*Commentary*

**Swimming, gliding, or hyphal riding? On microbial migration along the arbuscular mycorrhizal hyphal highway and functional consequences thereof**

Arbuscular mycorrhizal (AM) fungi colonize both roots and the surrounding soil, where they form dense hyphal networks extending the nutrient depletion zone surrounding the host root by up to several centimetres. This enables the efficient acquisition of mineral nutrients such as phosphorus (P) and nitrogen (N) from the soil environment, in addition to other effects being exerted on the soil itself, e.g. enmeshing soil particles to aggregates or reducing tortuosity for water and solutes flow. Due to their ancient origin, and possibly also due to the specific environmental niche that they inhabit, AM fungi lack efficient exoenzymes to mineralize complex organic compounds and hence cannot release the N and/or P resources that may be ‘held’ within such complex compounds directly by themselves (Tisserant *et al.*, 2013). Yet, AM fungal hyphae were experimentally demonstrated to acquire mineral N from complex organic N sources, leading to the postulation of a key role in hyphae-associated prokaryotes in ‘unlocking‘ otherwise unavailable resources for the AM fungi and their associated host plants (Hodge *et al.*, 2001; Hodge, 2014). It subsequently took a number of years to begin to decipher the exact players involved in the utilization of organic nutrients by AM fungi and, subsequently, their host plant. Interestingly, the first code broken was that of the players involved in the phosphate story (Zhang *et al.*, 2018). In the recently published article in *New Phytologist*, Jiang *et al*. (2020; doi: 10.1111/nph.17081) follow up on this exciting work. The authors ask how an associate to AM fungal hyphae, the flagellated prokaryote *Rahnella aquatilis*, as well as other P solubilizing bacteria, migrate towards a a localized source of organic P (phytate) along the AM fungal hyphae in order to fulfil their role in P solubilization. Using a series of four elegantly designed experiments and employing a whole array of methodical approaches, from microscopy, gfp tagging, qPCR, P analytics and traditional microbiology, coupled with a synthetic proxy for AM fungal hyphae, the authors conclude that the bacterium swims in a water film along the AM fungal hyphae, being nourished by hyphal exudates on its way towards the phytate patch, where it extends the functional capability of the fungus to efficiently utilize this otherwise inaccessible P source. The authors also extend their experimentation to another (non-flagellated) bacterium, which was not able to make it along the hyphal highway in a similar manner, supporting the notion that motile bacteria are at an advantage in arriving at the organic hotspot compared to sessile ones.

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Despite the obvious importance of biotic interactions in the AM fungal hyphosphere for global carbon and nutrient cycling and overall ecosystem functioning, this realm still largely remains an enigma (Hodge, 2014). Tight association between AM fungal hyphae and organic patches/materials in soil have been repeatedly observed (e.g., Mosse, 1959; Bunn *et al.*, 2019) and positive effects of proximity of AM fungal hyphae on mineralization of organic materials have been demonstrated (Hodge *et al.*, 2001). However, this has not always resulted in improved mineral nutrition (particularly of N) for the associated host plants (Hodge, 2001, 2014). Studies such as that by Jiang *et al.* (2020) are therefore urgently required to unravel this mysterious zone of interaction and decipher the key processes and players from the inevitable background system ‘noise‘ (Jansa *et al.*, 2019). Moreover, although the organic N story has received widespread attention over the last two decades, a smoking gun parallel to the association between *Rhizophagus* and *Rahnella* for utilization of P from phytate, has not yet been unearthed. The reasons for this may be multifold: N cycling involves many more, and partly competing, processes than P cycling; multiple different microbes fulfil the same function but at different temporal and/or spatial scales (functional redundancy); previous studies have tended to used chemically heterogeneous (though ecologically more relevant) N substrates, such as plant litter containing a variety of organic N compounds - proteins, nucleic acids and various secondary metabolites, to name just the more prominent ones.

