Review Article

Title

Ground-nesting ants as architects of microbial landscapes

Name(s) of author(s)

Jacob Longmeyer, Nikesh Dahal, Nicholas Medina, John Vandermeer

Key words: ants, soil, microbe, landscape, dispersal, ant-microbe interactions, zoogeochemistry, review

Address(es) of author(s)

Jacob Longmeyer (contact author), Department of Ecology & Evolutionary Biology, University of Michigan, Biological Sciences Building, 1105 North University Avenue, Ann Arbor, MI 48109. E-mail: jalo@umich.edu
Nikesh Dahal, Department of Ecology & Evolutionary Biology, University of Michigan, Biological Sciences Building, 1105 North University Avenue, Ann Arbor, MI 48109. E-mail: nikeshd@umich.edu
Nicholas Medina, Center for Tree Science, The Morton Arboretum, 4100 Illinois Route 53 Lisle, IL 60532. Department of Ecology & Evolutionary Biology, University of Michigan, Biological Sciences Building, 1105 North University Avenue, Ann Arbor, MI 48109. nmedina@mortonarb.org
John Vandermeer, Department of Ecology & Evolutionary Biology, University of Michigan, Biological Sciences Building, 1105 North University Avenue, Ann Arbor, MI 48109. E-mail: jvander@umich.edu

Number of pages: 32
Number of figures: 0
Number of tables: 1

Abstract

As soil ecosystem engineers, ground-nesting ants alter various physicochemical properties of soils globally. However, less is known about how ant behaviors including bioturbation and hygiene affect microbial communities, such as by altering habitat complexity, resource availability, and competitive dynamics. Interactions such as this are increasingly recognized as important components in shaping the structure and function of ecological communities. In this review, we summarize current knowledge on the ecological consequences of ant-driven processes for soil microbial biodiversity patterns and dynamics. We evaluate the impacts of ants on microbial communities across scales, from effects within the ant nest, beyond the nest into foraging areas, and ultimately across landscapes. We highlight that ants maintain close symbioses with various fungal and bacterial species, selectively employ species-specific antibiotics, maintain abiotic gradients through nest construction and foraging tunnel activities, create mosaics of differential selection on microbes across landscapes, and also act as dispersal agents for microbes. We also present commonly used methods in microbial ecology for capturing and quantifying microbial community characteristics, for myrmecologists interested in exploring new avenues of research and collaborations within this emerging field.

Introduction

Understanding how species interactions influence population dynamics and community diversity is a central goal of ecology (May 1972, Chesson 2000). In natural ecosystems, interactions occur across biological scales of organization, including animals and microorganisms, shaping species persistence and biodiversity patterns (Janzen 1977). Recent research has shown that microbial populations can affect animals by influencing the timing of key life-history events (Metcalf & al. 2019) and fitness consequences through interaction effects (Knutie & al. 2017, Gould & al. 2018). Similarly, microbial interactions themselves have long been recognized as critical for ecosystem functions, given their central roles in energy flow and matter cycling (Lindeman 1942, Azam & Malfatti 2007, Falkowski & al. 2008, Cordero & Datta 2016). However, the reciprocal aspect of these interactions – how do animals influence microbial community dynamics and diversity patterns? – remains poorly understood.

Animals play a key role in shaping ecosystem structure and function through both consumptive and non-consumptive effects. Historically, ecological theory emphasized the role of consumptive interactions like predation and herbivory in regulating population and diversity patterns (Hairston & al. 1960, Paine 1966, Oksanen & al. 1981). But more recent work has expanded on these frameworks to include non-consumptive effects, such as trait-mediated interactions and habitat modification, which can cascade through ecological communities and influence community structure and composition (Werner & Peacor 2003, Schmitz & al. 2004, Peckarsky & al. 2008, Schmitz 2017). These diffuse, cross-scale interactions are now central to the emerging field of *zoogeochemistry*, which examines the impacts of animals on microbially-mediated processes like carbon storage and nutrient cycling (Vanni 2002, Estes & al. 2011, Schmitz & al. 2018, Leroux & Schmitz 2025). Within this context, ants emerge as especially important organisms due to their remarkable abundance and widespread distribution. Recent estimates suggest that there are about 20 quadrillion ant individuals worldwide with a total biomass of 12 megatons dry carbon, which exceeds that of all wild birds and mammals combined (Schultheiss & al. 2022). These patterns, coupled with their status as ecosystem engineers through intimate associations with the biophysical matrix of the soil (Jones & al. 1996, Lavelle & al. 2006, Viles & al. 2021, Wu & al. 2025), position ants as key animals that may exert biotic controls on microbial diversity and processes via higher order interactions.

Yet, the outcomes of such animal-driven processes can vary widely depending on environmental context (Schmitz & al. 2015). As such, context-dependency, or the tendency for the strength and direction of interactions to vary based on environmental conditions, is increasingly recognized as a key feature of ecological systems (Bertness & Callaway 1994, Catford & al. 2022). In microbial ecology, community assembly processes and diversity patterns are often highly contingent on both biotic and abiotic factors (Hoek & al. 2016, Bittleston & al. 2020). This variability extends to animal-microbe systems, where local environmental conditions play a crucial role in shaping both the outcomes of species interactions (Dahal & al. 2023) and their broader ecosystem effects (Li & al. 2021, Meyer & Leroux 2023, Vanderploeg & al. 2023). For example, a global meta-analysis by (McCary & Schmitz 2021) found that invertebrate detritivores with bioturbating traits increased decomposition rates by 28% and soil nitrogen availability by 99%. But the effect sizes were found to be substantially variable, highlighting how ecological consequences of even functionally similar organisms can be strongly shaped by contingent factors. These sensitivities are particularly relevant for small-bodied organisms like invertebrates and microbes, whose population dynamics are more prone to nonlinear and potentially chaotic dynamics (Rogers & al. 2022). Such dynamic instability may help explain the pronounced context-dependency observed in these groups.

Among invertebrates, ants represent a particularly compelling yet underexplored model for studying the consequences of animal-driven processes on microbial community assembly, structure, and function. Through localized behaviors, such as bioturbation, nest construction, and foraging, ants alter habitat complexity, resource availability, and competitive dynamics, thereby providing a dynamic interface for exploring zoogeochemical processes (Hölldobler & Wilson 1990, Folgarait 1998, Jouquet & al. 2006, Frouz & Jilková 2008). Similarly, their high abundance and broad global biogeographic distribution affords both observational and experimental studies with natural populations across a range of environmental gradients (Schultheiss & al. 2022). Importantly, ants operate at scales large enough to produce landscape-level effects, yet small enough to allow spatiotemporal tracking, which may not be readily feasible when working with larger animals. Finally, extensive literature on ant natural history and ecology has revealed emergent properties, ranging from keystone intransitivities to spatially clustered networks of pest control, that makes ants ideal systems for probing the links between cross-scale interactions and biodiversity maintenance in highly heterogeneous and microbially diverse soil environments (Way & Khoo 1992, Vandermeer & al. 2008, Lavelle & al. 2016, Vandermeer & Perfecto 2023).

Ground-nesting ants are a diverse group of animals found across various ecosystems, ranging from forests and grasslands to deserts and urban areas, and include well-known genera such as *Solenopsis*, *Atta*, *Acromyrmex*, *Formica*, *Lasius*, *Pheidole*, *Pogonomyrmex*, and *Tetramorium*, among many others. These ants typically construct elaborate networks of subterranean tunnels and chambers that range from a few centimeters to several meters deep, and provide them protection from predators and environmental stressors (Tschinkel 2003, Tschinkel 2015). Additionally, ground-nesting ants play important ecological roles by influencing nutrient cycling, soil structure formation, seed dispersal, arthropod and plant community structure, crop yields, and various other ecosystem services (HANDEL & BEATTIE 1990, KRUSHELNYCKY & GILLESPIE 2008, EVANS & al. 2011, DEL TORO & al. 2012, OFFENBERG 2015, VILES & al. 2021, WU & al. 2025).

In this review, we summarize current knowledge on the ecological consequences of ant-driven processes for soil microbial biodiversity patterns and dynamics. We distinguish the impacts of ants within and beyond their nests, as most research to date has focused on the former, even though ant behaviors and activities differ dramatically over this dynamic boundary. We also present a table of commonly used methods in microbial ecology for capturing and quantifying microbial communities. This list is intended as a synthesized resource to guide myrmecologists who are interested in exploring and collaborating on various aspects of ant-microbial interactions. Finally, we also explore the implications of ant-microbial interactions for spatial patterns of microbe diversity in soil, underscoring the potential role of ants in microbial dispersal and metapopulation dynamics across heterogeneous landscapes.

1. Within The Nest: Ground-nesting ants most directly shape soil microbial communities within the bounds of their nests, via localized behaviors, namely hygiene and bioturbation of soils (WILKINSON & al. 2009). To regulate homeostatic nest conditions, ants modify the physical environment to create biogenic structures, gather food from foraging areas, engage in individual and peer hygienic behaviors, and use glands that produce antibiotic defense compounds. Ultimately, these mechanisms can alter local soil physical, chemical, and biological properties (URBAŃCZYK & SZULC 2023), including soil structure, resource availability, habitat complexity, and microbial community and/or biofilm dynamics. Additionally, in contrast to some traditional views in ecology that tend to ignore microbial diversity, we suggest how more detailed microbial community ecology can be important for overall soil functions and nutrient dynamics.

1.1. Fungus Cultivation: The classic and most well-studied example (CURRIE & al. 1999, CALDERA & al. 2009, SUEN & al. 2011, ALLEN & al. 2023) of ant effects on soil microbial communities is the mutualism that, similar to other insect-fungal mutualists (JOSEPH & KEYHANI 2021), co-evolved *ca.* 66 million years ago between fungus-growing ants (tribe Attini) and their cultivated nest symbiont fungi, which digest the leaf tissues gathered to then serve as ant food (HÖLLDOBLER & WILSON 1990, SCHULTZ & al. 2024). Ant-fungus mutualisms are widespread, present in over 200 species of ants, with the leaf-cutting ant group containing over 70 species, although much research has focused on *Atta* and *Acromyrmex* (CALDERA & al. 2009). Their basidiomycete fungal cultivars are similarly diverse, with nearly 300 ant-cultivated fungal species in the order Agaricales, and a notable focus on the Leucocoprinae fungal tribe (SCHULTZ & al. 2024). Past research has focused on ant-fungal co-evolution (NORTH & al. 1997), which finds interesting patterns: variable specificity in the mutualism pairing, ranging from the evolution of ant colony-level fungal lineages (MUELLER & al. 2011), the detection of broad fungal functional groups based on grass- or dicot-feeding ant preferences; and sister clades of fungi being cultured by closely-related non-leaf cutting ants, such as *Trachymyrmex* (MUELLER & al. 2017). Within a nest, fungal lineages are mostly cultivated as monocultures (MUELLER & al. 2010), which ant workers actively curate by maintaining hygiene of their body’s microbiome to prevent spoilage of their food.

Ant body microbiomes often contain components to detect (Goes & al. 2020) and defend against pathogens, including plant pathogens (Offenberg & Damgaard 2019) and pathogens specific to fungal cultivars, such as the ascomycete *Escovopsis*. The *Escovopsis* fungus is controlled via antibiotics that are produced by bacterial commensalists living on ant bodies, which include some lineages of Actinobacteria. The main Actinobacteria studied is *Pseudonocardia,* which can spread spores widely, yet its population structure mirrors that of its ant host colonies (Caldera & Currie 2012). The consequences of such complex ant-associated microbial interactions are the generation and maintenance of bacterial biodiversity at finer levels of strains within microbial species, despite natural selection also operating genus levels and coarser taxonomic resolutions. Finer strain-level diversity in bacteria may have implications for their activity and functions in soil, as strains are widely known to show different functions (Park & al. 2022, Anderson & Bisanz 2023), such as in disease (Yan & al. 2020, Hoque & al. 2021). Furthermore, the complex Attini-Agaricales-*Escovopsis-Pseudocardonia* relationship described here, and found with similar species (Gehring & Bennett 2009) in similar settings, can clearly intransitive interaction structures, and thus should be studied as such for more realistic understanding of long-term predictions in nature (Vandermeer 2011, Soliveres & al. 2018, Soliveres & Allan 2018, Lozano & al. 2019, Vandermeer & Perfecto 2023, Verdú & al. 2023), and we encourage this further research to uncover future community dynamics helpful for biological control.

1.2. Hygiene: Ant body microbiomes and hygiene are basic mechanisms that shape soil microbial communities, namely via ant metapleural glands. Metapleural glands distinguish ants from other Hymenoptera (Hölldobler & Wilson 1990) and function as a source of secreted chemicals that ants spread to groom themselves and larvae, in part due to antimicrobial effects of the secreted chemicals (Stow & Beattie 2008, Yek & Mueller 2011). Secreted chemicals are relatively acidic (Maschwitz 1974), and is a strategy promoted by eusociality (Stow & al. 2007) that is also implemented by Attine ants for gardening fungal monocultures (Fernández-Marín & al. 2006). Secretions from the metapleural gland have variable efficacy in preventing germination of fungal spores (Beattie & al. 1985, Beattie & al. 1986), which implies that metapleural secretions filter and/or select for certain microbial taxa. However, much remains unknown about this mechanism of action filtering soil microbial communities, such as whether metapleural secretions can be specific to certain microbes, and whether this specificity changes across ant species.

