***n***
Bacteria								
Year	1.13	0.04	0.297	26	1.87	0.04	0.084	42
Month	1.39	0.05	0.203		0.92	0.02	0.463	
Nest	3.39	0.12	**0.026**		4.16	0.02	**0.001**	
Fungi								
Year	1.17	0.04	0.295	26	8.39	0.14	**0.001**	53
Month	1.45	0.05	0.181		1.76	0.03	**0.041**	
Nest	3.49	0.12	**0.003**		2.11	0.03	**0.012**	

Note: df_{den} in all cases 1 (data in Table A1).
Bold values are significant *p*-values.

TABLE 2 MANOVA results for Shannon–Wiener diversity, and the number of bacterial and fungal OTUs and T-RFs (breakdown of averages per year and month are given in Table A2)

Overall mean			**OTU**			**T-RF**		
OTU	**T-RF**	**Effect**	***F***	***p***	***df***	***F***	***p***	***df***
Shannon-W. *H*								
Bacteria								
3.67 (+/-0.56)	2.45 (+/-0.58)	Month	1.84	0.193	2	0.84	0.442	2
		Year	0.9	0.519	6	3.27	**0.014**	6
Fungi								
2.54 (+/-0.55)	2.37 (+/-0.59)	Month	1.88	0.186	2	0.18	0.835	2
		Year	1.5	0.244	6	0.5	0.806	6
No. of taxa								
Bacteria								
176 (+/-60)	26 (+/-14)	Month	2.02	0.167	2	2.35	0.113	2
		Year	0.76	0.611	6	10.7	**0.001**	6
Fungi								
96 (+/-24)	25 (+/-13)	Month	2.63	0.104	2	0.7	0.504	2
		Year	1.56	0.227	6	1.25	0.304	6

Bold values are significant *p*-values.

TABLE 3 Turnover of bacterial and fungal GOH-taxa in nests in 2013–2015

	GOH-taxa		
Years	**Same**	**Different**	**Change (%)**
Bacteria			
2013–2014	304	34	10.06
2014–2015	254	112	30.60
2013–2015	262	116	30.69
Fungi			
2013–2014	197	74	27.31
2014–2015	161	107	39.93
2013–2015	156	113	42.01

and Solirubrobacterales (Order) (Table A3, Figure A1). Conversely, the 338 bacterial GOH taxa, which were found in fewer than 26 samples, encompassed 88% of all GOH taxa, but only 25% of the TA.

Sixteen fungal GOH taxa were present in all 26 nests. These encompassed 5% of all the fungal GOH taxa and 53% of the fungal TA (Table A4, Figure A1). Taxa belonging to the phylum Ascomycota accounted for 50% of the TA, whereas the phylum Basidiomycota comprised 1%, and unidentified fungi 2%, respectively, of the TA. The five most abundant GOH taxa, each of which encompassed more than 2% each of the TA, were Ascomycota (Phylum), *Cladosporium*, Leotiomycetes (Class), *Oidiodendron*, and Pleosporales (Order). The 278 fungal GOH taxa, which were found in fewer than 26 samples, encompassed 95% of all GOH taxa and 47% of the TA (Table A4).

When comparing the species composition of nests and reference soils, we found that 18 bacterial and 5 fungal GOH-taxa had a 1.5-fold or higher abundance in the nest material, compared to the reference soils (Tables A3 and A4, and Figure 2a,b). Conversely, eight bacterial taxa and one fungal taxon were enriched in the reference soil samples (fold difference <0.5). In bacteria, the difference was statistically significant, after correction for false discovery rate, in 19 cases, 11 of which were enriched in nests, and 8 in the reference soils

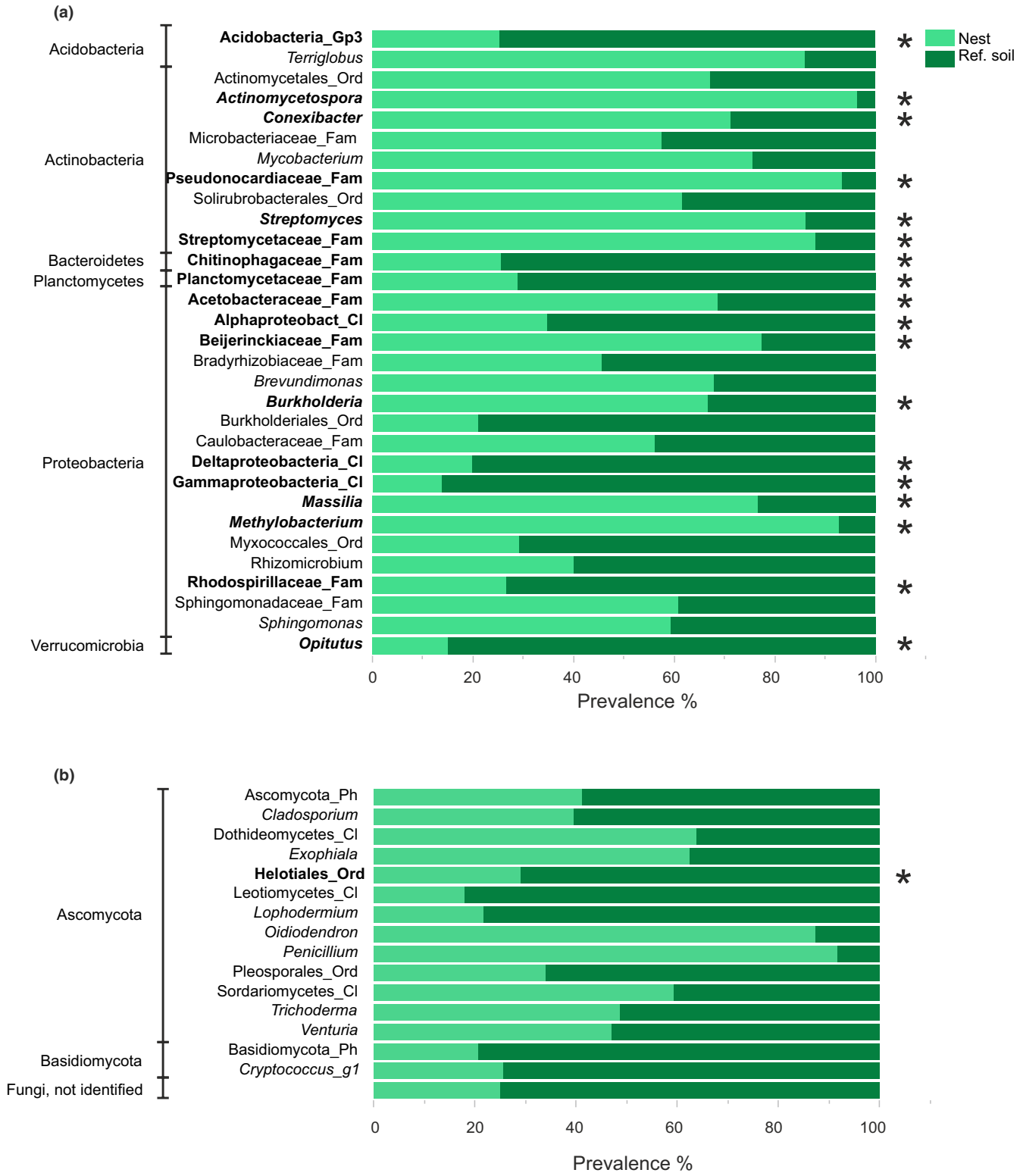

FIGURE 2 Relative representation of bacterial (a) and fungal (b) taxa in nests and reference soils, sampled in 2015. The *p*-values refer to repeated measures ANOVA, conducted on taxa that showed either a 1.5-fold or higher or a 0.5-fold or lower prevalence in nests than in reference soils. The asterisks indicate samples in which the differences were statistically significant after correction for false discovery rate.

(Repeated measures ANOVA: $F_{1,8} > 7.64$, $p < 0.025$). These included six Actinobacterial taxa (including the genera *Actinomycetospora*, *Streptomyces*, and *Conexibacter*), which together accounted for 7.7% of the TA, with an average fold difference of 10.1. Furthermore, five Proteobacterial taxa (including the genera *Methylobacterium*, *Massilia*, and *Burkholderia*, and the families *Beijerinckiaceae* and

Acetobacteraceae) accounted for 13.8% of the TA, with an average fold difference of 6.1. All six bacterial taxa that were absent from some of the reference soil samples belong to Actinobacteria or Proteobacteria (Table A3).