Mineralization of different classes of organic compounds may require different enzymes and/or microbes, and this may further complicate detailed insights. Simple observation of AM fungal hyphae recovered from unsterile soil-based potting substrate reveals a rich microbial life on their surfaces, forming biofilms with cells and with extracellular polymeric substances (Fig. 1). This means that the immediate vicinity of the AM fungal hyphae may consist of a variety of micro-environments, ranging from rather immobile gels to liquid water, all of which could be inhabited by a variety of different microbes showing varying motility, growth rates, functions and positions in soil food chains. Although some information is available on the strength of attachment of certain prokaryotic taxa to AM fungal hyphae (Toljander *et al.*, 2006) and the cascading effects of such interactions on the host plants (Artursson *et al.*, 2006), comprehensive knowledge of the composition of the hyphal microbiome of AM fungi, including upon its dynamics and functioning, is still largely absent. Temporal dynamics of liquid water films (particularly with respect to their stability and thickness) may further contribute to migration of microbes along the AM fungal hyphae, as also noted in the recent paper, although the diversity of micro-environmental conditions and the microbial life in the AM fungal hyphosphere certainly could further modulate many of the processes. Bacterial grazers like ciliated, amoeboid or gliding protists in particular, could contribute to both population control and dispersal of the different microbes in the AM fungal hyphosphere – especially because some of these eukaryotes could accommodate different microbes inside their cells for extensive periods of time, and release them back to the environment thereafter (e.g., Schulz *et al.*, 2014). Along the same lines, the potential role and impact of phages on microbial communities in the hyphosphere also needs be considered in future research.

The AM fungal highway could thus be used by disparate microbes for ease of movement across the soil environment in a variety of contrasting ways. The received wisdom is that as AM fungal hyphae show apical growth, some microbes at the very tip of the hyphae simply move passively through the soil attached to the hyphal tip, whereas those remaining alongside already established hypha largely remain there *in situ*. The exception of such a rule would be if the microbes proliferate and/or move (either randomly or chemotactically) towards the organic nutrient source (or other attractive targets), or are taxied there by means of other carriers such as protists. Separation of directed movement of individual cells from population growth along environmental gradients, and from local population booms following arriving at a nutrient-hotspot, is a challenge and one that could not yet be addressed in the current paper (Jiang *et al.*, 2020). However, as the authors clearly illustrate, the movement of *Rahnella* in the *Rhizophagus* hyphosphere was fuelled by energy derived from AM fungal exudates. This is potentially very important as it points to a possible control exerted on the prokaryote by the AM fungus. However, aside from addressing the possible control gears of the interaction between the AM fungus and the bacterium, further experimental attention is also required to unequivocally demonstrate bacterial chemotaxis towards the potential nutrient source. Likewise, it will also need further experimental effort to decipher the chicken-and-egg question involving feedback interactions between previously observed proliferation of the AM fungal hyphae in organic patches and presence/growth/metabolic activity of certain microbes in those patches (Nuccio *et al.*, 2013; Hodge, 2014). Importantly, both synergistic and antagonistic interactions have been reported between AM fungi and certain soil microbes (Artursson *et al.*, 2006; Svenningsen *et al.*, 2018). With current advancements in molecular, isotopic and imaging methods, we hope to see future breakthroughs in elucidating the structure, dynamics and assembly rules of AM fungal hyphosphere microbiomes. This exciting research will help in answering some of the long-standing questions as to who is actually responsible, and how these important, yet still poorly understood, processes of mycorrhiza-induced P and N mineralization and utilization of organic nutrient sources is achieved, using both simplified model systems as well as under complex field settings.

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**Fig. 1** Scanning electron microscopy (SEM) of biofilms containing microbial cells and extracellular polymeric matrix on surface of arbuscular mycorrhizal (*Rhizophagus irregularis*) fungal hyphae extracted from a root-free compartment of a pot experiment described previously by Bukovská *et al.* (2018) and fixed post-harvest in a glutaraldehyde-cacodylate buffer (pH 7.4). Fully hydrated samples were observed under environmental SEM (upper row of images), or post-fixed with osmium tetroxide, fully dehydrated and subsequently sputter-coated with 3 nm of platinum before observing in a high-vacuum SEM (bottom row of images), where the extracellular polymeric matrix inevitably collapsed in consequence to dehydration.