Overall, microbial filtering effects of ant sanitation and hygiene may work in opposition to microbial inoculation effects from ant diets, and together form dialectic processes shaping ant nest soil microbial community assembly. For example, venom production is another mechanism that can shape microbial communities, specifically inhibiting microbial biofilm formation in the fire ant *Solenopsis invicta* Buren, 1972 (Carvalho & al. 2019). Other glands can also produce antibiotic compounds (Offenberg & Damgaard 2019), which could scale up to shape plant disease dynamics (Offenberg 2015). Less is known about such effects of antibiotic compounds from other ant glands on specific microbial groups, as well as the collective sum of antibiotics used by a single ant species or how this might differ between ant species. Ant-derived antimicrobial compounds are selectively used when needed (Obin & Vander Meer 1985, Fernández-Marín & al. 2006). These compounds may also be tailored to nest pathogens, leading to disproportionate survival of microbes that do not pose direct threats to ants (Fernandes & al. 2018).

1.3. Nest Microbiome: Among the non-Attine ants, other ground-dwelling ants have been found to increase microbial diversity (Delgado-Baquerizo & al. 2019, Lindström & al. 2019), but studies have not tested explicit community assembly mechanisms (Stegen & al. 2013, Stegen & al. 2015, Tripathi & al. 2018). Specifically, patterns of microbial biodiversity in large and long-lived *Iridomyrmex purpureus* (Smith, F., 1858) ant nests include enrichment in opportunistic, faster-growing, and copiotrophic microbes, as well as those capable of antibiotic production (Delgado-Baquerizo & al. 2019). Fast-growing microbes included plant pathogens and bacteria in the phylum Bacteroidetes, as well as fungi in the division Chytridiomycota and archaea in the genus *Nitrososphaera*. Oligotrophic Acidobacteria abundances were reduced in nests, while decomposer fungi and plant root symbionts were not significantly different in ant nest soils. Additionally, harvester ant (*Veromessor andrei* (Mayr, 1886)) nests have shown enrichment in arbuscular mycorrhizal fungi (Friese & Allen 1993), which are critical for most plants' acquisition of phosphorus (Weber & al. 2025). Clearly, ant nests can serve as unique reservoirs for microbial biodiversity including archaea, which can scale up to affect plant primary production (Farji-Brener & Werenkraut 2017), such as in agricultural systems (Wu & al. 2025). Ultimately, however, research on the underlying mechanisms explaining these ant effects on microbial communities remain understudied and microbial community assembly processes should be explicitly tested (Nemergut & al. 2013).

1.4. Gut Microbiome: Many studies of ant microbiomes focus on internal gut community membership, which likely reflects both diet and genetic selection by the internal gut microenvironment. Ant diets come from variable resources (Rocha-Ortega & García-Martínez 2018) and can consist of macroinvertebrates varying widely in microbiome (Wagner & al. 1997). There is additional variation in core internal gut microbiome composition, such as in harvester ants (Gamboa & al. 2025) and the ant species *Diacamma* cf. *indicum* Santschi, 1920 in Japan, whose compositions are dominated by an unknown Firmicutes bacterial species (Shimoji & al. 2021). Furthermore, cephalotine ant gut bacterial communities were found to be more likely to be shared within trophic level, even across phylogenetically distinct lineages, suggesting stronger effects of diet and/or convergent evolution, as core community members (i.e. Rhizobiales bacteria) are likely key in aiding ant nitrogen metabolism (Anderson & al. 2012). Ant gut microbiomes can also affect nest soil microbiomes through the excretion of ant feces (Pereira & al. 2020, Cole & al. 2021), which serve as nutrient hotspots and inocula from which soil microbial community dynamics can then emerge.

1.5. Soil Microhabitats: Ants also shape soil microbial communities by modifying several aspects of soil microenvironments throughout their nests, although previous studies tend to focus on soil chemical variables. Overall, ant nests are built to maintain homeostasis via a regulated environment, which includes soil temperature and moisture (Jones & Oldroyd 2006, Bierbaß & al. 2015). Indeed, ground-nesting ants tend to have a protein-rich diet consisting of insects (Frizzi & al. 2020) as shown in their waste (Hudson & al. 2009), unlike leaf-dwelling ants that more likely focus on tending carbohydrate reservoirs (Vandermeer & al. 2010, Vandermeer & al. 2019). Accordingly, previous studies show lower, more acidic pH, and occasionally higher (Cammeraat & al. 2002) or lower (Dostál & al. 2005) organic carbon and inorganic nutrient concentrations, such as phosphorus and potassium in *Pachycondyla striata* Smith, F., 1858 nests (Almeida & al. 2019), but lower calcium and magnesium concentrations in grassland nests of the yellow meadow ant *Lasius flavus* (Fabricius, 1782) (Dostál & al. 2005). Nests of *Lasius flavus* have also been reported to be hotspots of nutrient cycling, showing lower carbon mineralization, but higher nitrogen mineralization and base cations, as well as higher leachable dissolved organic carbon and nitrogen (BIERBAß & al. 2015). Ant nest effects on soil chemistry can also vary by habitat, such as by elevation, where carbon and nitrogen are higher at low elevations but lower at higher elevations, possibly due to deeper nest architecture in drier soils (SANKOVITZ & PURCELL 2022). There may also be temporal legacy effects or soil memory of soil nutrient enrichment by ants, as found for *Atta colombica* GUÉRIN-MÉNEVILLE, 1844, where decomposition of ant waste material enriched soils in nitrogen and phosphorus up to one year after nest abandonment (HUDSON & al. 2009). In addition to nitrogen-rich insect protein diets, higher nutrient levels require higher mineralization rates by soil bacteria and fungi, but microbial activity is rarely measured (DAUBER & WOLTERS 2000). Ultimately, ant effects on soils can scale up to affect plant growth by stimulating both green shoot and root biomass (FARJI-BRENER & WERENKRAUT 2017), and even scale up to increase leaf nitrogen concentrations (WAGNER & FLEUR NICKLEN 2010).

There has been comparatively less focus on how ants modify soil structure (CAMMERAAT & RISCH 2008), despite the increasing recognition of soil structure as important for shaping soil microbial communities (KRAVCHENKO & al. 2014, BAVEYE & al. 2018, KRAVCHENKO & al. 2019) as well as nutrient cycling (KRAVCHENKO & GUBER 2017, VOGEL & al. 2022, MEDINA & VANDERMEER 2023). As part of their nest (and niche) construction process (VANDERMEER 2008, LALAND & al. 2016), ants are fundamentally integral parts of the soil fragmentation and formation process shaping overall soil geomorphology (WHITFORD & ELDRIDGE 2013). Intuitively, ant body size will correspond with mandible size (TSCHINKEL & al. 2003), which then corresponds with soil fragment size excavated and particles used for nest construction (AVILA-NÚÑEZ 2023), leading to changes in soil texture and bulk density (CAMMERAAT & RISCH 2008), microbial community compositional differences by specific ant colony identity (LUCAS & al. 2017), and possibly even colony developmental stage where microbial succession increases microbial diversity (NEPEL & al. 2023). This interspecific variation in excavation processes likely lead to differences in soil porosity, average pore size for workers to travel through, and pore network connectivity, all of which have significant impacts on soil microbial activity (KRAVCHENKO & GUBER 2017, KRAVCHENKO & al. 2019). However, it remains unclear how microbial community shifts underlie changes in nest architecture and geometry, which overall remains understudied (TSCHINKEL & al. 2003, RÖMER & ROCES 2014, FORTI & al. 2018).

Ant nest construction can also shape soil aggregation, which is also increasingly recognized as important for long-term soil fertility and organic matter storage (AHMADI & al. 2011, BLAUD & al. 2014, CHAPLOT & COOPER 2015, RILLIG & al. 2017, CHEN & al. 2022). During the nest construction process, in addition to preferring to use slightly harder soils, e.g. mineral or compacted, ants use their saliva to help cement soil particles together (BRIAN 1983). As a result, ant nests likely affect soil aggregate characteristics, such as stability and/or average diameter (ECHEZONA & IGWE 2012), but direct research on this aspect of soil development remains limited. Soil aggregation can shape microbial community diversity and composition (BACH & al. 2018), such as through biofilm formation (BÜKS & KAUPENJOHANN 2016, LEHMANN & al. 2017, WU & al. 2019) and modifying meta-population dynamics (JACKSON & al. 2014), but additional distinguishing underlying mechanisms that are specifically mediated by soil aggregates (BAILEY & al. 2013) remains an area for future study.

Fine-scale variation in bioturbation processes during ant nest construction then scales up to shape whole nest sizes and geometry. Broadly, ant nest architecture and geometry remains poorly understood, in part because ant nests and soil microbial communities are often studied separately (BOTTINELLI & al. 2015). However, specific examples of nest architecture exist for common ant genera including *Formica* (MIKHEYEV & TSCHINKEL 2004), *Camponotus* (TSCHINKEL 2005), *Odontomachus* (CERQUERA & TSCHINKEL 2010), and *Pogonomyrmex* harvester ants (TSCHINKEL 2004). Given that each chamber differs in function, such as to hold food or brood, chambers also very likely harbor different microbiomes, as shown in *Azteca trigona* EMERY, 1893 (LUCAS & al. 2019) and other invertebrates (ZHU & al. 2021). Over longer evolutionary time scales, ant nest-associated microbiomes may then feedback to shape the ant nest architecture, such as through constructing waste or brood chambers to have microclimatic properties minimizing pathogenic or encouraging beneficial microbial communities.

2. Beyond the Nest: Ants also impact microbial communities beyond their nests. Functional domains in soils, a concept coined by ANDERSON (1995) and elaborated on by LAVELLE (2002), is particularly useful in conceptualizing both the spatiotemporal aspects of regulation of microbial activity and soil processes, but also the hierarchical position that ants and other soil engineers assume when compared to the many other factors at play. Ants and other macrofaunal soil ecosystem engineers sit at a critical connecting point between micro and macro scales, wherein they determine the architecture of soils through the creation of aggregates and pores across plot scales, which then form the habitat and substrate access to microbial food webs (LAVELLE & al. 2006, LAVELLE & al. 2016). Different ant species have differing effects on microbial community composition and activity (DAUBER & WOLTERS 2000, BOOTS & al. 2012, FERNANDES & al. 2024), so when considering communities of ants, one can begin to visualize a mosaic of ant colonies, each assemblage uniquely affecting microbial communities in particular and potentially complementary ways. Here we highlight possible mechanisms of interest regarding the various ways through which ants may impact microbial communities beyond the nest.

2.1. Ant Mosaics: The concept of ant mosaics has already been well-characterized within the sphere of arboreal ants, and is defined by mutually exclusive territories of dominant ants that also house several co-occurring non-dominant or submissive ant species, thus creating unique assemblages of ant species (ROOM 1971, BLÜTHGEN & STORK 2007). Ant mosaics have been shown to structure arthropod communities and related ecosystem impacts, with resident ants shifting the biotic contexts of ecological interactions (LESTON 1973, DEJEAN & al. 1997, STÜBER & al. 2021). While ant mosaics have been traditionally applied to arboreal ants, we believe that this framework is useful for understanding not only the distributions of ground-nesting ants and arthropod communities, but also soil microbial communities and their subsequent impacts on ecosystem processes, particularly in environmental contexts where ant-mediated top-down controls are ecologically relevant.

Ant mosaics are spatiotemporally heterogeneous. With the passage of time, mosaics can either shift or remain static, and (PERFECTO & VANDERMEER 2013) suggested that competitive intransitivity drives shifting mosaics whereas competitive hierarchy drives static ones. Additionally, the presence of parasitoids can introduce nonlinear higher-order effects that complicate the spatial dynamics generated by intransitive competition, which has been shown both empirically and theoretically (VANDERMEER & PERFECTO 2020, VANDERMEER & PERFECTO 2024a). Contrary to traditional notions of ant mosaics, dominant ants can also drive the disassembly of co-occurrence patterns that would otherwise occur among subordinate ants (SANDERS & al. 2007). While ground ant nests may seem stationary, they are more mobile than conventionally thought, and nest relocation likely plays a role in mosaic re-formation as well, although nest movement is poorly characterized for most ant species and can be influenced by many non mutually-exclusive factors (MCGLYNN 2012). All of this can all affect the so-called 'pattern mobility' of ant mosaics through time, which will have important implications for temporal aspects of ant-microbe interactions through colony residence times and cultivation of species-specific microbial communities. For example, a mosaic with high pattern mobility may result in less heterogeneity over landscape scales, as (DAUBER & WOLTERS 2000) found that young *Lasius flavus* mounds lack the typical features characterizing the microbial communities of mature mounds, suggesting that nest residence time is an important factor in the alteration of microbial communities.