In fungi, five GOH-taxa belonging to the phylum Ascomycota, including the genera of *Oidiodendron*, *Exophiala*, *Penicillium*, and *Trichoderma*, showed a twofold or higher prevalence in the nests compared to reference soils. However, none of the differences were significant, given the extensive variation across nests and reference soils. The only fungal taxon to exceed 2% of the TA was the genus *Oidiodendron*, which alone comprised 17.7% of the TA, with a fold difference of 7.5. The remaining genera (*Exophiala*, *Penicillium*, *Trichoderma*, and an unidentified taxon) together represented 4.3% of the TA, with an average fold difference of 5.6 (Figure 2b, Table A4). Two of these, *Exophiala* and *Penicillium*, were absent from some of the reference soil samples from 2015. In the reference soils, four taxa showed enrichment, but only one of these was statistically significant (order Helotiales) and represented 1.82% of the TA (Table A4).

4 | DISCUSSION

4.1 | Community dynamics

Here, we show based on the sequenced OTUs that the composition of bacterial communities in ant nests remained fairly stable both within seasons and across years, yet varied significantly among nests. The fungal communities showed a similar, although less clear, pattern, as the T-RF data also signaled monthly and yearly variation. Consistently with this, the yearly turnover of fungal taxa in nests was higher than that of bacteria. Furthermore, the number of bacterial GOH taxa, consistently present in all samples, was almost twofold compared to the fungal taxa. This is consistent with a lower turnover of taxa and signals higher temporal stability of the bacterial communities. The diversity of the bacterial and fungal communities remained stable across both months and years as measured based on the OTU data. However, based on the T-RF data, bacterial diversity varied across years. The turnover of species does not necessarily affect the diversity, or the number of taxa, as long as the number of taxa that disappear each year is approximately the same as the number of new taxa. The difference between the OTU data and the T-RF data in the temporal variation of fungal community composition may also partly be explained by the smaller sample size of the OTU data set. Furthermore, as one taxon can be represented by several different T-RF peaks, and one T-RF peak can stand for several taxa, the estimates of diversity and species count based on T-RFLP may differ from those obtained based on the OTU data (Avis et al., 2006). The lower number of fungal taxa, compared to bacteria, may have many causes, but one potentially important factor is that the insides of *F. exsecta* nests are dry. Drought in general is associated with lower fungal diversity in soil (Bahram et al., 2018). However, fungi also respond quicker than bacteria to changes in humidity, which may contribute to their lower spatial and temporal stability (Hawkes et al., 2011).

4.2 | Community composition

The 45 consistent bacterial and 16 fungal taxa that were present in all nest samples throughout the sampling period belonged to eight bacterial (Acidobacteria, Actinobacteria, Armatimonadetes, Bacterioidetes, Planctomycetes, Proteobacteria, TM7, and Verrucomicrobia), and two fungal (Ascomycota and Basidiomycota) phyla. All these taxa were also detected in at least some of the reference soil samples. The overall number of taxa was also similar in the nests and the reference soils. However, the comprehensive sampling of the same nests across three years should reduce such errors. The abundances of some taxa may be under- or overestimated due to the limitations involved with the use of sequence read number as a measure for abundance (Degnan & Ochman, 2012), or some rarer taxa may have been overlooked due to the rarefying procedure (McMurdie & Holmes, 2014). Another potential caveat is that the nest samples covered several years, and thus a larger total number of samples than those from the reference soils, which were collected only in 2015. Thus, the total number of taxa is likely to be inflated in the nest samples, compared to the reference soil samples. However, the abundances (measured as the fraction of total abundance) differed in some cases between the nest and the reference soils, such that some taxa were enriched in the nests, compared to the reference soils. Given that the percentage of TA is corrected for sample size, the calculated differences in fold ratios should hold.

Of the bacterial taxa which we identified as consistent and enriched in the nests, all belonged to the phyla of Actinobacteria or Proteobacteria. Several studies have recorded close associations between ants and Actinobacteria (Barke et al., 2010; Lucas et al., 2017; Matarrita-Carranza et al., 2017; Seipke et al., 2012), and the high percentage of the enriched Actinobacterial taxa in the *F. exsecta* nests (covering over 18% of the TA) indeed suggests similar associations. Of the enriched bacterial taxa, four genera (*Actinomycetospora*, *Streptomyces*, *Methylobacterium*, and *Burkholderia*), and one unidentified taxon belonging to the family of Acetobacteraceae, have previously been recorded as core indicators of ant nests (Table A3 and references therein). Species belonging to Acetobacteraceae and *Burkholderia* have also been detected in the *F. exsecta* transcriptome (Johansson et al., 2013). A further two enriched bacteria (*Mycobacterium* and *Brevundimonas*) have known associations with ants and other social Hymenoptera, but also some of the non-enriched, but consistent taxa (*Conexibacter*, *Rhizomicrobium*, *Caulobacter*, *Phenylobacterium*, *Sphingomonas*, one unidentified genus belonging to Chitinophagaceae, and one to Bradyrhizobiaceae) had similar associations. The remaining bacterial taxa are mostly decomposers, or associated with plants or lichen, whereas the function of some remains undisclosed (Table A3 and references therein).

All consistent fungal taxa belonged to the phylum of Ascomycota, but somewhat surprisingly, none of the fungal taxa showed

significant enrichment in the nest material. This does not preclude the existence of important functions in ant nests and is consistent with our observation that fungi showed stronger temporal variation and signs of higher temporal turnover. Indeed, four of the fungal genera (*Oidiodendron*, *Exophiala*, *Cryptococcus*, and *Cladosporium*) have previously been identified as core indicators of the nests, and *Oidiodendron* represents a considerable fraction of the fungal community in this study (Table A4 and references therein). Furthermore, the genus *Cryptococcus* has previously been detected in the *F. exsecta* transcriptome (Johansson et al., 2013). Conversely, *Penicillium* and *Trichoderma* have no recorded associations with ants. Similar to bacteria, the function of some of the consistent fungal taxa is unknown.

4.3 | Characteristics and processes of the ant nests that potentially influence the microbes

We found the bacterial communities of the *F. exsecta* nests to be temporally stable, whereas the fungal communities showed more fluctuation across months and years. The temporal shifts in the microbial species composition and abundance in soils are considered to be primarily controlled by plants (Kaiser et al., 2016; Lata et al., 2010; Zhou et al., 2017). However, the nest mounds of *F. exsecta* nests are mainly void of live plants. The absence of plant material selects for microbiota, which is not dependent on shifts in plant root exudates, nor the resource-dependent cycles of fungal guilds. The cycles of saprotrophic taxa in forest soil follow the volume and quality of available litter, usually peaking in autumn (Žifčáková et al., 2016), but the litter input into ant nests does not follow the same cycle as the surrounding forest floor, as the ants actively add litter to the nest throughout the ant active season. Therefore, temporal fluctuations in the abundance of decomposers in the nests could be much lower than in the surrounding soil, which promotes the consistency of several microbial taxa. In general, fungi are the major decomposers in forest soil, often showing more distinct succession in litter-like material, compared to bacteria (Baldrian, 2017b), which could explain the somewhat higher temporal variability of fungi in *F. exsecta* nests. However, studies including reference soil samples from more years would be needed to determine this.

Temperature is considered to be another main driver of the structure and composition of microbial communities (Hawkes et al., 2011; Lladó et al., 2018; Rousk & Bååth, 2011). For example, the abundance of Acidobacteria and Proteobacteria increases with increased temperature (Lladó et al., 2017). In ant nests, the temperature is maintained on a steady and high level, for a longer period than in the surrounding forest floor (Frouz & Jilková, 2008; Katzerke et al., 2010). This is partly due to the insulating effects of the nest (Frouz & Jilková, 2008), selectively built-in spots of high solar radiation (Katzerke et al., 2010), but also as a result of the ongoing decomposition process (Laakso & Setälä, 1998). In boreal or subarctic soils, the nest could therefore affect the microbial communities, not only due to a generally higher and more stable temperature but also by prolonging the periods with above-zero temperatures, compared to the surrounding soil. Both the intensity and length of the varying temperature shape the patterns of microbial diversity (Margesin & Miteva, 2011). Moreover, ants can alter the levels of N, P, and C in the nests (Jurgensen et al., 2008; Lenoir et al., 2001). *Formica* ants prey on aphids and other invertebrates, and they also use honeydew from the aphids for food (Domisch et al., 2009). The residues of the food, together with ant excrements, affect the nutrient levels in the nests (Jílková et al., 2012; Kilpeläinen et al., 2007). This creates temporally different resource patterns for microbial communities inside the nest mounds compared to those prevailing in the surrounding soil.

