Commentary

Microbial villages in the geography of arbuscular mycorrhizal symbioses

Soil bacteria, archaea, and fungi provide critical ecosystem services but linking specific microorganisms with these services is challenging because their abundance and diversity are staggering. One gram of soil contains up to 1 billion bacterial cells with tens of thousands of taxa and up to 200 m of fungal hyphae (FAO *et al.*, 2020). How can the ecosystem roles of individual species be identified within this multitude?

'Arbuscular mycorrhizal hyphae provide an enormous yet invisible scaffolding upon which ecosystem and evolutionary processes occur.'

Controlled laboratory and greenhouse studies have clearly established that symbioses with arbuscular mycorrhizal (AM) fungi are critical for the normal phosphorus (P) nutrition of most plants (Treseder, 2013). Furthermore, studies show that 'hyphosphere bacteria' associated with AM fungal hyphae are needed to mineralize organic forms of P from the surrounding soil (Faghihinia *et al.*, 2022). Complex communities of hyphosphere microorganisms surround AM fungal hyphae that are distinct from those in bulk soil outside the zone of hyphal influence (Emmett *et al.*, 2021; Faghihinia *et al.*, 2022). The next step toward understanding how AM microbiomes contribute to plant P nutrition is to identify a core group of P-mineralizing bacteria *in situ*, in real-world hyphospheres.

This goal is daunting for several reasons. First, there is the challenge of collecting bacteria from hyphosphere microhabitats in field soils. Second, given the taxonomic and metabolic diversity of soil bacteria, it is difficult to ascertain which taxa are actively breaking down organic P on or near hyphae so that it becomes available for AM fungal uptake. Finally, a core consortium of P-solubilizing bacteria associated with AM hyphae must be distinguished from background site-specific variation that is inherent in soil microbial biogeography. The research pipeline developed by Wang *et al.* (2023; pp. 859–873) published in this issue of *New Phytologist* offers solutions to each of these challenges and provides sampling and analytical approaches to decipher patterns from background noise in

complex microbial communities. These advances will help us begin to understand the mechanisms by which bacteria associated with AM hyphae contribute to important ecosystem services.

Mycorrhizas are the basement of plant communities and manifestations of the geography of biomes (Read, 1991). Spatial structure is increasingly recognized for its role in facilitating species interactions and biogeochemical processes. Arbuscular mycorrhizal hyphae provide an enormous yet invisible scaffolding upon which ecosystem and evolutionary processes occur in the soils of most tropical and temperate ecosystems, including agroecosystems. This spatial and biological complexity of mycorrhizal symbioses generates emergent properties that may be missed in simplified laboratory and greenhouse studies. To address this problem, Wang *et al.* developed effective field-based methods to collect root-free AM fungal hyphae so that they could separately analyze microbial communities on hyphal surfaces and compare them with those in bulk soil. Furthermore, their experiments in arid, semi-arid, and mesic agroecosystems captured a wide range of environmental and biotic diversity. Wang and colleagues used network analysis to single out groups of interacting organisms from the microbial milieu and subsequent correlation analysis to link modules of interacting organisms with their capacity for P exploitation (higher phosphatase activity and lower total P remaining in soil). This approach also allowed them to identify bacterial 'guilds' (modules) specific to the AM hyphosphere. This narrowed the search and elucidated a core bacterial microbiome in the hyphosphere of a variety of different AM fungal taxa on maize and cotton at three different agricultural sites. This remarkable discovery was further substantiated in a greenhouse microcosm experiment using a single AM fungal isolate. The striking similarity of bacterial modules across different agroecosystems and in the microcosm experiment strongly suggests the existence of a conserved hyphosphere bacterial community and the potential importance of a hyphal scaffolding for microbiome assembly.

Environmental filtering, community interactions, and evolutionary processes can shape the geography of soil communities across a hierarchy of spatial and temporal scales (Chase *et al.*, 2021). Wang *et al.* observed distinctive AM fungal communities at their three study sites, which corroborates numerous field studies of large-scale environmental gradients that reveal spatial patterns in AM fungi based on climate, soil properties, and plant communities (e.g. Vasar *et al.*, 2022). The relative importance of biotic and abiotic filters varies among studies; nevertheless, these findings suggest niche differentiation among AM fungal species (Marro *et al.*, 2022). Is symbiotic function a driver of these patterns? There is evidence that plants select for AM fungi that deliver the most benefit (Bever, 2015) and furthermore, different AM fungal taxa select different bacterial communities (Zhou *et al.*, 2020; Emmett *et al.*, 2021) – even on the same host plant! The relevance of these micro-scale community interactions for understanding the

---

This article is a Commentary on Wang *et al.* (2023), **238**: 859–873.

function of mycorrhizal symbioses has yet to be fully explored but these discoveries are thought-provoking. Do plants actively cultivate networks of AM fungal hyphae to provide infrastructure for microbial processes that maximize resource exploitation in spatially and temporally heterogeneous soil? The lifespan of AM fungal hyphae is uncoupled from that of host plants, and relatively thick runner hyphae can persist long after roots die (Pepe *et al.*, 2018). Does the spatial structure created by residual AM hyphal networks influence evolutionary processes such as horizontal gene transfer and adaptation through multilevel selection? Future studies that utilize the spatially explicit field sampling methods developed by Wang *et al.* may help answer some of these questions.