LAVELLE & al. (2006) broaches the idea of mosaics of functional domains for soil invertebrates, which has been observed in earthworms, where different earthworm species assemblages formed distinct areas of influence, either increasing or decreasing bulk density of the soil (ROSSI 2003). Effects like this are likely most pronounced for organisms that are sessile or semi-sessile, like the iterative nesting behavior of social organisms, due to their ability to form physicochemical gradients over space. Through physical modification of soils and nutrient loading via nest-building activities, termites (*Odontotermes spp*.) support soil microbial activity, facilitating decomposition and mineralization of nutrients. These hotspots of microbial activity regulated by termite activity were found to create spatial structure and regulate ecosystem function of black cotton savannas in Kenya (FOX-DOBBS & al. 2010). Many ground-nesting ant species also maintain sizable and semi-sessile (MCGLYNN 2012) nests that could feasibly recreate similar patterns, although there is a dearth of literature assessing these local gradients outside of the nest context. Most work to date compares nest to non-nest (reference) soils in ants, which while useful, often assigns reference soils irrespective of the foraging activities of the colony of interest. This makes it difficult to ascertain any effect the ants may have throughout their foraging space, potentially ignoring the much larger footprint of influence that ants may have outside of their nest.

2.2. Tunnel Formation: For soil-dwelling ants, tunneling activity may represent an important behavior that modifies soil bacterial communities within foraging areas. *Solenopsis invicta*, the red imported fire ant, is a globally distributed invasive ant species that engages extensively in tunneling, which can be as deep as 11 centimeters (MARKIN & al. 1975a) and extend as far as 15 meters away from a nest (TSCHINKEL 2011). During tunnel construction, (MARKIN & al. 1975a) found that *S. invicta* first forms an aboveground ant trail, but soon excavates vertical shafts every 10 to 24 cm along that trail, which then branch into several more horizontal tunnels. These branching tunnels eventually meet tunnels from other vertical shafts, forming a contiguous underground tunnel to the food source. This process occurred over as little as 48 hours. Constant and rapid bioturbation throughout the colony's foraging area may enhance microbial activity, where mixing and aeration of soils by ants brings otherwise dormant microbes into contact with new digestible substrates, enhancing rates of chemical transformations (LAVELLE & al. 1995, LAVELLE & al. 2006). Tunnel excavation also oxygenates soils, which can alter microbial community composition and function (FENCHEL & FINLAY 2008), and increases soil porosity and hydraulic conductivity, which also increase microbial activity (TORBERT & AND WOOD 1992, WU & al. 2025). TRAVANTY & al. (2022) found that soils colonized by *S. invicta* had significantly different bacterial communities compared to unaffected soil, with elevated abundances of several taxa including

Actinobacteria, although this study investigated nest soil, so effects beyond the nest remain unknown. These ‘beyond-nest’ effects may also show intraspecific variation, where polygyne forms of *S. invicta* tend to form several smaller nests connected by tunnels (TSCHINKEL 2013), which may intensify effects of tunneling through inter-nest exchanges. Looking at ants more broadly, VILES & al. (2021) found that ants move a median of 1.5 tons of soil per ha annually and up to over 60 tons of soil per ha annually for species like *Aphaenogaster longiceps* (SMITH, F., 1858) in Australia and *Pogonomyrmex badius* (LATREILLE, 1802) in Florida, USA. There are few studies that investigate foraging tunnel formation behavior under natural conditions (MARKIN & al. 1975b, BERGHOFF & al. 2002), leaving traits like tunnel turnover, extent, and density largely unknown for most ant species, all of which could have varying effects on microbial activity and persistence throughout the landscape covered by ant foraging.

2.3. Indirect Effects: Throughout their foraging space, ants indirectly affect microbial communities through the plethora of interactions they hold with other non-microbial organisms. For example, ants may have indirect effects on microbial communities through suppression (DUNHAM & MIKHEYEV 2010) or augmentation (SANDERS & VAN VEEN 2011) of microbivore populations, such as springtails and mites, with consequences for nutrient cycling and litter decomposition. This effect is likely dependent on ant species, as wood ants (*Formica spp.*) were found to have minimal or no effect on soil faunal populations (LAAKSO 1999, LENOIR & al. 2003). However, wood ants may alter microbial populations through their effects on plant community composition, where WARDLE & al. (2011) found that long-term exclusion of the red wood ant (*Formica aquilonia* YARROW, 1955) increased herbaceous plant biomass, which in turn stimulated microbial biomass and activity and related microbial-driven processes of litter decomposition and C and N mineralization. Aside from indirect effects on microbes, ants can conversely have indirect effects on aboveground diversity by exerting controls on soil microbial diversity and dynamics. For example, FERNANDES & al. (2024) found that ant-handling of myrmecochorous seeds by leaf-cutting ants *Atta sexdens* (LINNAEUS, 1758) and *Acromyrmex subterraneus* (FOREL, 1893) on seeds of *Mabea fistulifera* resulted in different fungal communities based on the identity of the ants handling them. Seed manipulation by *Atta sexdens* is known to positively affect germination and handling by *Acromyrmex subterraneus* is known to have a negative effect on germination (FERNANDES & al. 2018). Ants can also affect microbial composition of floral nectar on plants that they defend. VANNETTE & al. (2017) found that aggressive ant *Azteca sericeasur* LONGINO, 2007 modified floral visitation to coffee (*Coffea arabica*) by pollinators and that plant association with *Azteca sericeasur* was correlated with distinct microbial community composition, which may be linked to later fruit set and weight (PHILPOTT & al. 2006).

Even arboreal ants can link aboveground and belowground communities by affecting soil microbial activity. In a study on the dominant arboreal ant, *Azteca trigona*, nutrient-enriched waste products ‘raining’ from their nests onto the forest floor resulted in a 2.4-fold increase in microbial detritivores and predators compared to an area 10 meters away, along with increases in decomposition of leaf litter as well (CLAY & al. 2013). These effects also extended up to a meter away from the nest. While this study did not directly address microbes, nutrient translocation through waste piles by social insects like ants is an important zoogeochemical process shaping microbial activity and community composition across soil ecosystems.

3. Commonly Used Microbial Quantification Methods: Quantifying microbial diversity is fundamentally challenging, first and foremost due to the ubiquitous yet invisible nature of microbial life forms. Large population sizes, short generational times, high levels of metabolic plasticity, and ability to share genes between distant lineages further complicate this task (POLZ & al. 2013, SOUCY & al. 2015, LENNON & DENEF 2016). Although tremendous progress has been made since the invention of the microscope—the event that led to the birth of microbiology as a modern scientific discipline—method selection can be overwhelming due to sheer number of tools and the varying levels of analytical depth and biological information they can offer.

Here, we list some commonly used methods today, organized into four categories based on the type of information they can generate: (1) *Taxonomic and genomic profiles*, (2) *Functional activity and expression*, (3) *Biomass and productivity*, and (4) *Spatial and in situ organization* (Table 1). Rather than providing an exhaustive catalogue, our list is designed to support hypothesis driven method selection—particularly for testing the assumptions and predictions of ecological theories in the context of cross-scale ant-microbe interactions—as well as to facilitate functional studies on how ants may alter microbial processes in soil environments. This synthesis is particularly relevant today, as the field of microbial ecology is increasingly data-rich yet lacking in cohesive theoretical foundations (NEMERGUT & al. 2013, ANTWIS & al. 2017, PROSSER & MARTINY 2020, HUG 2024).

{Insert Table 1 Here}

4. Ants & Microbial Dispersal: The most basic abstraction in community ecology, framed as the competitive exclusion principle, states that no two species overlapping in resource requirements or niche space can coexist, as the superior competitor will eventually drive the other to extinction (GAUSE 1934). Yet, empirically observed patterns of biodiversity often contradict these predictions (HUTCHINSON 1961). Among several mechanisms proposed to resolve this paradox, spatial structures, both within populations (e.g., Janzen-Connell effects) and between populations (e.g., metacommunity dynamics), have emerged as key ecological mechanisms driving species coexistence (JANZEN 1970, CONNELL 1971, MURRELL & LAW 2003, YITBAREK & VANDERMEER 2017). In line with this spatial framing, it is increasingly understood that natural ecosystems are rarely uniform or closed. Instead, they are embedded in a heterogeneous matrix of fluctuating environmental conditions and interaction networks (LEVIN 1992, PERFECTO & al. 2019). Species persistence, thus, is not only affected by birth and death rates within a patch but also by the migration of individuals between patches (LEVINS 1969, HANSKI 1998). This open-system, or metapopulation, perspective offers a way to understand how interdependent local and regional processes together influence community structure and dynamics. As VANDERMEER & PERFECTO (2024b) note in The Dialectical Agroecologist: *"biodiversity maintenance is a landscape affair, with populations and mixed species groups in a never-ending dance between local extinctions and regional movements"*.

While dispersal is a deceptively simple process involving movement of individuals from one patch to another, it can produce complex patterns of biodiversity. (VELLEND 2016) outlines how dispersal can be understood across scales (1) as a high level process influencing diversity alongside selection, drift, and speciation (2) as a process interacting with selection, and (3) as a low level process with spatially variable fitness effects. At broader spatial scales or higher level, dispersal can increase local diversity via introduction of immigrants from the regional species pool (DAMSCHEN & al. 2006). However, this increased local diversity can simultaneously homogenize the patches, thereby decreasing beta diversity (KNEITEL & MILLER 2003, PEDRUSKI & ARNOTT 2011). Moreover, when the magnitude of local advantage is allowed to vary across patches, the relationship between dispersal and diversity can interact with selection to become a non-linear and hump-shaped: dispersal can increase diversity at lower or intermediate rates due to rescue effects, but reduce diversity at high dispersal rates as a result of constant propagule influx through mass-effects, which effectively expands the local advantage throughout the metacommunity (MOUQUET & LOREAU 2003). Finally, dispersal traits themselves may evolve under spatially variable selection regimes. For example, the well known competition-colonization model posits that negative trait correlation between dispersal and local competitive abilities can enable species coexistence through negative frequency-dependent selection after disturbance (LEVINS & CULVER 1971, TILMAN 1994, CADOTTE & al. 2006).

These scale-dependent effects are particularly relevant for soil ecosystems, where inherent spatial heterogeneity forms a structurally complex and fragmented landscape (ZHOU & al. 2002, PHILLIPS 2017), with ants as a major driver. As ecosystem engineers, ground-dwelling ants actively modify microbial habitats in ways that may both constrain and facilitate microbial dispersal. As such, studying ant-microbe interactions is essential for informing the health and resilience of socio-ecologically vital soil ecosystems that underpin our food systems (EVANS & al. 2011, WU & al. 2025) and serve as major carbon pools.

4.1. Microbial Dispersal Across Scales: The importance of dispersal in microbial biodiversity maintenance depends on the degree to which populations are restricted in their movement. Historically, it has been assumed in microbial ecology that “everything is everywhere, but the environment selects” (BAAS-BECKING 1934), implying that microbial communities are not constrained by dispersal. However, recent research has now shown that microbes, much like larger organisms, also experience dispersal limitation (MARTINY & al. 2006, CUSTER & al. 2022). In contrast to continental-scale patterns predicted by edaphic factors, beta-diversity of soil microbial diversity at finer spatial scales is influenced by dispersal limitation (FIERER & JACKSON 2006, MARTINY & al. 2011). This underscores the scale-dependent nature of dispersal processes in microbial communities.

Experimental and modelling studies have confirmed that dispersal influences microbial community structure and dynamics in more complex and nuanced ways. For instance, ALBRIGHT & MARTINY (2018) found that altering dispersal rates affected richness, evenness, and composition of soil bacterial communities. Similarly, individual-based modeling of microbial decomposers has revealed that dispersal interacts with selection to shape community assembly (EVANS & al. 2017). Specifically, at low dispersal rates, communities were strongly influenced by stochastic processes, while at higher dispersal rates, there was increased environmental selection for many functional trait diversity, likely due to migration of specialist taxa (EVANS & al. 2017). Additionally, a unique aspect of applying metacommunity theory to microbes is widespread dormancy behavior, which allows microbes to persist under adverse conditions until they become favorable once again for growth (MCDONALD & al. 2023). Dormancy can alter traditional metacommunity paradigms depending on how much dormancy covaries with dispersal, and likely strengthens colonization legacies and priority effects, while also causing time lags for mass effects (WISNOSKI & al. 2019).

Beyond community assembly, dispersal has been shown to promote species coexistence, influence community recovery following disturbance events, and lead to modification of key ecosystem properties, such as leaf litter decomposition, mediated by the soil microbiome (KERR & al. 2002, EVANS & al. 2020, WALTERS & al. 2022). Notably, recent work has highlighted the zoogeochemical implications of microbial dispersal. HAWKINS & ZEGLIN (2022) experimentally showed that manipulating microbial dispersal using soil bags and bison dung deposition significantly increased diversity and homogenized community structure across varying land-use regimes. Taken together, these findings suggest that microbial dispersal is highly context dependent, and influenced by both biotic and abiotic factors. While a thorough review of microbial dispersal is beyond the scope of this text, we point readers towards CHOUDOIR & DEANGELIS (2022) and CUSTER & al. (2022) for comprehensive review of microbial dispersal modes, vectors, and their eco-evolutionary consequences. In the following section, we explore possible mechanisms through which ants may influence microbial dispersal.