5 | CONCLUSIONS

Our study shows that ant nests can provide an environment with microbial communities distinct from the surrounding soil, both in time and space. This differential is likely brought about by the activities of ants that on the one hand allow unique biochemical processes in the absence of plants, and on the other hand, create opportunities for symbiotic interactions between the ants and the microbes. The stable nest environment could thus act as a reservoir, where inocula of microbial taxa, less tolerant of climatic fluctuation, could survive through unfavorable seasons. Over time the microbial communities may come to diverge, due to drift and selection, especially given the potentially long lifespan of the ant colonies, up to 30 years (Pamilo, 1991; Sundström personal observation). Several of the taxa found in this study have been found in association with ants in general, and some specifically with *F. exsecta* (Johansson et al., 2013). We also found that a subset of the bacterial taxa was enriched in the nests, compared to the reference soils outside the nests, whereas other taxa, albeit consistently present, were not enriched. Taken together, these findings may reflect mutualistic interactions between the ants and the microbes, but with the present data further conjectures on this would be premature.

ACKNOWLEDGMENTS

We thank Dr. Helena Johansson for her invaluable help with the planning of the data analysis, and our technician Heini Ali-Kovero for her input during the laboratory assays. This work was funded by the Academy of Finland (grant numbers 252411 and 284666).

CONFLICT OF INTERESTS

None declared.

AUTHOR CONTRIBUTIONS

Stafva Lindström: Conceptualization (lead); Data curation (lead); Formal analysis (lead); Investigation (lead); Methodology (lead); Project administration (lead); Visualization (lead); Writing-original draft (lead); Writing-review & editing (lead). **Sari Timonen:** Conceptualization (equal); Formal analysis (equal); Investigation

(supporting); Methodology (equal); Supervision (equal); Writing-original draft (supporting). **Liselotte Sundström:** Conceptualization (supporting); Formal analysis (supporting); Funding acquisition (lead); Project administration (supporting); Resources (lead); Supervision (lead); Writing-original draft (supporting); Writing-review & editing (equal).

ETHICS STATEMENT

None required.

DATA AVAILABILITY STATEMENT

The unprocessed sequences generated and analyzed in the current study are available in NCBI Sequence Read Archive, BioProject number PRJNA399258: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA399258. The bacterial and fungal T-RFLP data are available in the figshare repository at https://doi.org/10.6084/m9.figshare.14547558

ORCID

Stafva Lindström https://orcid.org/0000-0002-2863-940X

REFERENCES

Anderson, K. E., Russell, J. A., Moreau, C. S., Kautz, S., Sullam, K. E., Hu, Y. I., Basinger, U., Mott, B. M., Buck, N., & Wheeler, D. E. (2012). Highly similar microbial communities are shared among related and trophically similar ant species. *Molecular Ecology*, *21*(9), 2282–2296. https://doi.org/10.1111/j.1365-294x.2011.05464.x

Aschenbrenner I. A., Cernava T., Erlacher A., Berg G., Grube M. (2017). Differential sharing and distinct co-occurrence networks among spatially close bacterial microbiota of bark, mosses and lichens. *Molecular Ecology*, *26*(10), 2826–2838. http://dx.doi.org/10.1111/mec.14070

Avis, P. G., Dickie, I. A., & Mueller, G. M. (2006). A "dirty" business: Testing the limitations of terminal restriction fragment length polymorphism (TRFLP) analysis of soil fungi. *Molecular Ecology*, *15*, 873–882. https://doi.org/10.1111/j.1365-294X.2005.02842.x

Ba A. S., & Phillips S. A. (1996). Yeast biota of the red imported fire ant. *Mycological Research*, *100*(6), 740–746. http://dx.doi.org/10.1016/s0953-7562(96)80208-5

Bahram, M., Hildebrand, F., Forslund, S. K., Anderson, J. L., Soudzilovskaia, N. A., Bodegom, P. M., Bengtsson-Palme, J., Anslan, S., Coelho, L. P., Harend, H., Huerta-Cepas, J., Medema, M. H., Maltz, M. R., Mundra, S., Olsson, P. A., Pent, M., Põlme, S., Sunagawa, S., Ryberg, M., … Bork, P. (2018). Structure and function of the global topsoil microbiome. *Nature*, *560*, 233–237. https://doi.org/10.1038/s41586-018-0386-6

Baldrian, P. (2017a). Forest microbiome: Diversity, complexity and dynamics. *FEMS Microbiology Reviews*, *41*, 109–130. https://doi.org/10.1093/femsre/fuw040

Baldrian, P. (2017b). Microbial activity and the dynamics of ecosystem processes in forest soils. *Current Opinion in Microbiology*, *37*, 128–134. https://doi.org/10.1016/j.mib.2017.06.008

Baldrian P., Kolařík M., Štursová M., Kopecký J., Valášková V., Větrovský T., Žifčáková L., Šnajdr J., Rídl J., Vlček Č., & Voříšková J. (2012). Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. *The ISME Journal*, *6*(2), 248–258. http://dx.doi.org/10.1038/ismej.2011.95

Barke, J., Seipke, R. F., Grüschow, S., Heavens, D., Drou, N., Bibb, M. J., Goss, R. J. M., Yu, D. W., & Hutchings, M. I. (2010). A mixed community of actinomycetes produce multiple antibiotics for the fungus farming ant *Acromyrmex octospinosus*. *BMC Biology*, *8*, 109. https://doi.org/10.1186/1741-7007-8-109

Beckers, B., De Beeck, M. O., Weyens, N., Boerjan, W., & Vangronsveld, J. (2017). Structural variability and niche differentiation in the rhizosphere and endosphere bacterial microbiome of field-grown poplar trees. *Microbiome*, *5*, 1–17. https://doi.org/10.1186/s40168-017-0241-2

Benjamini, Y, & Hochberg, Y (1995). Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society Series B*, *57*(1), 289–300.

Boots, B., Keith, A. M., Niechoj, R., Breen, J., Schmidt, O., & Clipson, N. (2012). Unique soil microbial assemblages associated with grassland ant species with different nesting and foraging strategies. *Pedobiologia (Jena)*, *55*, 33–40. https://doi.org/10.1016/j.pedobi.2011.10.004

Brandl, H. B., van Dongen, W. F. D., Darolová, A., Krištofík, J., Majtan, J., & Hoi, H. (2014). Composition of bacterial assemblages in different components of reed warbler nests and a possible role of egg incubation in pathogen regulation. *PLoS One*, *9*, e114861. https://doi.org/10.1371/journal.pone.0114861

Broussard, J. L., & Devkota, S. (2016). The changing microbial landscape of Western society: Diet, dwellings and discordance. *Molecular Metabolism*, *5*, 737–742. https://doi.org/10.1016/j.molmet.2016.07.007

Brown B. P., & Wernegreen J. J. (2016). Deep divergence and rapid evolutionary rates in gut-associated Acetobacteraceae of ants. *BMC Microbiology*, *16*(1). http://dx.doi.org/10.1186/s12866-016-0721-8

Brütsch, T., & Chapuisat, M. (2014). Wood ants protect their brood with tree resin. *Animal Behavior*, *93*, 157–161. https://doi.org/10.1016/j.anbehav.2014.04.024

Brütsch, T., Jaffuel, G., Vallat, A., Turlings, T. C. J., & Chapuisat, M. (2017). Wood ants produce a potent antimicrobial agent by applying formic acid on tree-collected resin. *Ecology and Evolution*, *7*, 2249–2254. https://doi.org/10.1002/ece3.2834

Callahan, B. J., McMurdie, P. J., & Holmes, S. P. (2017). Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *ISME Journal*, *11*, 2639–2643. https://doi.org/10.1038/ismej.2017.119

Chung, J., Ha, E.-S.-S., Park, H.-R.-R., & Kim, S. (2004). Isolation and characterization of Lactobacillus species inhibiting the formation of *Streptococcus mutans* biofilm. *Oral Microbiology and Immunology*, *19*, 214–216. https://doi.org/10.1111/j.0902-0055.2004.00137.x

Collingwood, C. (1979). The Formicidae (Hymenoptera) of Fennoscandia and Denmark. *Fauna Entomologica Scandinavica*, *8*, 1–174.

Coyte, K. Z., Schluter, J., & Foster, K. R. (2015). The ecology of the microbiome: Networks, competition, and stability. *Science*, *350*, 663–666. https://doi.org/10.1126/science.aad2602.

Czechowski, W., Radchenko, A., & Czhechowska, W. (2002). *The Ants (Hymenoptera, Formicidae) of Poland* (1st ed.). Museum of Institute of Zoology.

Davey M. L., & Currah R. S. (2006). Interactions between mosses (Bryophyta) and fungi. *Canadian Journal of Botany*, *84*(10), 1509–1519. http://dx.doi.org/10.1139/b06-120

Dean, W. R. J., Milton, S. J., & Klotz, S. (1997). The role of ant nest-mounds in maintaining small-scale patchiness in dry grasslands in Central Germany. *Biodiversity and Conservation*, *6*, 1293–1307.