All of this leads to an inevitable question, how do we define an evolutionary unit of selection when talking about AM fungi and the hyphosphere? Some authors have referred to the 'mycorrhizal collective' (plant plus symbiotic fungi) as an evolutionary individual (Molter, 2019), and discoveries by Wang *et al.* and Emmett *et al.* (2021) indicate that the mycorrhizal collective must include the bacterial microbiome associated with AM fungal hyphae. Evolution generates adaptations when there is phenotypic variation, differential fitness, and heritability (Lewontin, 1970), and these three requisites can occur at the scale of holobionts (Roughgarden *et al.*, 2018). Multilevel selection theory recognizes that natural selection often acts simultaneously on two or more levels of the biological hierarchy (Heisler & Damuth, 1987), with the same or different direction and strength (Marín, 2016). In this context, the fitness of an individual depends on its own traits plus the (average) traits of the surrounding intraspecific group, in addition to emergent traits, for example, biofilm formation when bacterial populations reach a threshold density (Marín, 2016). It has been argued that selfish individuals out-compete altruistic individuals, but altruistic collectives out-compete selfish groups (Wilson & Wilson, 2007). Does this kind of hierarchical selection also apply to interspecific teams comprised of a multitude of unrelated organisms? Since holobiont function is an emergent property of dynamic networks of interspecific interactions ranging from facilitation to antagonism, is the metaphor of 'selfish cheaters' obsolete? What are the roles of community assembly and genetic processes in the evolution of holobionts? More research is needed to address these questions, but there is already empirical evidence suggesting that microbiomes generate plant adaptation.

Multilevel selection may help explain how AM fungi and associated microbes can generate local adaptation in P-limited and water-limited ecosystems (Johnson *et al.*, 2010; Ji *et al.*, 2013; Remke *et al.*, 2020). A reciprocal inoculation experiment found that sympatric combinations of host plants, AM fungi, and associated soil microbes generated higher plant fitness compared with allopatric combinations (Johnson *et al.*, 2010). Subsequent analysis revealed that a single AM fungal species, *Gigaspora gigantea*, colonized plant roots inoculated with both allopatric and sympatric soil (Ji *et al.*, 2013). Could the unmeasured bacterial communities associated with sympatric ecotypes of *G. gigantea* be the conveyors of the observed local adaptation? This hypothesis is supported by the results of a field-based experiment showing that a

common soil bacterium can genetically adapt to its abiotic environment within just 18 months (Chase *et al.*, 2021). Could there be a similar bacterial adaptation to associate with resident ecotypes of AM fungi?

Further empirical work is needed to better understand the evolutionary dynamics of holobionts (Roughgarden *et al.*, 2018), and mycorrhizal microbiomes may provide an excellent model system for these studies. An effective approach for linking microbiome structure with its symbiotic function is to measure relative fitness and symbiotic, plant, and fungal traits (Chaudhary *et al.*, 2022) across natural resource/stress gradients. Another approach might be to use multigenerational microcosms and reciprocal transplant experiments in which AM fungi and mycorrhizal collectives are selected to perpetuate future generations according to their function with an unchanging plant host genotype (Swenson *et al.*, 2000). Realistic environmental conditions are essential to capture the emergent properties of mycorrhizal collectives, so whenever possible, studies should be conducted in the field or microcosms constructed with field-collected soils and corresponding microbial communities. Wang and colleagues have developed techniques for *in situ* collection of AM hyphae in the field and methods to elucidate patterns from complex datasets. Building upon these contributions will help advance our understanding of the roles of mycorrhizal microbiomes and other holobionts in natural and human-managed ecosystems.

ORCID

Nancy Collins Johnson ID https://orcid.org/0000-0001-7077-6232
César Marín ID https://orcid.org/0000-0002-2529-8929

Nancy Collins Johnson[1,2]* ID and César Marín[3,4] ID

[1]Department of Biological Sciences, Northern Arizona University, 617 S Beaver St., Flagstaff, AZ 86011, USA;
[2]School of Earth and Sustainability, Northern Arizona University, 624 S Knoles Dr., Flagstaff, AZ 86011, USA;
[3]Centro de Investigación e Innovación para el Cambio Climatico (CiiCC), Universidad Santo Tomás, Ave Ramón Picarte 1130, Valdivia, 5090000, Chile;
[4]Department of Ecological Sciences, Faculty of Earth and Life Sciences, Vrije Universiteit Amsterdam, de Boelelaan 1085, Amsterdam, 1081 HV, the Netherlands
(*Author for correspondence: email nancy.johnson@nau.edu)

References

Bever JD. 2015. Preferential allocation, physio-evolutionary feedbacks, and the stability and environmental patterns of mutualism between plants and their root symbionts. *New Phytologist* **205**: 1503–1514.