4.2. Ant Impacts on Microbial Dispersal: Ants can influence local microbial diversity through impacting microbial dispersal rates, altering their ability to colonize new patches across landscapes. Perhaps at the largest scale is through nuptial flights, an important stage of the ant colony life cycle where new queens and males leave the nest to mate and found new colonies. Information on flight ranges during nuptial flights is particularly sparse, although MARKIN & al. (1971) found that 99% of *Solenopsis invicta* queens landed within 1.6 kilometers of the nest location, but a small proportion of queens dispersed 11-16 kilometers away from the nest. Additionally, *Atta texana* (BUCKLEY, 1860), *Atta cephalotes* (LINNAEUS, 1758), and *Atta sexdens* were observed to disperse maximally from 9.6 to 11 kilometers away from the nest site (FOWLER & al. 1986). Transportation of microbes during nuptial flights has only been well-studied within the fungus-farming ants (tribe Attini). New gynes possess an infrabuccal pocket in which they store a piece of the fungus from the mother colony, and use it to start a new fungal garden after the nuptial flight (BOULOGNE & al. 2014). These ants are also important for dispersal of their Actinobacteria symbiont, *Pseudonocardia*, which helps to combat fungal pathogens within the nest. Recombination by *Pseudonocardia* is constrained by association with their ant hosts, showing dispersal limitation even over relatively small scales (CALDERA & CURRIE 2012). Importantly, ants likely possess some amount of control over which microbial species they do and do not disperse, where PAGNOCCA & al. (2008) failed to find any *Escovopsis*, the specialized fungal pathogen that attacks the ant's fungal garden, or the entomopathogenic fungi *Beauveria sp.* or *Metarhizium sp.* on or within gynes, suggesting effective sanitation methods to prevent dispersal of pathogens to new nests. They did find, however, several types of filamentous fungi and yeasts on the gynes, including those in the genera *Cladosporium*, *Aureobasidium*, *Candida*, and *Cryptococcus*, suggesting that they do passively disperse other fungal taxa. Beyond the fungus-farming ants, not much is known about the direct transmission of microbes by gynes during nuptial flights.

In addition to larger scales of dispersal for microbes, ants also serve as dispersal agents on a finer scale within their nest structures, across different areas of soil that represent relatively distinct patches. Firstly, ants create different chambers in their nests for different uses such as food storage, waste management, and brood rearing, which develop different soil microclimates and also harbor different microbial communities (LUCAS & al. 2019), and thereby establishes fine-scale spatial organization and potential meta-community dynamics of ant nest-associated microbial populations. Second, as discussed above, ant bodies have their own selective microbiome that they maintain with hygienic behavior (Offenberg & Damgaard 2019). Third, ant mandibles move around fragmented pieces of soil during the nest construction process, which can be relatively frequent in semi-sessile species. Nest construction often involves excavating soil fragments from deeper soil layers, ranging from a typical 30 cm down to 12 meters depth (Tschinkel 2004). Compared to soils without ant activity, ant workers likely increase microbial dispersal rates, and therefore may exaggerate mass effects for microbes. Stronger mass effects would lead to more homogenization, but also more lags, variation, and/or stochasticity in competitive patch dynamics. Additionally, ant foraging and tunneling can also facilitate microbial dispersal throughout patches of soil, as foraging extents can cover relatively large areas, such as in *Solenopsis invicta* where foraging tunnels can extend up to 15 meters from the nest (Tschinkel 2011, Vandermeer & al. 2022). Ant tunneling may be an important mechanism of microbial dispersal, as tunnels are widely separated in space across many directions, and long-distance dispersal is often rare across taxa (Jordano 2017). Tunnel distributions will shift based on available food resources and may represent a source of mass effects of microbial dispersal as workers move and transport soil fragments, food throughout the tunnels. Through these activities, ants likely disperse at least a selected portion of soil microbes across finer scales within their nest structures.

Landscape-scale ant mosaics provide one source of habitat heterogeneity that could provide differing selective regimes on microbial communities, through their multitude of effects on the physical and chemical properties of soils, hygienic behavior, and translocation of nutrient-rich substrates into and out of nests. An extreme example of species sorting includes the fungal lineages maintained by fungus-farming ants (tribe Attini) which are very well conserved from generation to generation, where Poulsen & Boomsma (2005) found that the monocultured fungus in a colony was able to reject mycelial components from neighboring colonies with high fidelity. However, whereas the basidiomycetous fungi of fungus-farming ants is intimately tied to their survival, the activities of ants also influence microbial diversity and community composition for a multitude of species that do not directly or clearly impact the ants' survival (Clay & al. 2013, Fernandes & al. 2018, Delgado-Baquerizo & al. 2019). This mosaic of selective regimes may function to preserve landscape-level biodiversity of microbial communities, and this effect is likely stronger within static mosaics or those with low pattern mobility.

Ant-mediated dispersal and sorting of microbial communities serves to structure microbial communities and associated functions across spatial and temporal scales, from within and beyond the nest to the shifting mosaics of ant territories through time. Of importance as well is dormancy, which contributes to the maintenance of microbial biodiversity (Jones & Lennon 2010). It is not known, however, how the changes in environmental cues associated with ant activities may mediate dormancy patterns within microbial communities and its impact on microbial biodiversity.

5. Future Directions & Conclusion: Within the emerging field of zoogeochemistry, there remains much to explore regarding ant-microbe interactions and their subsequent effects on population dynamics, community diversity, and ecosystem function. Although we do know that metapleural secretions and other antibiotic compounds produced by ants can have species-specific effects, this has only been tested for a handful of bacterial and fungal species. Better characterization of antibiotic specificity, to what extent they are produced and used, and under what conditions they are used will all be helpful in understanding better the role of ants in shaping microbial community composition within their functional domains. This also applies to the microbial selection that occurs for gynes during ant nuptial flights, which for the microbial species is likely an important long-distance dispersal event. Another deserving avenue of research is the connection between nest architecture and microbial diversity and function. To what extent do distinct nest structures house distinct microbial assemblages, and through what mechanisms are these biodiversity and community patterns maintained?

Although the majority of previous work focuses on the impacts of ants within their nests, the total area influenced by ants throughout their functional domains extends substantially farther beyond the nest. However, since very few studies have addressed this broader spatial scale, many questions remain unanswered with regards to beyond-nest effects on soil microbial community composition and functions. Firstly, it remains unclear which behaviors have significant impacts on microbial community structure, and whether or not these effects translate into shifts in community function, as functional redundancy is common in microbial communities (TALBOT & al. 2014). Spatiotemporal aspects of the ant mosaic are also of interest, as ant nests and associated foraging areas are very dynamic – constantly appearing, disappearing, advancing, and retreating – which presents the question of the consequences of legacy memory effects of ant activities for microbial community reassembly and soil processes (CANARINI & al. 2021, MEDINA & VANDERMEER 2023).

Lastly, much of the work in zoogeochemistry treats microbial community diversity as a black box (SCHMITZ & al. 2018), but recent methodological advances present a plethora of ways to characterize soil microbial diversity and dynamics, which can help us develop new testable hypotheses from diverse theoretical frameworks, ultimately better linking cross-scale species interactions, involving animals and microbes, to modified ecosystem processes. Since many aspects of ant-microbe interactions are spatially explicit, we also encourage dedicating analytical and sampling resources to investigating spatial effects.

Acknowledgments

We would like to thank Vincent Denef for assistance during idea development and feedback on written portions, as well as department staff and funding supports.

References

AHMADI, A., NEYSHABOURI, M.R., ROUHIPOUR, H. & ASADI, H. 2011: Fractal dimension of soil aggregates as an index of soil erodibility. – Journal of Hydrology 400: 305-311.

ALBRIGHT, M.B.N. & MARTINY, J.B.H. 2018: Dispersal alters bacterial diversity and composition in a natural community. – The ISME Journal 12: 296-299.

ALLEN, M.F., SHULMAN, H., RUNDEL, P.W., HARMON, T.C. & ARONSON, E.L. 2023: Leaf-cutter ants – mycorrhizal fungi: observations and research questions from an unexpected mutualism. – Frontiers in Fungal Biology 4.

ALMEIDA, F.S., ELIZALDE, L., SILVA, L.M.S. & QUEIROZ, J.M. 2019: The effects of two abundant ant species on soil nutrients and seedling recruitment in Brazilian Atlantic Forest. – Revista Brasileira de Entomologia 63: 296-301.

ANDERSON, B.D. & BISANZ, J.E. 2023: Challenges and opportunities of strain diversity in gut microbiome research. – Frontiers in Microbiology 14.

Anderson, J.M. 1995: Soil Organisms as Engineers: Microsite Modulation of Macroscale Processes. In: Linking Species & Ecosystems. – Springer, Boston, MA, pp. 94-106.

Anderson, K.E., Russell, J.A., Moreau, C.S., Kautz, S., Sullam, K.E., Hu, Y., 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: 2282-2296.

Antwis, R.E., Griffiths, S.M., Harrison, X.A., Aranega-Bou, P., Arce, A., Bettridge, A.S., Brailsford, F.L., De Menezes, A., Devaynes, A., Forbes, K.M., Fry, E.L., Goodhead, I., Haskell, E., Heys, C., James, C., Johnston, S.R., Lewis, G.R., Lewis, Z., Macey, M.C., McCarthy, A., McDonald, J.E., Mejia-Florez, N.L., O'Brien, D., Orland, C., Pautasso, M., Reid, W.D.K., Robinson, H.A., Wilson, K. & Sutherland, W.J. 2017: Fifty important research questions in microbial ecology. – FEMS Microbiology Ecology 93.

Avila-Núñez, J.L. 2023: Ants foraging in sidewalks: particle-use behavior of the fire ant *Solenopsis geminata* Fabricius (Hymenoptera: Formicidae). – Insectes Sociaux 70: 357-364.

Azam, F. & Malfatti, F. 2007: Microbial structuring of marine ecosystems. – Nature Reviews Microbiology 5: 782-791.

Baas-Becking, L.G.M. 1934: Geobiologie; of inleiding tot de milieukunde. – WP Van Stockum & Zoon NV.

Bach, E.M., Williams, R.J., Hargreaves, S.K., Yang, F. & Hofmockel, K.S. 2018: Greatest soil microbial diversity found in micro-habitats. – Soil Biology and Biochemistry 118: 217-226.

Bailey, V.L., Fansler, S.J., Stegen, J.C. & McCue, L.A. 2013: Linking microbial community structure to β-glucosidic function in soil aggregates. – The ISME Journal 7: 2044-2053.

Baveye, P.C., Otten, W., Kravchenko, A., Balseiro-Romero, M., Beckers, É., Chalhoub, M., Darnault, C., Eickhorst, T., Garnier, P., Hapca, S., Kiranyaz, S., Monga, O., Mueller, C.W., Nunan, N., Pot, V., Schlüter, S., Schmidt, H. & Vogel, H.-J. 2018: Emergent Properties of Microbial Activity in Heterogeneous Soil Microenvironments: Different Research Approaches Are Slowly Converging, Yet Major Challenges Remain. – Frontiers in Microbiology 9: 1929.

Beattie, A.J., Turnbull, C., Hough, T., Jobson, S. & Knox, R.B. 1985: The Vulnerability of Pollen and Fungal Spores to Ant Secretions: Evidence and Some Evolutionary Implications. – American Journal of Botany 72: 606-614.

Beattie, A.J., Turnbull, C.L., Hough, T. & Knox, R.B. 1986: Antibiotic Production: a Possible Function for the Metapleural Glands of Ants (Hymenoptera: Formicidae). – Annals of the Entomological Society of America 79: 448-450.

Berghoff, S.M., Weissflog, A., Linsenmair, K.E., Hashim, R. & Maschwitz, U. 2002: Foraging of a hypogaeic army ant: a long neglected majority. – Insectes Sociaux 49: 133-141.

Bertness, M.D. & Callaway, R. 1994: Positive interactions in communities. – Trends in Ecology & Evolution 9: 191-193.

Bierbaß, P., Gutknecht, J.L.M. & Michalzik, B. 2015: Nest-mounds of the yellow meadow ant (*Lasius flavus*) at the "Alter Gleisberg", Central Germany: Hot or cold spots in nutrient cycling? – Soil Biology and Biochemistry 80: 209-217.

Bittleston, L.S., Gralka, M., Leventhal, G.E., Mizrahi, I. & Cordero, O.X. 2020: Context-dependent dynamics lead to the assembly of functionally distinct microbial communities. – Nature Communications 11: 1440.

Blaud, A., Chevallier, T., Virto, I., Pablo, A.L., Chenu, C. & Brauman, A. 2014: Bacterial community structure in soil microaggregates and on particulate organic matter fractions located outside or inside soil macroaggregates. – Pedobiologia 57: 191-194.

Blüthgen, N. & Stork, N.E. 2007: Ant mosaics in a tropical rainforest in Australia and elsewhere: A critical review. – Austral Ecology 32: 93-104.

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 55: 33-40.

Bottinelli, N., Jouquet, P., Capowiez, Y., Podwojewski, P., Grimaldi, M. & Peng, X. 2015: Why is the influence of soil macrofauna on soil structure only considered by soil ecologists? – Soil and Tillage Research 146: 118-124.

Boulogne, I., Ozier-Lafontaine, H. & Loranger-Merciris, G. 2014: Leaf-Cutting Ants, Biology and Control. In: Lichtfouse, E. (Eds.): Sustainable Agriculture Reviews: Volume 13. – Springer International Publishing, Cham, pp. 1-17.

Brian, M.V. 1983: Cavity nests and soil mounds. In: Social Insects. – Springer, Dordrecht, pp. 74-93.

Büks, F. & Kaupenjohann, M. 2016: Enzymatic biofilm digestion in soil aggregates facilitates therelease of particulate organic matter by sonication. – SOIL 2: 499-509.