Degnan, P. H., & Ochman, H. (2012). Illumina-based analysis of microbial community diversity. *ISME Journal*, *674*, 183–194. https://doi.org/10.1038/ismej.2011.74

Domisch, T., Finér, L., Neuvonen, S., Niemelä, P., Risch, A. C., Kilpeläinen, J., Ohashi, M., & Jurgensen, M. F. (2009). Foraging activity and dietary spectrum of wood ants (*Formica rufa* group) and their role in nutrient fluxes in boreal forests. *Ecological Entomology*, *34*, 369–377.

Dostál, P., Březnová, M., Kozlíčková, V., Herben, T., & Kovář, P. (2005). Ant-induced soil modification and its effect on plant below-ground

biomass. *Pedobiologia (Jena)*, *49*, 127–137. https://doi.org/10.1016/j.pedobi.2004.09.004

Douwes, P., Abenius, J., Cederberg, B., & Wahlstedt, U. (2012). *Nationalnyckeln till Sveriges flora och fauna*. Steklar: Myror-getingar. Hymenoptera: Formicidae-Vespidae, Uppsala: ArtDatabanken, SLU.

Druzhinina I. S., Seidl-Seiboth V., Herrera-Estrella A., Horwitz B. A., Kenerley C. M., Monte E., Mukherjee P. K., Zeilinger S., Grigoriev I. V., & Kubicek C. P. (2011). Trichoderma: the genomics of opportunistic success. *Nature Reviews Microbiology*, *9*(10), 749–759. http://dx.doi.org/10.1038/nrmicro2637

Duarte A. P. M., Attili-Angelis D., Baron N. C., Forti L. C., & Pagnocca F. C. (2014). Leaf-cutting ants: an unexpected microenvironment holding human opportunistic black fungi. *Antonie van Leeuwenhoek*, *106*(3), 465–473. http://dx.doi.org/10.1007/s10482-014-0215-3

Duff, L. B., Urichuk, T. M., Hodgins, L. N., Young, J. R., & Untereiner, W. A. (2016). Diversity of fungi from the mound nests of *Formica ulkei* and adjacent non-nest soils. *Canadian Journal of Microbiology*, *62*, 562–571.

Edgar, R. C. (2013). UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nature Methods*, *10*, 996–998. https://doi.org/10.1038/nmeth.2604

Edgar, R. C. (2018). Updating the 97% identity threshold for 16S ribosomal RNA OTUs. *Bioinformatics*, *34*, 2371–2375. https://doi.org/10.1093/bioinformatics/bty113

Evans, S. E., & Wallenstein, M. D. (2014). Climate change alters ecological strategies of soil bacteria. *Ecology Letters*, *17*, 155–164. https://doi.org/10.1111/ele.12206

Frouz, J., & Jilková, V. (2008). The effect of ants on soil properties and processes (Hymenoptera: Formicidae). *Myrmecological News*, *11*, 191–199.

Gardes, M., & Bruns, T. D. (1993). ITS primers with enhanced specificity for basidiomycetes, application to the identification of mycorrihiza and rusts. *Molecular Ecology*, *2*, 113–118.

Haag-Liautard, C., Vitikainen, E., Keller, L., & Sundstroem, L. (2009). Fitness and the level of homozygosity in a social insect. *Journal of Evolutionary Biology*, *22*, 134–142. https://doi.org/10.1111/j.1420-9101.2008.01635.x.

Hallett, L. M., Jones, S. K., MacDonald, A. A. M., Jones, M. B., Flynn, D. F. B., Ripplinger, J., Slaughter, P., Gries, C., & Collins, S. L. (2016). codyn: An r package of community dynamics metrics. *Methods in Ecology and Evolution*, *7*, 1146–1151.

Hastings, A., Byers, J. E., Crooks, J. A., Cuddington, K., Jones, C. G., Lambrinos, J. G., Talley, T. S., & Wilson, W. G. (2007). Ecosystem engineering in space and time. *Ecology Letters*, *10*, 153–164. https://doi.org/10.1111/j.1461-0248.2006.00997.x

Hawkes, C. V., Kivlin, S. N., Rocca, J. D., Huguet, V., Thomsen, M. A., & Suttle, K. B. (2011). Fungal community responses to precipitation. *Global Change Biology*, *17*, 1637–1645. https://doi.org/10.1111/j.1365-2486.2010.02327.x

Holec, M., & Frouz, J. (2006). The effect of two ant species *Lasius niger* and *Lasius flavus* on soil properties in twoncontrasting habitats. *European Journal of Soil Biology*, *42*, S213–S217.

Ishak H. D., Plowes R., Sen R., Kellner K., Meyer E., Estrada D. A., Dowd S. E., & Mueller U. G. (2011). Bacterial Diversity in Solenopsis invicta and Solenopsis geminata Ant Colonies Characterized by 16S amplicon 454 Pyrosequencing. *Microbial Ecology*, *61*(4), 821–831. http://dx.doi.org/10.1007/s00248-010-9793-4

Jaffe K., Caetano F. H., Sánchez P., Hernández J. V., Caraballo L., Vitelli-Flores J., Monsalve W., Dorta B., & Lemoine V. R. (2001). Sensitivity of ant (*Cephalotes*) colonies and individuals to antibiotics implies feeding symbiosis with gut microorganisms. *Canadian Journal of Zoology*, *79*(6), 1120–1124. http://dx.doi.org/10.1139/cjz-79-6-1120

Jílková, V., Pech, P., Mihaljevič, M., & Frouz, J. (2017). Effects of the ants *Formica sanguinea*, *Lasius niger*, and *Tetramorium* cf. *caespitum* on soil properties in an ore-washery sedimentation basin. *Journal of Soils and Sediments*, *17*, 2127–2135. https://doi.org/10.1007/s11368-017-1667-7

Jílková, V., Šebek, O., & Frouz, J. (2012). Mechanisms of pH change in wood ant (*Formica polyctena*) nests. *Pedobiologia (Jena)*, *55*, 247–251. https://doi.org/10.1016/j.pedobi.2012.04.002

Johansson, H., Dhaygude, K., Lindström, S., Helanterä, H., Sundström, L., Trontti, K. (2013). A Metatranscriptomic Approach to the Identification of Microbiota Associated with the Ant *Formica exsecta*. *PLoS One*, *8*, e79777. https://doi.org/10.1371/journal.pone.0079777

Jurgensen, M. F., Finér, L., Domisch, T., Kilpeläinen, J., Punttila, P., Ohashi, M., Niemelä, P., Sundström, L., Neuvonen, S., & Risch, A. C. (2008). Organic mound-building ants: Their impact on soil properties in temperate and boreal forests. *Journal of Applied Entomology*, *132*, 266–275. https://doi.org/10.1111/j.1439-0418.2008.01280.x

Kaiser, K., Wemheuer, B., Korolkow, V., Wemheuer, F., Nacke, H., Schöning, I., Schrumpf, M., & Daniel, R. (2016). Driving forces of soil bacterial community structure, diversity, and function in temperate grasslands and forests. *Scientific Reports*, *6*, 33696. https://doi.org/10.1038/srep33696

Katzerke, A., Bliss, P., & Moritz, R. F. A. (2010). Size matters: mole (*Talpa europaea*) hills and nest-site selection of the ant *Formica exsecta*. *Population Ecology*, *52*, 271–277. https://doi.org/10.1007/s10144-009-0180-2

Kautz S., Rubin B. E. R., Russell J. A., & Moreau C. S. (2013). Surveying the Microbiome of Ants: Comparing 454 Pyrosequencing with Traditional Methods To Uncover Bacterial Diversity. *Applied and Environmental Microbiology*, *79*(2), 525–534. http://dx.doi.org/10.1128/aem.03107-12

Kilpeläinen, J., Finér, L., Niemelä, P., Domisch, T., Neuvonen, S., Ohashi, M., Risch, A. C., & Sundström, L. (2007). Carbon, nitrogen and phosphorus dynamics of ant mounds (*Formica rufa* group) in managed boreal forests of different successional stages. *Applied Soil Ecology*, *36*, 156–163. https://doi.org/10.1016/j.apsoil.2007.01.005

Kõljalg, U., Nilsson, R. H., Abarenkov, K., Tedersoo, L., Taylor, A. F. S., Bahram, M., Bates, S. T., Bruns, T. D., Bengtsson-Palme, J., Callaghan, T. M., Douglas, B., Drenkhan, T., Eberhardt, U., Dueñas, M., Grebenc, T., Griffith, G. W., Hartmann, M., Kirk, P. M., Kohout, P., … Larsson, K.-H. (2013). Towards a unified paradigm for sequence-based identification of fungi. *Molecular Ecology*, *22*, 5271–5277. https://doi.org/10.1111/mec.12481

Korkama-Rajala T., Müller M. M., & Pennanen T. (2008). Decomposition and Fungi of Needle Litter from Slow- and Fast-growing Norway Spruce (Picea abies) Clones. *Microbial Ecology*, *56*(1), 76–89. http://dx.doi.org/10.1007/s00248-007-9326-y

Koskella, B., & Bergelson, J. (2020). The study of host-microbiome (co) evolution across levels of selection. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *375*, 20190604. https://doi.org/10.1098/rstb.2019.0604

Laakso, J., Setälä, H., & Setala, H. (1998). Composition and trophic structure of detrital food web in ant nest mounds of *Formica aquilonia* and in the surrounding forest soil. *Oikos*, *81*, 266–278. https://doi.org/10.2307/3547047

Lata, J.-C., Ranjard, L., Dufrene, E., Maron, P.-A., Soudani, K., Damesin, C., Soudani, K., & Lata, J.-C. (2010). Seasonal dynamics of the bacterial community in forest soils under different quantities of leaf litter. *Applied Soil Ecology*, *47*, 14–23.