Chase AB, Weihe C, Martiny JB. 2021. Adaptive differentiation and rapid evolution of a soil bacterium along a climate gradient. *Proceedings of the National Academy of Sciences, USA* **118**: e2101254118.

Chaudhary VB, Holland EP, Charman-Anderson S, Guzman A, Bell-Dereske L, Cheeke TE, Corrales A, Duchicela J, Egan C, Gupta MM *et al.* 2022. What are mycorrhizal traits? *Trends in Ecology & Evolution* **37**: 573–581.

Emmett BD, Lévesque-Tremblay V, Harrison MJ. 2021. Conserved and reproducible bacterial communities associate with extraradical hyphae of arbuscular mycorrhizal fungi. *The ISME Journal* **15**: 2276–2288.

Faghihinia M, Jansa J, Halverson LJ, Staddon PL. 2022. Hyphosphere microbiome of arbuscular mycorrhizal fungi: a realm of unknowns. *Biology and Fertility of Soils* **59**: 17–34.

FAO, ITPS, GSBI, CBD, EC. 2020. *State of knowledge of soil biodiversity – status, challenges and potentialities, report 2020.* Rome, Italy: FAO.

Heisler IL, Damuth J. 1987. A method for analyzing selection in hierarchically structured populations. *The American Naturalist* **130**: 582–602.

Ji B, Gehring CA, Wilson GWT, Miller RM, Flores-Rentería L, Johnson NC. 2013. Patterns of diversity and adaptation in Glomeromycota from three prairie grasslands. *Molecular Ecology* **22**: 2573–2587.

Johnson NC, Wilson GW, Bowker MA, Wilson JA, Miller RM. 2010. Resource limitation is a driver of local adaptation in mycorrhizal symbioses. *Proceedings of the National Academy of Sciences, USA* **107**: 2093–2098.

Lewontin RC. 1970. The units of selection. *Annual Review of Ecology and Systematics* **1**: 1–18.

Marín C. 2016. The levels of selection debate: taking into account existing empirical evidence. *Acta Biológica Colombiana* **21**: 467–472.

Marro N, Grilli G, Soteras F, Caccia M, Longo S, Cofré N, Borda V, Burni M, Janoušková M, Urcelay C. 2022. The effects of arbuscular mycorrhizal fungal species and taxonomic groups on stressed and unstressed plants: a global meta-analysis. *New Phytologist* **235**: 320–332.

Molter DJ. 2019. On mycorrhizal individuality. *Biology and Philosophy* **34**: 52.

Pepe A, Giovannetti M, Sbrana C. 2018. Lifespan and functionality of mycorrhizal fungal mycelium are uncoupled from host plant lifespan. *Scientific Reports* **8**: 10235.

Read DJ. 1991. Mycorrhizas in ecosystems. *Experientia* **47**: 376–391.

Remke MJ, Johnson NC, Wright J, Williamson M, Bowker MA. 2020. Sympatric pairings of dryland grass populations, mycorrhizal fungi, and associated soil biota enhance mutualism and ameliorate drought stress. *Journal of Ecology* **109**: 1210–1223.

Roughgarden J, Gilbert SF, Rosenberg E, Zilber-Rosenberg I, Lloyd EA. 2018. Holobionts as units of selection and a model of their population dynamics and evolution. *Biological Theory* **13**: 44–65.

Swenson W, Wilson DS, Elias R. 2000. Artificial ecosystem selection. *Proceedings of the National Academy of Sciences, USA* **97**: 9110–9114.

Treseder KK. 2013. The extent of mycorrhizal colonization of roots and its influence on plant growth and phosphorus content. *Plant and Soil* **371**: 1–13.

Vasar M, Davison J, Sepp SK, Oja J, Al-Quraishy S, Bueno CG, Cantero JJ, Fabiano EC, Decocq G, Fraser L *et al.* 2022. Global taxonomic and phylogenetic assembly of AM fungi. *Mycorrhiza* **32**: 135–144.

Wang L, Zhang L, George TS, Feng G. 2023. A core microbiome in the hyphosphere of arbuscular mycorrhizal fungi has functional significance in organic phosphorus mineralization. *New Phytologist* **238**: 859–873.

Wilson DS, Wilson EO. 2007. Rethinking the theoretical foundation of sociobiology. *The Quarterly Review of Biology* **82**: 327–348.

Zhou J, Chai X, Zhang L, George TS, Wang F, Feng G. 2020. Different arbuscular mycorrhizal fungi co-colonizing on a single plant root system recruit distinct microbiomes. *mSystems* **5**: e00929-20.