Cadotte, M.W., Mai, D.V., Jantz, S., Collins, M.D., Keele, M. & Drake, J.A. 2006: On Testing the Competition-Colonization Trade-Off in a Multispecies Assemblage. – The American Naturalist 168: 704-709.

Caldera, E.J. & Currie, C.R. 2012: The Population Structure of Antibiotic-Producing Bacterial Symbionts of *Apterostigma dentigerum* Ants: Impacts of Coevolution and Multipartite Symbiosis. – The American Naturalist 180: 604-617.

Caldera, E.J., Poulsen, M., Suen, G. & Currie, C.R. 2009: Insect Symbioses: A Case Study of Past, Present, and Future Fungus-growing Ant Research. – Environmental Entomology 38: 78-92.

Cammeraat, E.L.H. & Risch, A.C. 2008: The impact of ants on mineral soil properties and processes at different spatial scales. – Journal of Applied Entomology 132: 285-294.

Cammeraat, L.H., Willott, S.J., Compton, S.G. & Incoll, L.D. 2002: The effects of ants' nests on the physical, chemical and hydrological properties of a rangeland soil in semi-arid Spain. – Geoderma 105: 1-20.

Canarini, A., Schmidt, H., Fuchslueger, L., Martin, V., Herbold, C.W., Zezula, D., Gündler, P., Hasibeder, R., Jecmenica, M., Bahn, M. & Richter, A. 2021: Ecological memory of recurrent drought modifies soil processes via changes in soil microbial community. – Nature Communications 12: 5308.

Carvalho, D.B.D., Fox, E.G.P., Santos, D.G.D., Sousa, J.S.D., Freire, D.M.G., Nogueira, F.C.S., Domont, G.B., Castilho, L.V.A.D. & Machado, E.D.A. 2019: Fire ant venom alkaloids inhibit biofilm formation. – Toxins 11.

Catford, J.A., Wilson, J.R.U., Pyšek, P., Hulme, P.E. & Duncan, R.P. 2022: Addressing context dependence in ecology. – Trends in Ecology & Evolution 37: 158-170.

Cerquera, L.M. & Tschinkel, W.R. 2010: The Nest Architecture of the Ant *Odontomachus brunneus*. – Journal of Insect Science 10: 1-12.

Chaplot, V. & Cooper, M. 2015: Soil aggregate stability to predict organic carbon outputs from soils. – Geoderma 243-244: 205-213.

Chen, J., Song, D., Liu, D., Sun, J., Wang, X., Zhou, W. & Liang, G. 2022: Soil Aggregation Shaped the Distribution and Interaction of Bacterial-Fungal Community Based on a 38-Year Fertilization Experiment in China. – Frontiers in Microbiology 13.

Chesson, P. 2000: Mechanisms of Maintenance of Species Diversity. – Annual Review of Ecology, Evolution, and Systematics 31: 343-366.

Choudoir, M.J. & DeAngelis, K.M. 2022: A framework for integrating microbial dispersal modes into soil ecosystem ecology. – iScience 25.

Clay, N.A., Lucas, J., Kaspari, M. & Kay, A.D. 2013: Manna from heaven: Refuse from an arboreal ant links aboveground and belowground processes in a lowland tropical forest. – Ecosphere 4.

Cole, M.E., Ceja-Navarro, J.A. & Mikaelyan, A. 2021: The power of poop: Defecation behaviors and social hygiene in insects Jabra-Rizk, M.A. – PLOS Pathogens 17.

Connell, J.H. 1971: On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees. In: Boer, J.P. den & Gradwell, G.R. (Eds.): Dynamics of populations : proceedings of the advanced study institute on Dynamics of numbers in populations, Oosterbeek, the Netherlands. – Wageningen : Centre for agricultural publishing and documentation, pp. 298-312.

Cordero, O.X. & Datta, M.S. 2016: Microbial interactions and community assembly at microscales. – Current Opinion in Microbiology 31: 227-234.

Currie, C.R., Mueller, U.G. & Malloch, D. 1999: The agricultural pathology of ant fungus gardens. – Proceedings of the National Academy of Sciences 96: 7998-8002.

Custer, G.F., Bresciani, L. & Dini-Andreote, F. 2022: Ecological and Evolutionary Implications of Microbial Dispersal. – Frontiers in Microbiology 13.

Dahal, N., Glyshaw, P., Carter, G., Vanderploeg, H.A. & Denef, V.J. 2023: Impacts of an invasive filter-feeder on bacterial biodiversity are context dependent. – FEMS Microbiology Ecology 99.

Damschen, E.I., Haddad, N.M., Orrock, J.L., Tewksbury, J.J. & Levey, D.J. 2006: Corridors Increase Plant Species Richness at Large Scales. – Science 313: 1284-1286.

Dauber, J. & Wolters, V. 2000: Microbial activity and functional diversity in the mounds of three different ant species. – Soil Biology and Biochemistry 32: 93-99.

Dejean, A., Djieto-Lordon, C. & Durand, J.L. 1997: Ant Mosaic in Oil Palm Plantations of the Southwest Province of Cameroon: Impact on Leaf Miner Beetle (Coleoptera: Chrysomelidae). – Journal of Economic Entomology 90: 1092-1096.

Del Toro, I., Ribbons, R.R. & Pelini, S.L. 2012: The little things that run the world revisited: A review of ant-mediated ecosystem services and disservices (Hymenoptera: Formicidae). – Myrmecological News 17: 133-146.

Delgado-Baquerizo, M., Eldridge, D.J., Hamonts, K. & Singh, B.K. 2019: Ant colonies promote the diversity of soil microbial communities. – The ISME Journal 5: 4-8.

Dostál, P., Březnová, M., Kozlíčková, V., Herben, T. & Kovář, P. 2005: Ant-induced soil modification and its effect on plant below-ground biomass. – Pedobiologia 49: 127-137.

Dunham, A.E. & Mikheyev, A.S. 2010: Influence of an invasive ant on grazing and detrital communities and nutrient fluxes in a tropical forest. – Diversity and Distributions 16: 33-42.

Echezona, B.C. & Igwe, C.A. 2012: Stabilities of ant nests and their adjacent soils. – International Agrophysics 26: 355-363.

Estes, J.A., Terborgh, J., Brashares, J.S., Power, M.E., Berger, J., Bond, W.J., Carpenter, S.R., Essington, T.E., Holt, R.D., Jackson, J.B.C., Marquis, R.J., Oksanen, L., Oksanen, T., Paine, R.T., Pikitch, E.K., Ripple, W.J., Sandin, S.A., Scheffer, M., Schoener, T.W., Shurin, J.B., Sinclair, A.R.E., Soulé, M.E., Virtanen, R. & Wardle, D.A. 2011: Trophic Downgrading of Planet Earth. – Science 333: 301-306.

Evans, S., Martiny, J.B.H. & Allison, S.D. 2017: Effects of dispersal and selection on stochastic assembly in microbial communities. – The ISME Journal 11: 176-185.

Evans, S.E., Bell-Dereske, L.P., Dougherty, K.M. & Kittredge, H.A. 2020: Dispersal alters soil microbial community response to drought. – Environmental Microbiology 22: 905-916.

Evans, T.A., Dawes, T.Z., Ward, P.R. & Lo, N. 2011: Ants and termites increase crop yield in a dry climate. – Nature Communications 2: 262.

Falkowski, P.G., Fenchel, T. & Delong, E.F. 2008: The Microbial Engines That Drive Earth's Biogeochemical Cycles. – Science 320: 1034-1039.

Farji-Brener, A.G. & Werenkraut, V. 2017: The effects of ant nests on soil fertility and plant performance: a meta-analysis. – Journal of Animal Ecology 86: 866-877.

Fenchel, T. & Finlay, B. 2008: Oxygen and the Spatial Structure of Microbial Communities. – Biological Reviews 83: 553-569.

Fernandes, T.V., Fernandes, O.L., Gomes, I.J.M.T., Solar, R.R.C. & Campos, R.I. 2024: Ant identity determines the fungi richness and composition of a myrmecochorous seed. – PLOS ONE 19.

Fernandes, T.V., Paolucci, L.N., Carmo, F.M.S., Sperber, C.F. & Campos, R.I. 2018: Seed manipulation by ants: disentangling the effects of ant behaviours on seed germination. – Ecological Entomology 43: 712-718.

Fernández-Marín, H., Zimmerman, J.K., Rehner, S.A. & Wcislo, W.T. 2006: Active use of the metapleural glands by ants in controlling fungal infection. – Proceedings of the Royal Society B: Biological Sciences 273: 1689-1695.

Fierer, N. & Jackson, R.B. 2006: The diversity and biogeography of soil bacterial communities. – Proceedings of the National Academy of Sciences 103: 626-631.

Folgarait, P.J. 1998: Ant biodiversity and its relationship to ecosystem functioning: a review. – Biodiversity and Conservation 7: 1221-1244.

Forti, L.C., Moreira, A.A., Camargo, R. da S., Caldato, N. & Castellani, M.A. 2018: Nest architecture development of grass-cutting ants. – Revista Brasileira de Entomologia 62: 46-50.

Fowler, H.G., Pereira-da-Silva, V., Forti, L.C. & Saes, N.B. 1986: Population Dynamics of Leaf-Cutting Ants: A Brief Review. In: Fire Ants And Leaf-cutting Ants. – CRC Press, 23 pp.

Fox-Dobbs, K., Doak, D.F., Brody, A.K. & Palmer, T.M. 2010: Termites create spatial structure and govern ecosystem function by affecting N_2 fixation in an East African savanna. – Ecology 91: 1296-1307.

Friese, C.F. & Allen, M.F. 1993: The Interaction of Harvester Ants and Vesicular–Arbuscular Mycorrhizal Fungi in a Patchy Semi-Arid Environment: The Effects of Mound Structure on Fungal Dispersion and Establishment. – Functional Ecology 7: 13-20.

Frizzi, F., Masoni, A., Migliorini, M., Fanciulli, P.P., Cianferoni, F., Balzani, P., Giannotti, S., Davini, G., Frasconi Wendt, C. & Santini, G. 2020: A comparative study of the fauna associated with nest mounds of native and introduced populations of the red wood ant *Formica paralugubris.* – European Journal of Soil Biology 101.

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

Gamboa, D.A., Flynn, P.J., Horna-Lowell, E.S. & Pinter-Wollman, N. 2025: Social organization and physical environment shape the microbiome of harvester ants. – Animal Microbiome 7: 29.

Gause, G.F. 1934: Experimental Analysis of Vito Volterra's Mathematical Theory of the Struggle for Existence. – Science 79: 16-17.

Gehring, C. & Bennett, A. 2009: Mycorrhizal Fungal–Plant–Insect Interactions: The Importance of a Community Approach. – Environmental Entomology 38: 93-102.

Goes, A.C., Barcoto, M.O., Kooij, P.W., Bueno, O.C. & Rodrigues, A. 2020: How Do Leaf-Cutting Ants Recognize Antagonistic Microbes in Their Fungal Crops? – Frontiers in Ecology and Evolution 8.

Gould, A.L., Zhang, V., Lamberti, L., Jones, E.W., Obadia, B., Korasidis, N., Gavryushkin, A., Carlson, J.M., Beerenwinkel, N. & Ludington, W.B. 2018: Microbiome interactions shape host fitness. – Proceedings of the National Academy of Sciences 115: E11951-E11960.

Hairston, N.G., Smith, F.E. & Slobodkin, L.B. 1960: Community Structure, Population Control, and Competition. – The American Naturalist 94: 421-425.

Handel, S.N. & Beattie, A.J. 1990: Seed Dispersal by Ants. – Scientific American 263: 76-83B.

Hanski, I. 1998: Metapopulation dynamics. – Nature 396: 41-49.

Hawkins, J.H. & Zeglin, L.H. 2022: Microbial Dispersal, Including Bison Dung Vectored Dispersal, Increases Soil Microbial Diversity in a Grassland Ecosystem. – Frontiers in Microbiology 13.

Hoek, T.A., Axelrod, K., Biancalani, T., Yurtsev, E.A., Liu, J. & Gore, J. 2016: Resource Availability Modulates the Cooperative and Competitive Nature of a Microbial Cross-Feeding Mutualism. – PLOS Biology 14.

Hölldobler, B. & Wilson, E.O. 1990: The Ants. – Belknap Press of Harvard University Press, Cambridge, Mass, 732 pp.

Hoque, M.N., Rahman, M.S., Ahmed, R., Hossain, Md.S., Islam, Md.S., Islam, T., Hossain, M.A. & Siddiki, A.Z. 2021: Diversity and genomic determinants of the microbiomes associated with COVID-19 and non-COVID respiratory diseases. – Gene Reports 23.

Hudson, T.M., Turner, B.L., Herz, H. & Robinson, J.S. 2009: Temporal patterns of nutrient availability around nests of leaf-cutting ants (*Atta colombica*) in secondary moist tropical forest. – Soil Biology and Biochemistry 41: 1088-1093.

Hug, L.A. 2024: The ever-changing tree of life. – Nature Microbiology 9: 1906-1908.

Hutchinson, G.E. 1961: The Paradox of the Plankton. – The American Naturalist 95: 137-145.

Jackson, D., Allen, D., Perfecto, I. and Vandermeer, J. 2014: Self-organization of background habitat determines the nature of population spatial structure – Oikos 123: 751-761.