Lauber, C. L., Strickland, M. S., Bradford, M. A., & Fierer, N. (2008). The influence of soil properties on the structure of bacterial and fungal communities across land-use types. *Soil Biology & Biochemistry*, *40*, 2407–2415. https://doi.org/10.1016/j.soilbio.2008.05.021

Legendre, P., & Anderson, M. J. (1999). Distance-based redundancy analysis: Testing multispecies responses in multifactorial ecological experiments. *Ecological Monographs*, *69*, 1–24.

Lenoir, L., Persson, T., & Bengtsson, J. (2001). Wood ant nests as potential hot spots for carbon and nitrogen mineralisation. *Biology and Fertility of Soils*, *34*, 235–240. https://doi.org/10.1007/s003740100405

Lester P. J., Sébastien A., Suarez A. V., Barbieri R. F., & Gruber M. A. M. (2017). Symbiotic bacterial communities in ants are modified by invasion pathway bottlenecks and alter host behavior. *Ecology*, *98*(3), 861–874. http://dx.doi.org/10.1002/ecy.1714

Lindström, S., Rowe, O., Timonen, S., Sundström, L., & Johansson, H. (2018). Trends in bacterial and fungal communities in ant nests observed with Terminal-Restriction Fragment Length Polymorphism (T-RFLP) and Next Generation Sequencing (NGS) techniques—validity and compatibility in ecological studies. *PeerJ*, *6*, e5289. https://doi.org/10.7717/peerj.5289

Lindström, S., Timonen, S., Sundström, L., & Johansson, H. (2019). Ants reign over a distinct microbiome in forest soil. *Soil Biology & Biochemistry*, *139*, e107529. https://doi.org/10.1016/j.soilbio.2019.107529

Littlewood, N. A., & Young, M. R. (2008). A habitat suitability model for the narrow-headed ant, *Formica exsecta*, evaluated against independent data. *Insect Conservation and Diversity*, *1*, 108–113. https://doi.org/10.1111/j.1752-4598.2008.00014.x

Liu, W. T., Marsh, T. L., Cheng, H., & Forney, L. J. (1997). Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. *Applied and Environmental Microbiology*, *63*, 4516–4522. https://doi.org/10.1128/AEM.63.11.4516-4522.1997

Lladó, S., López-Mondéjar, R., & Baldrian, P. (2017). Forest soil bacteria: Diversity, involvement in ecosystem processes, and response to global change. *Microbiology and Molecular Biology Reviews*, *81*, e00063-16. https://doi.org/10.1128/MMBR.00063-16

Lladó, S., López-Mondéjar, R., & Baldrian, P. (2018). Drivers of microbial community structure in forest soils. *Applied Microbiology and Biotechnology*, 4331–4338. https://doi.org/10.1007/s00253-018-8950-4

Lucas, J., Bill, B., Stevenson, B., & Kaspari, M. (2017). The microbiome of the ant-built home: the microbial communities of a tropical arboreal ant and its nest. *Ecosphere*, *8*, e01639. https://doi.org/10.1002/ecs2.1639

Madden A. A., Grassetti A., Soriano J-A. N., & Starks P. T. (2013). Actinomycetes with Antimicrobial Activity Isolated from Paper Wasp (Hymenoptera: Vespidae: Polistinae) Nests. *Environmental Entomology*, *42*(4), 703–710. http://dx.doi.org/10.1603/en12159

Marchesi, J. R., Sato, T., Weightman, A. J., Martin, T. A., Fry, J. C., Hiom, S. J., & Wade, W. G. (1998). Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. *Applied and Environment Microbiology*, *64*, 795–799. https://doi.org/10.1128/AEM.64.2.795-799.1998

Margesin, R., & Miteva, V. (2011). Diversity and ecology of psychrophilic microorganisms. *Research in Microbiology*, *162*, 346–361. https://doi.org/10.1016/j.resmic.2010.12.004

Matarrita-Carranza, B., Moreira-Soto, R. D., Murillo-Cruz, C., Mora, M., Currie, C. R., & Pinto-Tomas, A. A. (2017). Evidence for widespread associations between neotropical hymenopteran insects and Actinobacteria. *Frontiers in Microbiology*, *8*, 1–17. https://doi.org/10.3389/fmicb.2017.02016

Mattoso T. C., Moreira D. D. O., & Samuels R. I. (2012). Symbiotic bacteria on the cuticle of the leaf-cutting ant Acromyrmex subterraneus protect workers from attack by entomopathogenic fungi. *Biology Letters*, *8*(3), 461–464. http://dx.doi.org/10.1098/rsbl.2011.0963

McMurdie, P. J., & Holmes, S. (2014). Waste not, want not: Why rarefying microbiome data is inadmissible. *PLoS Computational Biology*, *10*, e100531. https://doi.org/10.1371/journal.pcbi.1003531

Moreira, E. A., Alvarez, T. M., Persinoti, G. F., Paixão, D. A. A., Menezes, L. R., Cairo, J. P. F., Squina, F. M., Costa-Leonardo, A. M., Carrijo, T., & Arab, A. (2018). Microbial Communities of the Gut and Nest of the Humus- and Litter-Feeding Termite *Procornitermes araujoi* (Syntermitinae). *Current Microbiology*, *75*, 1609–1618. https://doi.org/10.1007/s00284-018-1567-0

Nacke, H., Goldmann, K., Schöning, I., Pfeiffer, B., Kaiser, K., Villamizar, G. A. C., Schrumpf, M., Buscot, F., Daniel, R., & Wubet, T. (2016). Fine spatial scale variation of soil microbial communities under European beech and Norway spruce. *Frontiers in Microbiology*, *7*, article 2067.

Oksanen, J., Blanchet, G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P., O'Hara, R., Simpson, G., Solymos, P., Stevens, H., Szoecs, E., & Wagner, H. (2017). *Community Ecology Package 'vegan' v. 2.4-5*. https://github.com/vegandevs/vegan

Pagnocca F. C., Rodrigues A., Nagamoto N. S., & Bacci M. (2008). Yeasts and filamentous fungi carried by the gynes of leaf-cutting ants. *Antonie van Leeuwenhoek*, *94*(4), 517–526. http://dx.doi.org/10.1007/s10482-008-9268-5

Pamilo, P. (1991). Life span of queens in the ant *Formica exsecta*. *Insectes Sociaux*, *38*, 111–119. https://doi.org/10.1007/BF01240961

Prescott, C. E., & Grayston, S. J. (2013). Tree species influence on microbial communities in litter and soil: Current knowledge and research needs. *Forest Ecology and Management*, *309*, 19–27. https://doi.org/10.1016/j.foreco.2013.02.034

Pershina E. V., Ivanova E. A., Korvigo I. O., Chirak E. L., Sergaliev N. H., Abakumov E. V., Provorov N. A., & Andronov E. E. (2018). Investigation of the core microbiome in main soil types from the East European plain. *Science of The Total Environment*, *631-632*, 1421–1430. http://dx.doi.org/10.1016/j.scitotenv.2018.03.136

Promnuan Y., Kudo T., & Chantawannakul P. (2009). Actinomycetes isolated from beehives in Thailand. *World Journal of Microbiology and Biotechnology*, *25*(9), 1685–1689. http://dx.doi.org/10.1007/s11274-009-0051-1

Purahong W., Wubet T., Lentendu G., Schloter M., Pecyna M. J., Kapturska D., Hofrichter M., Krüger D., & Buscot F. (2016). Life in leaf litter: novel insights into community dynamics of bacteria and fungi during litter decomposition. *Molecular Ecology*, *25*(16), 4059–4074. http://dx.doi.org/10.1111/mec.13739

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research*, *41*, 590–596. https://doi.org/10.1093/nar/gks1219

Rankinen, K., Karvonen, T., & Butterfield, D. (2004). A simple model for predicting soil temperature in snow-covered and seasonally frozen soil. *Hydrology and Earth System Sciences*, *8*, 706–716.