Janzen, D.H. 1970: Herbivores and the Number of Tree Species in Tropical Forests. – The American Naturalist 104: 501-528.

Janzen, D.H. 1977: Why Fruits Rot, Seeds Mold, and Meat Spoils. – The American Naturalist 111: 691-713.

Jones, C.G., Lawton, J.H. & Shachak, M. 1996: Organisms as Ecosystem Engineers. In: Samson, F.B. & Knopf, F.L. (Eds.): Ecosystem Management: Selected Readings. – Springer, New York, NY, pp. 130-147.

Jones, J.C. & Oldroyd, B.P. 2006: Nest Thermoregulation in Social Insects. In: Simpson, S.J. (Eds.): Advances in Insect Physiology. – Academic Press, pp. 153-191.

Jones, S.E. & Lennon, J.T. 2010: Dormancy contributes to the maintenance of microbial diversity. – Proceedings of the National Academy of Sciences of the United States of America 107: 5881-5886.

Jordano, P. 2017: What is long-distance dispersal? And a taxonomy of dispersal events. – Journal of Ecology 105: 75-84.

Joseph, R. & Keyhani, N.O. 2021: Fungal mutualisms and pathosystems: life and death in the ambrosia beetle mycangia. – Applied Microbiology and Biotechnology 105: 3393-3410.

Jouquet, P., Dauber, J., Lagerlöf, J., Lavelle, P. & Lepage, M. 2006: Soil invertebrates as ecosystem engineers: Intended and accidental effects on soil and feedback loops. – Applied Soil Ecology 32: 153-164.

Kerr, B., Riley, M.A., Feldman, M.W. & Bohannan, B.J.M. 2002: Local dispersal promotes biodiversity in a real-life game of rock–paper–scissors. – Nature 418: 171-174.

KNEITEL, J.M. & MILLER, T.E. 2003: Dispersal Rates Affect Species Composition in Metacommunities of *Sarracenia purpurea* Inquilines. – The American Naturalist 162: 165-171.

KNUTIE, S.A., WILKINSON, C.L., KOHL, K.D. & ROHR, J.R. 2017: Early-life disruption of amphibian microbiota decreases later-life resistance to parasites. – Nature Communications 8.

KRAVCHENKO, A.N. & GUBER, A.K. 2017: Soil pores and their contributions to soil carbon processes. – Geoderma 287: 31-39.

KRAVCHENKO, A.N., GUBER, A.K., RAZAVI, B.S., KOESTEL, J., QUIGLEY, M.Y., ROBERTSON, G.P. & KUZYAKOV, Y. 2019: Microbial spatial footprint as a driver of soil carbon stabilization. – Nature Communications 10.

KRAVCHENKO, A.N., NEGASSA, W.C., GUBER, A.K., HILDEBRANDT, B., MARSH, T.L. & RIVERS, M.L. 2014: Intra-aggregate Pore Structure Influences Phylogenetic Composition of Bacterial Community in Macroaggregates. – Soil Science Society of America Journal 78: 1924-1939.

KRUSHELNYCKY, P.D. & GILLESPIE, R.G. 2008: Compositional and Functional Stability of Arthropod Communities in the Face of Ant Invasions. – Ecological Applications 18: 1547-1562.

LAAKSO, J. 1999: Short-term effects of wood ants (*Formica aquilonia* YARR.) on soil animal community structure. – Soil Biology and Biochemistry 31: 337-343.

LALAND, K., MATTHEWS, B. & FELDMAN, M.W. 2016: An introduction to niche construction theory. – Evolutionary Ecology 30: 191-202.

LAVELLE, P. 2002: Functional domains in soils. – Ecological Research 17: 441-450.

LAVELLE, P., DECAËNS, T., AUBERT, M., BAROT, S., BLOUIN, M., BUREAU, F., MARGERIE, P., MORA, P. & ROSSI, J.-P. 2006: Soil invertebrates and ecosystem services. – European Journal of Soil Biology 42: S3-S15.

LAVELLE, P., LATTAUD, C., TRIGO, D. & BAROIS, I. 1995: Mutualism and biodiversity in soils. In: The Significance and Regulation of Soil Biodiversity. – Springer, Dordrecht, pp. 23-33.

LAVELLE, P., SPAIN, A., BLOUIN, M., BROWN, G., DECAËNS, T., GRIMALDI, M., JIMÉNEZ, J.J., MCKEY, D., MATHIEU, J., VELASQUEZ, E. & ZANGERLÉ, A. 2016: Ecosystem Engineers in a Self-organized Soil: A Review of Concepts and Future Research Questions. – Soil Science 181.

LEHMANN, A., ZHENG, W. & RILLIG, M.C. 2017: Soil biota contributions to soil aggregation. – Nature Ecology & Evolution 1: 1828-1835.

LENNON, J.T. & DENEF, V.J. 2016: Evolutionary Ecology of Microorganisms: From the Tamed to the Wild. In: Manual of Environmental Microbiology. – John Wiley & Sons, Ltd, p. 4.1.2-1-4.1.2-12.

LENOIR, L., BENGTSSON, J. & PERSSON, T. 2003: Effects of *Formica* ants on soil fauna-results from a short-term exclusion and a long-term natural experiment. – Oecologia 134: 423-430.

LEROUX, S.J. & SCHMITZ, O.J. 2025: Integrating Network and Meta-Ecosystem Models for Developing a Zoogeochemical Theory. – Ecology Letters 28.

LESTON, D. 1973: The Ant Mosaic - Tropical Tree Crops and the Limiting of Pests and Diseases. – PANS Pest Articles & News Summaries 19: 311-341.

LEVIN, S.A. 1992: The Problem of Pattern and Scale in Ecology: The Robert H. MacArthur Award Lecture. – Ecology 73: 1943-1967.

LEVINS, R. 1969: Some Demographic and Genetic Consequences of Environmental Heterogeneity for Biological Control. – Bulletin of the Entomological Society of America 15: 237-240.

LEVINS, R. & CULVER, D. 1971: Regional Coexistence of Species and Competition between Rare Species. – Proceedings of the National Academy of Sciences 68: 1246-1248.

Li, J., Ianaiev, V., Huff, A., Zalusky, J., Ozersky, T. & Katsev, S. 2021: Benthic invaders control the phosphorus cycle in the world's largest freshwater ecosystem. – Proceedings of the National Academy of Sciences 118.

Lindeman, R.L. 1942: The Trophic-Dynamic Aspect of Ecology. – Ecology 23: 399-417.

Lindström, S., Timonen, S., Sundström, L. & Johansson, H. 2019: Ants reign over a distinct microbiome in forest soil. – Soil Biology and Biochemistry 139.

Lozano, G.L., Bravo, J.I., Diago, M.F.G., Park, H.B., Hurley, A., Peterson, S.B., Stabb, E.V., Crawford, J.M., Broderick, N.A. & Handelsman, J. 2019: Introducing THOR, a model microbiome for genetic dissection of community behavior. – mBio 10: 1-14.

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.

Lucas, J.M., Madden, A.A., Penick, C.A., Epps, M.J., Marting, P.R., Stevens, J.L., Fergus, D.J., Dunn, R.R. & Meineke, E.K. 2019: *Azteca* ants maintain unique microbiomes across functionally distinct nest chambers. – Proceedings of the Royal Society B: Biological Sciences 286.

Markin, G.P., Dillier, J.H., Hill, S.O., Blum, M.S. & Hermann, H.R. 1971: Nuptial flight and flight ranges of the imported fire ant, *Solenopsis saevissima richteri* (Hymenoptera : Formicidae). – Journal of the Georgia Entomological Society 6: 145-156.

Markin, G.P., O'Neal, J. & Dillier, J. 1975a: Foraging Tunnels of the Red Imported Fire Ant, *Solenopsis invicta* (Hymenoptera: Formicidae). – Journal of the Kansas Entomological Society 48: 83-89.

Markin, G.P., O'Neal, J. & Dillier, J. 1975b: Foraging Tunnels of the Red Imported Fire Ant, *Solenopsis invicta* (Hymenoptera: Formicidae). – Journal of the Kansas Entomological Society 48: 83-89.

Martiny, J.B.H., Bohannan, B.J.M., Brown, J.H., Colwell, R.K., Fuhrman, J.A., Green, J.L., Horner-Devine, M.C., Kane, M., Krumins, J.A., Kuske, C.R., Morin, P.J., Naeem, S., Øvreås, L., Reysenbach, A.-L., Smith, V.H. & Staley, J.T. 2006: Microbial biogeography: putting microorganisms on the map. – Nature Reviews Microbiology 4: 102-112.

Martiny, J.B.H., Eisen, J.A., Penn, K., Allison, S.D. & Horner-Devine, M.C. 2011: Drivers of bacterial β-diversity depend on spatial scale. – Proceedings of the National Academy of Sciences 108: 7850-7854.

Maschwitz, U. 1974: Vergleichende Untersuchungen zur Funktion der Ameisenmetathorakaldrüse. – Oecologia 16: 303-310.

May, R.M. 1972: Will a Large Complex System be Stable? – Nature 238: 413-414.

McCary, M.A. & Schmitz, O.J. 2021: Invertebrate functional traits and terrestrial nutrient cycling: Insights from a global meta-analysis. – Journal of Animal Ecology 90: 1714-1726.

McDonald, M.D., Owusu-Ansah, C., Ellenbogen, J.B., Malone, Z.D., Ricketts, M.P., Frolking, S.E., Ernakovich, J.G., Ibba, M., Bagby, S.C. & Weissman, J.L. 2023: What is microbial dormancy? – Trends in Microbiology 32: 142-150.

McGlynn, T.P. 2012: The Ecology of Nest Movement in Social Insects. – Annual Review of Entomology 57: 291-308.

Medina, N. & Vandermeer, J. 2023: Developing systems theory in soil agroecology: incorporating heterogeneity and dynamic instability. – Frontiers in Environmental Science 11.

Metcalf, C.J.E., Henry, L.P., Rebolleda-Gómez, M. & Koskella, B. 2019: Why Evolve Reliance on the Microbiome for Timing of Ontogeny? – mBio 10.

Meyer, G.A. & Leroux, S.J. 2023: Towards a mechanistic understanding of animal–ecosystem interactions. – Journal of Animal Ecology 92: 2244-2247.

Mikheyev, A.S. & Tschinkel, W.R. 2004: Nest architecture of the ant *Formica pallidefulva* : structure, costs and rules of excavation. – Insectes Sociaux 51: 30-36.

Mouquet, N. & Loreau, M. 2003: Community Patterns in Source-Sink Metacommunities. – The American Naturalist 162: 544-557.

Mueller, U.G., Ishak, H.D., Bruschi, S.M., Smith, C.C., Herman, J.J., Solomon, S.E., Mikheyev, A.S., Rabeling, C., Scott, J.J., Cooper, M., Rodrigues, A., Ortiz, A., Brandão, C.R.F., Lattke, J.E., Pagnocca, F.C., Rehner, S.A., Schultz, T.R., Vasconcelos, H.L., Adams, R.M.M., Bollazzi, M., Clark, R.M., Himler, A.G., LaPolla, J.S., Leal, I.R., Johnson, R.A., Roces, F., Sosa-Calvo, J., Wirth, R. & Bacci Jr, M. 2017: Biogeography of mutualistic fungi cultivated by leafcutter ants. – Molecular Ecology 26: 6921-6937.

Mueller, U.G., Mikheyev, A.S., Solomon, S.E. & Cooper, M. 2011: Frontier mutualism: coevolutionary patterns at the northern range limit of the leaf-cutter ant–fungus symbiosis. – Proceedings of the Royal Society B: Biological Sciences 278: 3050-3059.

Mueller, U.G., Scott, J.J., Ishak, H.D., Cooper, M. & Rodrigues, A. 2010: Monoculture of Leafcutter Ant Gardens Dornhaus, A. – PLoS ONE 5.

Murrell, D.J. & Law, R. 2003: Heteromyopia and the spatial coexistence of similar competitors. – Ecology Letters 6: 48-59.

Nemergut, D.R., Schmidt, S.K., Fukami, T., O'Neill, S.P., Bilinski, T.M., Stanish, L.F., Knelman, J.E., Darcy, J.L., Lynch, R.C., Wickey, P. & Ferrenberg, S. 2013: Patterns and processes of microbial community assembly. – Microbiology and Molecular Biology Reviews 77: 342-356.

Nepel, M., Mayer, V.E., Barrajon-Santos, V. & Woebken, D. 2023: Bacterial diversity in arboreal ant nesting spaces is linked to colony developmental stage. – Communications Biology 6: 1-12.

North, R.D., Jackson, C.W. & Howse, P.E. 1997: Evolutionary aspects of ant-fungus interactions in leaf-cutting ants. – Trends in Ecology & Evolution 12: 386-389.

Obin, M.S. & Vander Meer, R.K. 1985: Gaster flagging by fire ants (*Solenopsis* spp.): Functional significance of venom dispersal behavior. – Journal of Chemical Ecology 11: 1757-1768.

Offenberg, J. 2015: Ants as tools in sustainable agriculture. – Journal of Applied Ecology 52: 1197-1205.

Offenberg, J. & Damgaard, C. 2019: Ants suppressing plant pathogens: a review. – Oikos 128: 1691-1703.