Rasche, F., Knapp, D., Kaiser, C., Koranda, M., Kitzler, B., Zechmeister-Boltenstern, S., Richter, A., & Sessitsch, A. (2011). Seasonality and resource availability control bacterial and archaeal communities in soils of a temperate beech forest. *ISME Journal*, *5*, 389–402. https://doi.org/10.1038/ismej.2010.138

Ratzke, C., Denk, J., & Gore, J. (2018). Ecological suicide in microbes. *Nature Ecology & Evolution*, *2*, 867–872. https://doi.org/10.1038/s41559-018-0535-1

Reber, A., Purcell, J., Buechel, S. D., Buri, P., & Chapuisat, M. (2011). The expression and impact of antifungal grooming in ants. *Journal of Evolutionary Biology*, *24*, 954–964. https://doi.org/10.1111/j.1420-9101.2011.02230.x

Reyes R. D. H., & Cafaro M. J. (2015). Paratrechina longicornisants in a tropical dry forest harbor specific Actinobacteria diversity. *Journal of Basic Microbiology*, *55*(1), 11–21. http://dx.doi.org/10.1002/jobm.201300785

Rosengren, R., Fortelius, W., Lindström, K., & Luther, A. (1987). Phenology and causation of nest heating and thermoregulation in red wood ants of the *Formica rufa* group studied in coniferous forest habitats in southern Finland. *Annales Zoologici Fennici*, *24*, 147–155.

Rousk, J., & Bååth, E. (2011). Growth of saprotrophic fungi and bacteria in soil. *FEMS Microbiology Ecology*, *78*, 17–30. https://doi.org/10.1111/j.1574-6941.2011.01106.x.

Santalahti, M., Sun, H., Jumpponen, A., Pennanen, T., & Heinonsalo, J. (2016). Vertical and seasonal dynamics of fungal communities in boreal Scots pine forest soil of fungi in boreal forests. *FEMS Microbiology Ecology*, *92*, 1–31. https://doi.org/10.1093/femsec/fiw170

Santos A. V., Dillon R. J., Dillon V. M., Reynolds S. E., & Samuels R. I. (2004). Ocurrence of the antibiotic producing bacterium Burkholderia sp. in colonies of the leaf-cutting ant Atta sexdens rubropilosa. *FEMS Microbiology Letters*, *239*(2), 319–323. http://dx.doi.org/10.1016/j.femsle.2004.09.005

Schimel, J. P., & Schaeffer, S. M. (2012). Microbial control over carbon cycling in soil. *Frontiers in Microbiology*, *3*, 1–11. https://doi.org/10.3389/fmicb.2012.00348

Seifert, B. (2011). *A taxonomic revision of the* Formica cinerea *group (Hymenoptera: Formicidae)* (1st ed.). Nabu Press.

Seipke, R. F., Kaltenpoth, M., & Hutchings, M. I. (2012). Streptomyces as symbionts: An emerging and widespread theme? *FEMS Microbiology Reviews*, *36*, 862–876.

Shade, A., & Handelsman, J. (2012). Beyond the Venn diagram: The hunt for a core microbiome. *Environmental Microbiology*, *14*, 4–12. https://doi.org/10.1111/j.1462-2920.2011.02585.x

Sietiö, O. M., Tuomivirta, T., Santalahti, M., Kiheri, H., Timonen, S., Sun, H., Fritze, H., & Heinonsalo, J. (2018). Ericoid plant species and *Pinus sylvestris* shape fungal communities in their roots and surrounding soil. *New Phytologist*, *218*, 738–751.

Silvia, P., Actis-Perino, E., Perugini, J., & Bonfante, P. (1995). Molecular diversity of ericoid mycorrhizal fungi. *Canadian Journal of Botany*, *5*, 123–131.

Sorensen, P. O., Germino, M. J., & Feris, K. P. (2013). Microbial community responses to 17 years of altered precipitation are seasonally dependent and coupled to co-varying effects of water content on vegetation and soil C. *Soil Biology & Biochemistry*, *64*, 155–163. https://doi.org/10.1016/j.soilbio.2013.04.014

Strickland, M. S., Lauber, C., Fierer, N., & Bradford, M. A. (2009). Testing the functional significance of microbial community composition. *Ecology*, *90*, 441–451. https://doi.org/10.1890/08-0296.1

Sundstrom, L., Chapuisat, M., & Keller, L. (1996). Conditional manipulation of sex ratios by ant workers: A test of kin selection theory. *Science*, *274*, 993–995. https://doi.org/10.1126/science.274.5289.993

Tecon, R., & Or, D. (2017). Biophysical processes supporting the diversity of microbial life in soil. *FEMS Microbiology Reviews*, *39*, 599–623. https://doi.org/10.1093/femsre/fux039

Timonen, S., Sinkko, H., Sun, H., Sietiö, O. M., Rinta-Kanto, J. M., Kiheri, H., & Heinonsalo, J. (2017). Ericoid roots and mycospheres govern plant-specific bacterial communities in boreal forest humus. *Microbial Ecology*, *73*, 939–953. https://doi.org/10.1007/s00248-016-0922-6

Ugelvig, L. V., Kronauer, D. J. C., Schrempf, A., Heinze, J., & Cremer, S. (2010). Rapid anti-pathogen response in ant societies relies on high genetic diversity. *Proceedings of the Royal Society B: Biological Sciences*, *277*, 2821–2828. https://doi.org/10.1098/rspb.2010.0644

VanBorm S., Buschinger A., Boomsma J. J., & Billen J. (2002). Tetraponeraants have gut symbionts related to nitrogen–fixing root–nodule bacteria. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *269*(1504), 2023–2027. http://dx.doi.org/10.1098/rspb.2002.2101

Vander Meer, R. (2012). Ant interactions with soil organisms and associated semiochemicals. *Journal of Chemical Ecology*, *38*, 728–745. https://doi.org/10.1007/s10886-012-0140-8

Vitikainen, E., Haag-Liautard, C., & Sundström, L. (2011). Inbreeding and reproductive investment in the ant *Formica exsecta*. *Evolution*, *65*, 2026–2037. https://doi.org/10.1111/j.1558-5646.2011.01273.x

Vitikainen, E. I. K., Haag-Liautard, C., & Sundström, L. (2015). Natal dispersal, mating patterns, and inbreeding in the ant *Formica exsecta*. *American Naturalist*, *186*, 716–727.

Voulgari-Kokota, A., Grimmer, G., Steffan-Dewenter, I., & Keller, A. (2019). Bacterial community structure and succession in nests of two megachilid bee genera. *FEMS Microbiology Ecology*, *95*, fiy218. https://doi.org/10.1093/femsec/fiy218

Weisburg, W. G., Barns, S. M., Pelletier, D. A., & Lane, D. J. (1991). 16S ribosomal DNA amplification for phylogenetic study. *Journal of Bacteriology*, *173*, 697–703. https://doi.org/10.1128/JB.173.2.697-703.1991

White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Gelfard, J. J. Sninsky, & T. White (Eds.), *PCR protocols: A guide to methods and application* (pp. 315–322). Academic Press.

Yamoah E., Jones E. E., Weld R. J, Suckling D. M., Waipara N., Bourdôt G. W, Hee A. K. W., & Stewart A. (2008). Microbial population and diversity on the exoskeletons of four insect species associated with gorse (Ulex europaeus L.). *Australian Journal of Entomology*, *47*(4), 370–379. http://dx.doi.org/10.1111/j.1440-6055.2008.00655.x

Zhou, X., Guo, Z., Chen, C., & Jia, Z. (2017). Soil microbial community structure and diversity are largely influenced by soil pH and nutrient quality in 78-year-old tree plantations. *Biogeosciences*, *14*, 2101–2111. https://doi.org/10.5194/bg-14-2101-2017

Žifčáková, L., Větrovský, T., Howe, A., & Baldrian, P. (2016). Microbial activity in forest soil reflects the changes in ecosystem properties between summer and winter. *Environmental Microbiology*, *18*, 288–301. https://doi.org/10.1111/1462-2920.13026

How to cite this article: Lindström, S., Timonen, S., & Sundström, L. (2021). The bacterial and fungal community composition in time and space in the nest mounds of the ant *Formica exsecta* (Hymenoptera: Formicidae). *MicrobiologyOpen*. 2021;10:e1201. https://doi.org/10.1002/mbo3.1201

APPENDIX 1

TABLE A1 The number of reads, the number of OTUs identified from the reads, and the inferred number of taxa identified at the level of genus or any higher taxonomic level (GOH)

	Bacteria		Fungi	
	Nests	Soil	Nests	Soil
Reads				
Unrarefied	1,371,127	1,061,232	1,765,128	199,875
Rarefied	633,204	219,186	382,018	131,017
OTUs				
2013	11,248		2435	
2014	12,484		3132	
2015	9848	3776	2922	3764
Total	33,580	3776	8489	3764
No. GOH taxa				
2013	331		233	
2014	311		235	
2015	309	340	192	216
Unique	383		294	

TABLE A2 Average (SD) species diversity and richness indices across years or months

	Shannon–Wiener *H′*		Number		
Sampling time	OTU	T-RF	Taxa (GOH)	T-RFs	OTUs
Bacteria					
2013	3.81 (0.59)	2.21 (0.93)	187 (66)	20 (12)	1406 (601)
2014	3.62 (0.59)	2.73 (0.29)	176 (61)	39 (12)	1387 (551)
2015	3.61 (0.55)	2.37 (0.24)	166 (60)	18 (6)	1094 (392)
May (2013–2015)	3.78 (0.61)	2.35 (0.62)	184 (61)	22 (14)	1432 (584)
June (2013–2015)	3.74 (0.60)	2.45 (0.76)	188 (66)	28 (17)	1436 (559)
August (2013–2015)	3.50 (0.48)	2.55 (0.24)	157 (56)	28 (11)	1023 (318)
Fungi					
2013	2.82 (0.48)	2.20 (0.62)	96 (29)	19 (12)	304 (127)
2014	2.56 (0.57)	2.52 (0.34)	104 (25)	30 (13)	348 (127)
2015	2.28 (0.51)	2.37 (0.72)	87 (16)	27 (11)	325 (66)
May (2013–2015)	2.30 (0.71)	2.40 (0.71)	96 (30)	28 (14)	330 (114)
June (2013–2015)	2.64 (0.46)	2.31 (0.40)	107 (23)	23 (11)	383 (89)
August (2013–2015)	2.70 (0.39)	2.39 (0.63)	85 (15)	26 (13)	272 (96)

TABLE A3 Bacterial taxa present in all nest samples (45 taxa, $n = 26$) 2013–2015, compared to their presence in reference soil samples ($n = 9$) in 2015, and, according to literature, the association of the taxa with ants

					2013–15 Nests		Enrichment		2015 Reference soils			
Phylum	Class	Order	Family	Genus	Reads	% of TA	Fold difference	*p*-Value	Presence	Reads	% of TA	Association
Acidobacteria	Acidobacteria_Gp1			Acidobacteria_Gp1	48,732	7.7	0.95		9/9	17,796	8.12	Decomposers of cellulose and chitin[1]
Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae	Granulicella	28,160	4.45	0.60		9/9	16,326	7.45	Decomposers of cellulose and chitin[1]
Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae	Terriglobus	3449	0.54	18.00	0.35	9/9	72	0.03	Decomposers of cellulose and chitin[1]
Acidobacteria	Acidobacteria_Gp3			Acidobacteria_Gp3	1981	0.31	0.40	**0.006**	9/9	1703	0.78	Decomposers of cellulose and chitin[1]
Actinobacteria	Actinobacteria	Acidimicrobiales	Acidimicrobineae I.s.	Aciditerrimonas	1382	0.22	0.51		9/9	953	0.43	
Actinobacteria	Actinobacteria	Acidimicrobiales			4773	0.75	0.63		9/9	2604	1.19	
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae		7962	1.26	2.00	0.07	9/9	1375	0.63	
Actinobacteria	Actinobacteria	Actinomycetales	Mycobacteriaceae	Mycobacterium	28,739	4.54	2.55	0.14	9/9	3898	1.78	Ants, bees, paper wasps[2, 3, 4, 5, 6, 7, 8]
Actinobacteria	Actinobacteria	Actinomycetales	Pseudonocardiaceae	Actinomycetospora	2277	0.36	36.00	**<0.0001**	3/9	19	0.01	Ants; core indicators of ant nests; bees, paper wasps[2, 3, 4, 5, 6, 7, 8, 9]
Actinobacteria	Actinobacteria	Actinomycetales	Pseudonocardiaceae		8122	1.28	7.11	**0.005**	5/9	396	0.18	
Actinobacteria	Actinobacteria	Actinomycetales	Streptomycetaceae	Streptomyces	10,230	1.62	8.10	**0.001**	5/9	447	0.2	Ants; core indicators of ant nests; bees, paper wasps [2, 3, 4, 5, 6, 7, 8, 9]
Actinobacteria	Actinobacteria	Actinomycetales	Streptomycetaceae		6300	0.99	6.19	**0.004**	6/9	350	0.16	
Actinobacteria	Actinobacteria	Actinomycetales			50,575	7.99	2.26	0.03	9/9	7748	3.53	
Actinobacteria	Actinobacteria	Solirubrobacterales	Conexibacteraceae	Conexibacter	1965	0.31	1.82	**0.001**	9/9	376	0.17	Ants, bees, paper wasps [2, 3, 4, 5, 6, 7, 8]
Actinobacteria	Actinobacteria	Solirubrobacterales			20,050	3.17	1.54	**0.021**	9/9	4508	2.06	
Actinobacteria	Actinobacteria				6943	1.1	0.71		9/9	3408	1.55	
Armatimonadetes	Armatimonadia	Armatimonadales	Armatimonadaceae	Armatimonadetes_gp1	2339	0.37	1.12		9/9	732	0.33	
Bacteroidetes	Sphingobacteria	Sphingobacteriales	Chitinophagaceae		8156	1.29	0.37	**0.006**	9/9	7563	3.45	Ants[10]
Bacteroidetes	Sphingobacteria	Sphingobacteriales	Sphingobacteriaceae	Mucilaginibacter	12,200	1.93	0.93		9/9	4543	2.07	Leaf and litter decomposer[11]
Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	Singulisphaera	466	0.07	0.88		9/9	181	0.08	
Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae		353	0.06	0.35	**0.011**	9/9	365	0.17	
Proteobacteria	Alphaproteobacteria	Alphaproteobacteria I.s.	Alphaproteobacteria I.s.	Rhizomicrobium	10,123	1.6	0.43	0.10	9/9	8184	3.73	Ants[3, 10, 12, 13]
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Brevundimonas	3385	0.53	2.79	0.09	9/9	423	0.19	Ants[3, 10, 12, 13]
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	11,615	1.83	0.85		9/9	4718	2.15	Ants[3, 10, 12, 13]

(Continues)

TABLE A3 (Continued)

Phylum	Class	Order	Family	Genus	2013–15 Nests Reads	2013–15 Nests % of TA	Enrichment Fold difference	Enrichment *p*-Value	2015 Reference soils Presence	2015 Reference soils Reads	2015 Reference soils % of TA	Association
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Phenylobacterium	5595	0.88	0.62		9/9	3090	1.41	Ants[3, 10, 12, 13]
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae		9489	1.5	1.09		9/9	3025	1.38	Some species symbiotic with ants[9, 13, 14]
Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae		2047	0.32	4.57	**0.001**	4/9	145	0.07	Plants and lichen[15, 16, 17]
Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae		18,649	2.95	0.63		9/9	10,191	4.65	Some species symbiotic with ants; ass w plants and lichen[13, 14, 15, 16, 17]
Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	Methylobacterium	6228	0.98	16.33	**<0.00011**	9/9	123	0.06	Ants; core indicator of ant nests [3, 9, 10, 12, 13,
Proteobacteria	Alphaproteobacteria	Rhizobiales			13,811	2.18	0.54		9/9	8925	4.07	
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae		36,034	5.69	2.47	**0.009**	9/9	5039	2.3	Some species symbiotic with ants; core indicators of nest; in transcriptome [9, 13, 14, 18]
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae		643	0.1	0.25	**0.018**	9/9	875	0.4	Plants and lichen [15, 16, 17]
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	9864	1.56	1.58	0.43	9/9	2165	0.99	Ants [3, 10, 12, 13]
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae		5931	0.94	1.84	0.48	9/9	1107	0.51	
Proteobacteria	Alphaproteobacteria				12,624	1.99	0.42	**0.024**	9/9	10,447	4.77	
Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	29,332	4.63	3.24	**0.024**	9/9	3145	1.43	Ants; core indicator of nests; in transcriptome[4, 9, 18, 19, 20]
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Massilia	14,075	2.22	3.70	**0.001**	4/9	1314	0.6	Decomposer[21]
Proteobacteria	Betaproteobacteria	Burkholderiales			1916	0.3	0.53	0.05	9/9	1259	0.57	
Proteobacteria	Deltaproteobacteria	Myxococcales			2720	0.43	0.44	0.05	9/9	2133	0.97	
Proteobacteria	Deltaproteobacteria				341	0.05	0.18	**0.0005**	9/9	611	0.28	
Proteobacteria	Gammaproteobacteria				2301	0.36	0.13	**<0.0001**	9/9	6196	2.83	
Proteobacteria					3947	0.62	0.42		9/9	3245	1.48	
TM7				TM7_genera I.s.	3386	0.53	0.85		9/9	1349	0.62	
Verrucomicrobia	Opitutae	Opitutales	Opitutaceae	Opitutus	1379	0.22	0.19	**0.001**	9/9	2563	1.17	Symbiotic, N2 fixation[22]
Not identified					14,528	2.29	0.55		9/9	9064	4.14	

Note: The *p*-values refer to repeated measures ANOVA based on data from 2015 only and conducted on taxa that showed either a 1.5-fold or higher, or a 0.5-fold or lower prevalence in nests than in reference soils. Significant differences after correction for false discovery rate are in boldface.