Oksanen, L., Fretwell, S.D., Arruda, J. & Niemela, P. 1981: Exploitation Ecosystems in Gradients of Primary Productivity. – The American Naturalist 118: 240-261.

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: 517-526.

Paine, R.T. 1966: Food Web Complexity and Species Diversity. – The American Naturalist 100: 65-75.

Park, S.-Y., Rao, C., Coyte, K.Z., Kuziel, G.A., Zhang, Y., Huang, W., Franzosa, E.A., Weng, J.-K., Huttenhower, C. & Rakoff-Nahoum, S. 2022: Strain-level fitness in the gut microbiome is an emergent property of glycans and a single metabolite. – Cell 185: 513-529.

Peckarsky, B.L., Abrams, P.A., Bolnick, D.I., Dill, L.M., Grabowski, J.H., Luttbeg, B., Orrock, J.L., Peacor, S.D., Preisser, E.L., Schmitz, O.J. & Trussell, G.C. 2008: Revisiting the Classics: Considering Nonconsumptive Effects in Textbook Examples of Predator–Prey Interactions. – Ecology 89: 2416-2425.

Pedruski, M.T. & Arnott, S.E. 2011: The effects of habitat connectivity and regional heterogeneity on artificial pond metacommunities. – Oecologia 166: 221-228.

Pereira, H., Jossart, M. & Detrain, C. 2020: Waste management by ants: the enhancing role of larvae. – Animal Behaviour 168: 187-198.

Perfecto, I. & Vandermeer, J. 2013: Ant Assemblage on a Coffee Farm: Spatial Mosaic Versus Shifting Patchwork. – Environmental Entomology 42: 38-48.

Perfecto, I., Vandermeer, J. & Wright, A. 2019: Nature's Matrix: Linking Agriculture, Biodiversity Conservation and Food Sovereignty. 2nd edition . – Routledge, New York, 316 pp.

Phillips, J.D. 2017: Soil Complexity and Pedogenesis. – Soil Science 182: 117.

Philpott, S.M., Uno, S. & Maldonado, J. 2006: The importance of ants and high-shade management to coffee pollination and fruit weight in Chiapas, Mexico. In: Arthropod Diversity and Conservation. – Springer, Dordrecht, pp. 473-487.

Polz, M.F., Alm, E.J. & Hanage, W.P. 2013: Horizontal gene transfer and the evolution of bacterial and archaeal population structure. – Trends in Genetics 29: 170-175.

Poulsen, M. & Boomsma, J.J. 2005: Mutualistic Fungi Control Crop Diversity in Fungus-Growing Ants. – Science 307: 741-744.

Prosser, J.I. & Martiny, J.B.H. 2020: Conceptual challenges in microbial community ecology. – Philosophical Transactions of the Royal Society B: Biological Sciences 375.

Rillig, M.C., Muller, L.A. & Lehmann, A. 2017: Soil aggregates as massively concurrent evolutionary incubators. – The ISME Journal 11: 1943-1948.

Rocha-Ortega, M. & García-Martínez, M.Á. 2018: Importance of Nesting Resources and Soil Conditions for the Recovery of Ant Diversity During Secondary Succession in a Tropical Rainforest. – Tropical Conservation Science 11.

Rogers, T.L., Johnson, B.J. & Munch, S.B. 2022: Chaos is not rare in natural ecosystems. – Nature Ecology & Evolution 6: 1105-1111.

Römer, D. & Roces, F. 2014: Nest enlargement in leaf-cutting ants: Relocated brood and fungus trigger the excavation of new chambers. – PLoS ONE 9.

Room, P.M. 1971: The Relative Distributions of Ant Species in Ghana's Cocoa Farms. – Journal of Animal Ecology 40: 735-751.

Rossi, J.-P. 2003: The spatiotemporal pattern of a tropical earthworm species assemblage and its relationship with soil structure: The 7th international symposium on earthworm ecology · Cardiff · Wales · 2002. – Pedobiologia 47: 497-503.

Sanders, D. & Veen, F.J.F. van 2011: Ecosystem engineering and predation: the multi-trophic impact of two ant species. – Journal of Animal Ecology 80: 569-576.

Sanders, N.J., Crutsinger, G.M., Dunn, R.R., Majer, J.D. & Delabie, J.H.C. 2007: An Ant Mosaic Revisited: Dominant Ant Species Disassemble Arboreal Ant Communities but Co-occur Randomly. – Biotropica 39: 422-427.

Sankovitz, M. & Purcell, J. 2022: Ant nests differentially affect soil chemistry across elevational gradients. – Insectes Sociaux 69: 293-298.

Schmitz, O. 2017: Predator and prey functional traits: understanding the adaptive machinery driving predator–prey interactions. – F1000 Research 6.

Schmitz, O.J., Buchkowski, R.W., Burghardt, K.T. & Donihue, C.M. 2015: Chapter Ten - Functional Traits and Trait-Mediated Interactions: Connecting Community-Level Interactions with Ecosystem Functioning. In: Pawar, S., Woodward, G. & Dell, A.I. (Eds.): Advances in Ecological Research. – Trait-Based Ecology - From Structure to Function. Academic Press, pp. 319-343.

Schmitz, O.J., Krivan, V. & Ovadia, O. 2004: Trophic cascades: the primacy of trait-mediated indirect interactions. – Ecology Letters 7: 153-163.

Schmitz, O.J., Wilmers, C.C., Leroux, S.J., Doughty, C.E., Atwood, T.B., Galetti, M., Davies, A.B. & Goetz, S.J. 2018: Animals and the zoogeochemistry of the carbon cycle. – Science 362.

Schultheiss, P., Nooten, S.S., Wang, R., Wong, M.K.L., Brassard, F. & Guénard, B. 2022: The abundance, biomass, and distribution of ants on Earth. – Proceedings of the National Academy of Sciences 119.

Schultz, T.R., Sosa-Calvo, J., Kweskin, M.P., Lloyd, M.W., Dentinger, B., Kooij, P.W., Vellinga, E.C., Rehner, S.A., Rodrigues, A., Montoya, Q.V., Fernández-Marín, H., Ješovnik, A., Niskanen, T., Liimatainen, K., Leal-Dutra, C.A., Solomon, S.E., Gerardo, N.M., Currie, C.R., Bacci, M., Vasconcelos, H.L., Rabeling, C., Faircloth, B.C. & Doyle, V.P. 2024: The coevolution of fungus-ant agriculture. – Science 386: 105-110.

Shimoji, H., Itoh, H., Matsuura, Y., Yamashita, R., Hori, T., Hojo, M.K. & Kikuchi, Y. 2021: Worker-dependent gut symbiosis in an ant. – ISME Communications 1: 1-10.

Soliveres, S. & Allan, E. 2018: Everything you always wanted to know about intransitive competition but were afraid to ask Rees, M. – Journal of Ecology 106: 807-814.

Soliveres, S., Lehmann, A., Boch, S., Altermatt, F., Carrara, F., Crowther, T.W., Delgado-Baquerizo, M., Kempel, A., Maynard, D.S., Rillig, M.C., Singh, B.K., Trivedi, P. & Allan, E. 2018: Intransitive competition is common across five major taxonomic groups and is driven by productivity, competitive rank and functional traits Rees, M. – Journal of Ecology 106: 852-864.

Soucy, S.M., Huang, J. & Gogarten, J.P. 2015: Horizontal gene transfer: building the web of life. – Nature Reviews Genetics 16: 472-482.

Stegen, J.C., Lin, X., Fredrickson, J.K., Chen, X., Kennedy, D.W., Murray, C.J., Rockhold, M.L. & Konopka, A. 2013: Quantifying community assembly processes and identifying features that impose them. – The ISME journal 7: 2069-2079.

Stegen, J.C., Lin, X., Fredrickson, J.K. & Konopka, A.E. 2015: Estimating and mapping ecological processes influencing microbial community assembly. – Frontiers in Microbiology 6.

Stow, A. & Beattie, A. 2008: Chemical and genetic defenses against disease in insect societies. – Brain, Behavior, and Immunity 22: 1009-1013.

Stow, A., Briscoe, D., Gillings, M., Holley, M., Smith, S., Leys, R., Silberbauer, T., Turnbull, C. & Beattie, A. 2007: Antimicrobial defences increase with sociality in bees. – Biology Letters 3: 422-424.

Stüber, M., Tack, A.J.M., Zewdie, B., Mendesil, E., Shimales, T., Ayalew, B., Nemomissa, S., Sjögren, J., Vesterinen, E., Wezel, A. & Hylander, K. 2021: Multi-scale mosaics in top-down pest control by ants from natural coffee forests to plantations. – Ecology 102.

Suen, G., Teiling, C., Li, L., Holt, C., Abouheif, E., Bornberg-Bauer, E., Bouffard, P., Caldera, E.J., Cash, E., Cavanaugh, A., Denas, O., Elhaik, E., Favé, M.-J., Gadau, J., Gibson, J.D., Graur, D., Grubbs, K.J., Hagen, D.E., Harkins, T.T., Helmkampf, M., Hu, H., Johnson, B.R., Kim, J., Marsh, S.E., Moeller, J.A., Muñoz-Torres, M.C., Murphy, M.C., Naughton, M.C., Nigam, S., Overson, R., Rajakumar, R., Reese, J.T., Scott, J.J., Smith, C.R., Tao, S., Tsutsui, N.D., Viljakainen, L., Wissler, L., Yandell, M.D., Zimmer, F., Taylor, J., Slater, S.C., Clifton, S.W., Warren, W.C., Elsik, C.G., Smith, C.D., Weinstock, G.M., Gerardo, N.M. & Currie, C.R. 2011: The Genome Sequence of the Leaf-Cutter Ant *Atta cephalotes* Reveals Insights into Its Obligate Symbiotic Lifestyle. – PLOS Genetics 7.

Talbot, J.M., Bruns, T.D., Taylor, J.W., Smith, D.P., Branco, S., Glassman, S.I., Erlandson, S., Vilgalys, R., Liao, H.-L., Smith, M.E. & Peay, K.G. 2014: Endemism and functional convergence across the North American soil mycobiome. – Proceedings of the National Academy of Sciences 111: 6341-6346.

TILMAN, D. 1994: Competition and Biodiversity in Spatially Structured Habitats. – Ecology 75: 2-16.

TORBERT, H.A. & WOOD, C.W. AND 1992: Effects of soil compaction and water-filled pore space on soil microbial activity and N losses. – Communications in Soil Science and Plant Analysis 23: 1321-1331.

TRAVANTY, N.V., VARGO, E.L., APPERSON, C.S. & PONNUSAMY, L. 2022: Colonization by the Red Imported Fire Ant, *Solenopsis invicta*, Modifies Soil Bacterial Communities. – Microbial Ecology 84: 240-256.

TRIPATHI, B.M., STEGEN, J.C., KIM, M., DONG, K., ADAMS, J.M., KYUNG, Y. & ADAMS, J.M. 2018: Soil pH mediates the balance between stochastic and deterministic assembly of bacteria. – The ISME Journal 12: 1072-1083.

TSCHINKEL, W.R. 2003: Subterranean ant nests: trace fossils past and future? – Palaeogeography, Palaeoclimatology, Palaeoecology 192: 321-333.

TSCHINKEL, W.R. 2015: The architecture of subterranean ant nests: beauty and mystery underfoot. – Journal of Bioeconomics 17: 271-291.

TSCHINKEL, W.R. 2013: The Fire Ants. – Harvard University Press, 748 pp.

TSCHINKEL, W.R. 2005: The nest architecture of the ant, *Camponotus socius*. – Journal of Insect Science 5.

TSCHINKEL, W.R. 2004: The nest architecture of the Florida harvester ant, *Pogonomyrmex badius*. – Journal of Insect Science 4.

TSCHINKEL, W.R. 2011: The Organization of Foraging in the Fire Ant, *Solenopsis invicta*. – Journal of Insect Science 11: 1-30.

TSCHINKEL, W.R., MIKHEYEV, A.S. & STORZ, S.R. 2003: Allometry of Workers of the Fire Ant, *Solenopsis invicta*. – Journal of Insect Science 3.

URBAŃCZYK, D.A. & SZULC, W. 2023: Effect of anthills on changes in the physico-chemical properties in sandy soil. – Soil Science Annual 74: 1-6.

VANDERMEER, J. 2011: Intransitive loops in ecosystem models: From stable foci to heteroclinic cycles. – Ecological Complexity 8: 92-97.

VANDERMEER, J. 2008: The niche construction paradigm in ecological time. – Ecological Modelling 214: 385-390.

VANDERMEER, J., ARMBRECHT, I., MORA, A. DE LA, ENNIS, K.K., FITCH, G., GONTHIER, D.J., HAJIAN-FOROOSHANI, Z., HSIEH, H.-Y., IVERSON, A., JACKSON, D., JHA, S., JIMÉNEZ-SOTO, E., LOPEZ-BAUTISTA, G., LARSEN, A., LI, K., LIERE, H., MACDONALD, A., MARIN, L., MATHIS, K.A., MONAGAN, I., MORRIS, J.R., ONG, T., PARDEE, G.L., RIVERA-SALINAS, I.S., VAIYDA, C., WILLIAMS-GUILLEN, K., YITBAREK, S., UNO, S., ZEMENICK, A., PHILPOTT, S.M. & PERFECTO, I. 2019: The Community Ecology of Herbivore Regulation in an Agroecosystem: Lessons from Complex Systems. – BioScience 69: 974-996.

VANDERMEER, J., FLORES, J., LONGMEYER, J., PERFECTO, I. & MERCADER, R. 2022: Spatiotemporal Foraging Dynamics of *Solenopsis invicta* (Hymenoptera: Formicidae) and its Potential Effects on the Spatial Structure of Interspecific Competition. – Environmental Entomology: 8.

VANDERMEER, J. & PERFECTO, I. 2024A: Combining intransitive and higher-order effects in a coupled oscillator framework: A case study of an ant community. – Ecology 105.

VANDERMEER, J. & PERFECTO, I. 2020: Endogenous spatial pattern formation from two intersecting ecological mechanisms: the dynamic coexistence of two noxious invasive ant species in Puerto Rico. – Proceedings of the Royal Society B: Biological Sciences 287.

VANDERMEER, J. & PERFECTO, I. 2023: Intransitivity as a dynamic assembly engine of competitive communities. – Proceedings of the National Academy of Sciences 120.

VANDERMEER, J. & PERFECTO, I. 2024b: The Dialectical Agroecologist. 1st edition . – Cambridge University Press, 225 pp.

VANDERMEER, J., PERFECTO, I. & PHILPOTT, S. 2010: Ecological Complexity and Pest Control in Organic Coffee Production: Uncovering an Autonomous Ecosystem Service. – BioScience 60: 527-537.

VANDERMEER, J., PERFECTO, I. & PHILPOTT, S.M. 2008: Clusters of ant colonies and robust criticality in a tropical agroecosystem. – Nature 451: 457-459.

VANDERPLOEG, H.A., GLYSHAW, P.W., CARRICK, H.J., CARTER, G.S., DAHAL, N., DENEF, V.J., FANSLOW, D.L. & GODWIN, C.M. 2023: Seasonal interactions between Quagga Mussel grazing and phytoplankton in western Lake Erie: The view from different measuring technologies. – Aquatic Ecosystem Health & Management 26: 111-119.

VANNETTE, R.L., BICHIER, P. & PHILPOTT, S.M. 2017: The presence of aggressive ants is associated with fewer insect visits to and altered microbe communities in coffee flowers. – Basic and Applied Ecology 20: 62-74.

VANNI, M.J. 2002: Nutrient Cycling by Animals in Freshwater Ecosystems. – Annual Review of Ecology, Evolution, and Systematics 33: 341-370.

VELLEND, M. 2016: The Theory of Ecological Communities. – Princeton University Press, 248 pp.

VERDÚ, M., ALCÁNTARA, J.M., NAVARRO-CANO, J.A. & GOBERNA, M. 2023: Transitivity and intransitivity in soil bacterial networks. – The ISME Journal 17: 2135-2139.

VILES, H.A., GOUDIE, A.S. & GOUDIE, A.M. 2021: Ants as geomorphological agents: A global assessment. – Earth-Science Reviews 213.

VOGEL, H.-J., BALSEIRO-ROMERO, M., KRAVCHENKO, A., OTTEN, W., POT, V., SCHLÜTER, S., WELLER, U. & BAVEYE, P.C. 2022: A holistic perspective on soil architecture is needed as a key to soil functions. – European Journal of Soil Science 73.

WAGNER, D., BROWN, M.J.F. & GORDON, D.M. 1997: Harvester ant nests, soil biota and soil chemistry. – Oecologia 112: 232-236.

WAGNER, D. & FLEUR NICKLEN, E.F. 2010: Ant nest location, soil nutrients and nutrient uptake by ant-associated plants: does extrafloral nectar attract ant nests and thereby enhance plant nutrition? – Journal of Ecology 98: 614-624.

WALTERS, K.E., CAPOCCHI, J.K., ALBRIGHT, M.B.N., HAO, Z., BRODIE, E.L. & MARTINY, J.B.H. 2022: Routes and rates of bacterial dispersal impact surface soil microbiome composition and functioning. – The ISME Journal 16: 2295-2304.

WARDLE, D.A., HYODO, F., BARDGETT, R.D., YEATES, G.W. & NILSSON, M.-C. 2011: Long-term aboveground and belowground consequences of red wood ant exclusion in boreal forest. – Ecology 92: 645-656.

WAY, M.J. & KHOO, K.C. 1992: Role of Ants in Pest Management. – Annual Review of Entomology 37: 479-503.

WEBER, S.E., BASCOMPTE, J., KAHMEN, A. & NIKLAUS, P.A. 2025: AMF diversity promotes plant community phosphorus acquisition and reduces carbon costs per unit of phosphorus. – New Phytologist.

WERNER, E.E. & PEACOR, S.D. 2003: A Review of Trait-Mediated Indirect Interactions in Ecological Communities. – Ecology 84: 1083-1100.

WHITFORD, W.G. & ELDRIDGE, D.J. 2013: Effects of Ants and Termites on Soil and Geomorphological Processes. – Treatise on Geomorphology 12: 281-292.

WILKINSON, M.T., RICHARDS, P.J. & HUMPHREYS, G.S. 2009: Breaking ground: Pedological, geological, and ecological implications of soil bioturbation. – Earth-Science Reviews 97: 257-272.

WISNOSKI, N.I., LEIBOLD, M.A. & LENNON, J.T. 2019: Dormancy in Metacommunities. – The American Naturalist 194: 135-151.
WU, D., DU, E., EISENHAUER, N., MATHIEU, J. & CHU, C. 2025: Global engineering effects of soil invertebrates on ecosystem functions. – Nature 640: 120-129.
WU, Y., CAI, P., JING, X., NIU, X., JI, D., ASHRY, N.M., GAO, C. & HUANG, Q. 2019: Soil biofilm formation enhances microbial community diversity and metabolic activity. – Environment International 132.
YAN, Y., NGUYEN, L.H., FRANZOSA, E.A. & HUTTENHOWER, C. 2020: Strain-level epidemiology of microbial communities and the human microbiome. – Genome Medicine 12.
YEK, S.H. & MUELLER, U.G. 2011: The metapleural gland of ants. – Biological Reviews 86: 774-791.
YITBAREK, S. & VANDERMEER, J.H. 2017: Reduction of species coexistence through mixing in a spatial competition model. – Theoretical Ecology 10: 443-450.
ZHOU, J., XIA, B., TREVES, D.S., WU, L.Y., MARSH, T.L., O'NEILL, R.V., PALUMBO, A.V. & TIEDJE, J.M. 2002: Spatial and Resource Factors Influencing High Microbial Diversity in Soil. – Applied and Environmental Microbiology 68: 326-334. F
ZHU, D., DELGADO-BAQUERIZO, M., DING, J., GILLINGS, M.R. & ZHU, Y.G. 2021: Trophic level drives the host microbiome of soil invertebrates at a continental scale. – Microbiome 9.

Table and figure captions

Tab. 1: Commonly used methods today to support hypothesis driven method selection related to ant-microbe interactions. Columns are organized into four categories based on the type of information they can generate: (1) *Taxonomic and genomic profiles*, (2) *Functional activity and expression*, (3) *Biomass and productivity*, and (4) *Spatial and in situ organization*.

Taxonomic and Genomic Profiles

Method	***Microbial Metric Quantified***	***Key Applications***	***Methodological Considerations***	***Example Questions***
16S rRNA gene sequencing	Abundance of 16S rRNA gene	- Estimating bacterial/archaeal community diversity (structure & composition) based on taxonomy - Constructing phylogenies using a single gene	- Cost effective & lower learning curve compared to metagenomics - Low phylogenetic resolution due to reliance on a single, slowly evolving marker gene - Ideal for community surveys without functional data - More effective than metagenomics for detecting both dominant & rare taxa	Who is there? What genes do they carry? *- Do ants exert top-down controls on microbial community (re)assembly through trait-mediated effects?* *- Are ant nests hotspots of horizontal gene transfers due to higher level of microbial diversity/abundance?* *- Do ants alter functional trait distribution in microbial communities?*
ITS sequencing	Abundance of ITS gene marker	- Estimating fungal community diversity (structure & composition) based on taxonomy - Constructing phylogenies using a single gene		
Metagenomics	Gene content and abundance at the population & community level	- Characterizing functional potential & trait diversity across all domains - Tracking populations through space & time, including evolutionary change - Constructing phylogenies using selected genes	- High resolution data for bacteria, archaea, fungi, & viruses - Higher costs & computational demands than 16s or ITS - Requires complex bioinformatic pipelines & has a steeper learning curve - Ideal for capturing dominant populations (though higher sequencing depth can capture some rare members)	

Functional Activity and Expression

Method	***Microbial Metric Quantified***	***Key Applications***	***Methodological Considerations***	***Example Questions***
Meta-transcriptomics	Gene expression patterns at the population &	- Identifying active members based on transcript abundance profiles	- Highly sensitive to technical errors due to short half-life of RNA	What are the microbes doing functionally & metabolically?

	community level	- Characterizing community level activities in response to environmental changes	- Requires quick sample preservation, high sequencing depth, & complex analysis - Can be paired with other ‘omics data for integrated insights about expressed functions/niche differentiation	*- Do the nutrient pulses driven by ant activities (tunnelling, foraging, excretion etc.) significantly shift patterns of microbial gene expression?* *- Do microbial communities in ant nests exhibit greater functional diversity or enhanced nutrient cycling?* *- What is the relationship between microbial diversity & function in ant nests compared to surrounding soil environments?*
Metaproteomics	Protein content & abundance at the community level	- Identifying proteins responsible for catalyzing ecosystem functions - Inferring contributions of member species to community functions based on expressed gene products	- Complex pipelines - Closer functional proxy given that protein abundance is measured - Can identify post-translational modifications that are key to functional regulation in some cases	
Metabolomics	Metabolite profiles & abundance at the community level	- Characterizing metabolic states - Characterizing small molecule sets to infer species interactions	- Sensitive to sample processing & extraction - Difficult to get reliable classifications (though software improvements have significantly helped broad identification of chemically defined molecule classes) - Ideal for physiological profiling at high resolution	
Community-level physiological profiling (CLPP) analysis	Utilization patterns of various carbon substrates	- Assessing functional diversity across microbial community samples - Profiling carbon substrate preferences and activities at the community level	- Simple & cost-effective compared to ‘omics methods - Aggregated community level measurements that cannot be linked to taxonomic data - Ideal for rapid & high throughput screening of functional shifts	
Biomass and Productivity				
Method	***Microbial Metric Quantified***	***Key Applications***	***Methodological Considerations***	***Example Questions***

Flow cytometry	Cell counts, phenotypic features, & nucleic acid content	- Estimating total abundance - Estimating phenotypic diversity (community structure & composition) - Estimating metabolic population structures (active vs inactive cells)	- Cheap, easy learning curve, & high-throughput screening - Samples that will be compared need to be run in the same machine - Taxonomic & phenotypic features can be correlated but *cannot* be linked	How do the patterns of biomass, population abundance, & community turnover shift over time & space? - *How do the patterns of resistance & resilience of microbial communities differ inside vs outside ant nests?* - *Do ants alter dispersal rates and routes of soil microbial populations?* - *Can microbial growth efficiency be linked to stoichiometric shifts caused by ant-driven nutrient inputs?*
Leucine Incorporation Assay	Rate of incorporation of radiolabeled leucine into newly synthesized proteins	- Estimating heterotrophic production rates - Estimating bacterial growth efficiency if combined with respiration assay	- Standard & cost-effective proxy for bacterial production - Does not target autotrophs or fungi	
Phospholipid fatty acid (PFLA) analysis	Concentration of phospholipid fatty acids	- Estimating microbial biomass based on biomarker that breaks down quickly when a cell dies - Profiling shifts in redox states & activity, which can then be used to assess soil health	- Cannot resolve taxa beyond broad groups - Ideal for high throughput screening of active microbial biomass and activity	
Potential Mineralizable Carbon (PMC)	Rate of CO2 production from respiration of soil organic carbon (SOC)	- Estimating decomposition potential of SOC - Inferring carbon cycling rates based on microbial activity	- Highly sensitive to lab incubation conditions - Widely used indicator of soil biological activity and health	

Spatial and In Situ Organization

Method	***Microbial Metric Quantified***	***Key Applications***	***Methodological Considerations***	***Example Questions***
NanoSIMS	Uptake & flow of isotopically labeled substrates (e.g., ^{13}C, ^{15}N)	- Visualizing enrichment of specific isotopes at high resolution - Assessing spatial interactions of metabolite exchanges	- Technically complex & costly - Ideal for high resolution enrichment analysis at the cellular levels	How & where are microbial populations interacting at finer spatial scales? - *How do ants alter fine-scale spatial organization patterns*

Fluorescence in situ hybridization (FISH)	Abundance & spatial distribution of taxa using fluorescently labeled probes	- Quantifying abundance of targeted taxa - Visualizing spatial organization & co-localization patterns	- Limited sensitivity to populations with low activity or low ribosome content - Significantly easier to learn and use compared to nanoSIMS - Ideal for locating & identifying particular taxa of interest	*in heterogeneous soil matrices?* *- Do microbial populations exhibit higher levels of metabolic cross-feeding within ant-modified habitats?* *- Are certain nest structures hotspots of biogeochemical activity compared to others?*