1. Baldrian et al. (2012); 2. Barke et al. (2010); 3. Ishak et al. (2011); 4. Kautz et al. (2013); 5. Mattoso et al. (2012); 6. Reyes and Cafaro (2015); 7. Promnuan et al. (2009); 8. Madden et al. (2013); 9. Lindström et al. (2019); 10. Lucas et al. (2017); 11. Nacke et al. (2016); 12. Jaffe et al. (2001); 13. Lester et al. (2017); 14. Brown and Wernegreen (2016); 15. Aschenbrenner et al. (2017); 16. Pershina et al.; 2018. Sietiö et al. (2018); 18. Johansson et al. (2013); 19. Santos et al. (2004); 20. Van Borm et al. (2002); 21. Purahong et al. (2016); 22. Anderson et al. (2012).

TABLE A4 Fungal taxa present in all nest samples (16 taxa, *n* = 26) in 2013–2015, compared to their presence in reference soil samples (*n* = 9) in 2015; and according to literature, the association of the taxa with ants

					2013–2015 Nests		Enrichment		2015 Reference soils			
Phylum	Class	Order	Family	Genus	Reads	% of TA	Fold difference	*p*-Value	Presence	Reads	% of TA	Association
Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae	Cladosporium	9866	2.58	1.06		9/9	3187	2.43	Exoskeleton, core indicator of nests[1, 2]
Ascomycota	Dothideomycetes	Dothideomycetes l.s.	Myxotrichaceae	Oidiodendron	67,417	17.65	7.48	0.20	9/9	3096	2.36	Decomposer of recalcitrant litter; core indicator of ant nests[2, 3, 4]
Ascomycota	Dothideomycetes	Pleosporales	Venturiaceae	Venturia	5097	1.33	0.88		9/9	1981	1.51	Soil[5,6]
Ascomycota	Dothideomycetes	Pleosporales			8365	2.19	1.20		6/9	2395	1.83	
Ascomycota	Dothideomycetes				3101	0.81	1.25		8/9	847	0.65	
Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	Exophiala	2415	0.63	3.71	0.39	6/9	224	0.17	Exoskeleton of ants; core indicator of ant nests[2,7]
Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	Penicillium	5182	1.36	12.36	0.12	8/9	144	0.11	Soil
Ascomycota	Leotiomycetes	Helotiales			6935	1.82	0.42	0.002	9/9	5653	4.31	
Ascomycota	Leotiomycetes	Rhytismatales	Rhytismataceae	Lophodermium	1411	0.37	0.64		9/9	756	0.58	Endophytes of spruce needles [8]
Ascomycota	Leotiomycetes				8386	2.2	0.19	0.09	9/9	14881	11.36	
Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	4164	1.09	4.19	0.45	9/9	345	0.26	Soil[5,6]
Ascomycota	Sordariomycetes				4576	1.2	2.03	0.92	9/9	771	0.59	
Ascomycota					635,57	16.64	0.77		9/9	28,366	21.65	
Basidiomycota	Tremellomycetes	Tremellales	Tremellales l.s.	Cryptococcus_g1	2817	0.74	0.20	0.12	9/9	4797	3.66	Ants; in transcriptome[9, 10, 11]
Basidiomycota					1821	0.48	0.68		9/9	924	0.71	
Not identified					6927	1.81	0.19		9/9	12619	9.63	

Note: The *p*-values refer to repeated measures ANOVA based on data from 2015 only and conducted on taxa that showed either a 1.5-fold or higher, or a 0.5-fold or lower prevalence in nests than in reference soils. Significant differences after correction for false discovery rate are in boldface.

1. Yamoah et al. (2008); 2. Lindström et al. (2019); 3. Davey and Currah (2006); 4. Silvia et al. (1995); 5. Druzhinina et al. (2011); 6. Duff et al. (2016); 7. Duarte et al. (2014); 8. Korkama-Rajala et al. (2008); 9. Ba and Phillips (1996); 10. Johansson et al. (2013); 11. Pagnocca et al. (2008).

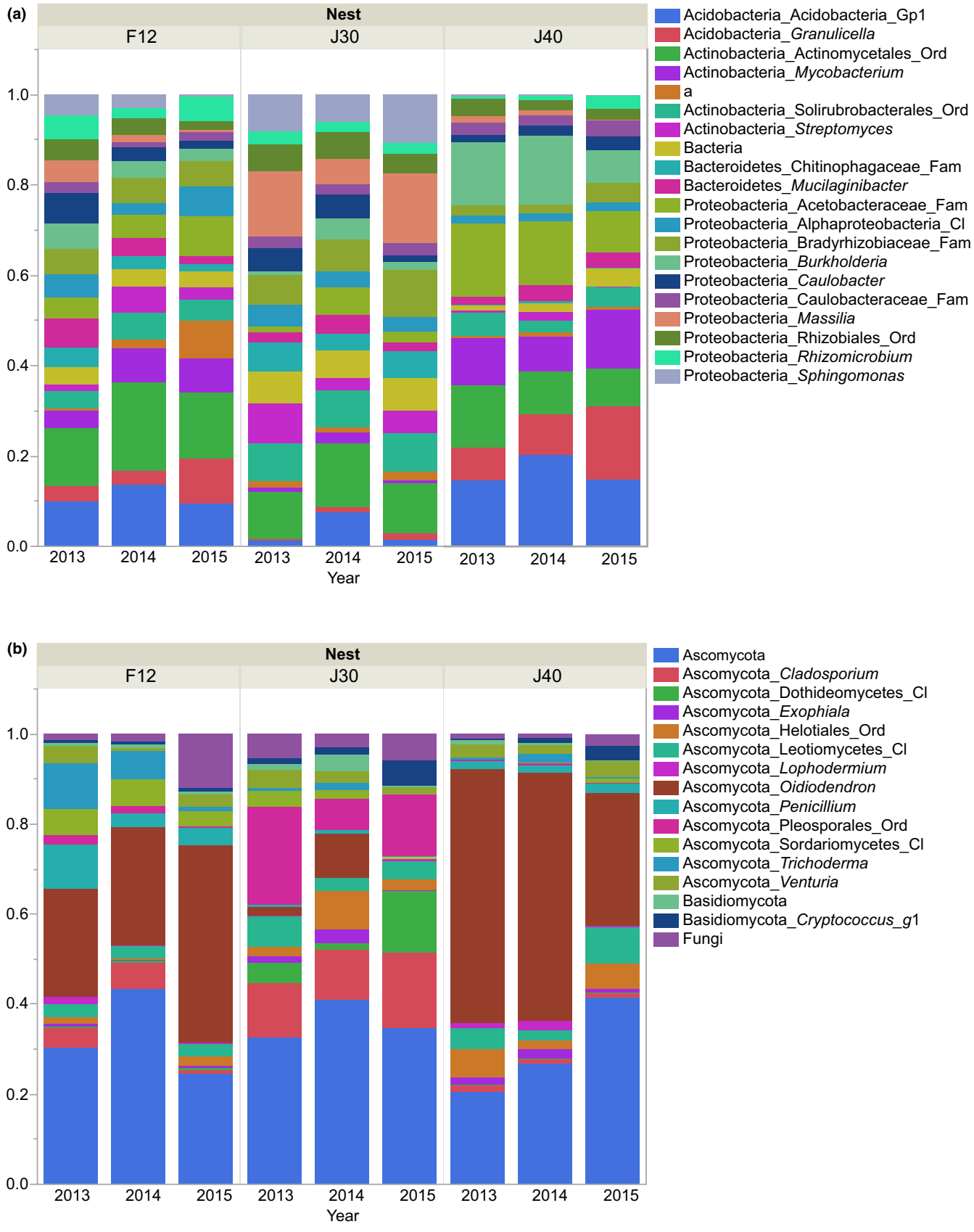

FIGURE A1 Proportional abundances of the 20 most abundant bacterial (a), and all fungal (b) GOH-taxa, that were consistently detected in all nest samples or reference soil samples.