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Commentary

Fungi in ant–plant interactions: a key to enhancing plant nutrient-acquisition strategies

To survive and reproduce as sessile organisms, plants often rely on mutually beneficial interactions with partners from different kingdoms. Plants known as myrmecophytes form mutually beneficial interactions with ants, and such interactions play an important role in tropical ecosystems. The plant provides food and shelter to the ant colony. In return, the ants provide several benefits including contributing to the nutrient needs of their host plant, through the accumulation of wastes from the ant colony. Such myrmecotrophy (meaning 'ant-feeding') is of low cost for the ants, as it is a by-product of their activities, but it can be of considerable benefit to the nutrient budget of the host plants, particularly in nutrient-poor environments (Dejean *et al.*, 2012). In ground-rooted ant–plant systems, the transfer of nutrients from ant wastes to their host plants can be mediated by fungi (Defossez *et al.*, 2011; Leroy *et al.*, 2011) or by bacteria (Lucas *et al.*, 2018). However, nothing is known about the role of endophytic fungi in nutrient transfer in epiphytic ant–plants, which do not have direct contact with the soil and suffer from limited availability of mineral nutrients. In this issue of *New Phytologist*, Gegenbauer *et al.* (2023; pp. 2210–2223) 'Exo- and endophytic fungi enable rapid transfer of nutrients from ant waste to orchid tissue' report on a fascinating way in which the epiphytic orchid, *Caularthron bilamellatum*, benefits from additional nutrients from ant wastes. This orchid has a storage organ known as a pseudobulb, formed from a thickening of the stem, which houses its ant colony. Specifically, this study investigates whether fungi present in the pseudobulb of the orchid are involved in the translocation of nutrients into the orchid tissues. The authors used an impressive set of innovative analytical techniques to test for fungi-mediated nitrogen fluxes from ant wastes to the plant tissues. They provide the first clear evidence that a tropical epiphyte obtain nitrogen from the activity of mutualistic ants through the synergism between two types of phylogenetically unrelated fungi. The study by Gegenbauer *et al.* (2023) is a significant contribution to our understanding of complex interactions in tropical ecosystems, and specifically, of the diversity of strategies used by plants to acquire nutrients.

The transfer of nutrients from ant wastes to myrmecophytic plants (known as myrmecotrophy) has been known since the 1970s for various ecological types of tropical plants worldwide, including trees (e.g. Defossez *et al.*, 2011; Dejean *et al.*, 2012), epiphytes (e.g. Watkins *et al.*, 2008; Gegenbauer *et al.*, 2012; Chomicki & Renner, 2019) and carnivorous plants (Bazile *et al.*, 2012). In myrmecophytes, ants accumulate organic wastes in their nesting sites inside the host plant (e.g. Defossez *et al.*, 2011; Lucas *et al.*, 2018). Stable isotopes have played a key role in quantifying the transfer of nitrogen from ant wastes to plant tissues. Food exchange has been directly demonstrated through labelling with enriched stable isotopes (e.g. Leroy *et al.*, 2011; Dejean *et al.*, 2012) or indirectly through natural abundance isotopic modelling (e.g. Treseder *et al.*, 1995; Watkins *et al.*, 2008). However, until recently, the underlying mechanisms of nutrient transfers remained unknown. Recent research has shown that in some systems the nutritional service provided by mutualistic ants to host plants is mediated by specific fungi (Defossez *et al.*, 2011; Leroy *et al.*, 2011).

> *'While studies on nutrient transfer via mycorrhiza in roots are numerous, much less is known about the role of endophytic fungi in the stem of ant-plants for nutrient provision.'*

It is only in the past 20 years, with the development of molecular approaches, that the identification and the extent of specificity of the fungal communities have been studied. Fungal strains isolated from the small chambers that house ants have been found to be both ubiquitous and not specific to either the ant or the plant species (e.g. Nepel *et al.*, 2016), while other strains have been found to show a high degree of ant-host specificity (e.g. Ruiz-González *et al.*, 2011; Blatrix *et al.*, 2013). The dominant fungi isolated from ant–plant systems studied so far are 'black yeasts' from the orders Chaetothyriales and Capnodiales of phylum Ascomycota (Voglmayr *et al.*, 2011). Ant-associated fungi are usually restricted to ant wastes and dead cells and do not penetrate living plant cells (Defossez *et al.*, 2011; Voglmayr *et al.*, 2011). The first evidence of the presence of hyphae inside plant tissues was found in the ground-rooted myrmecophyte *Hirtella physophora* (Leroy *et al.*, 2017). In that case, endophytic fungi (likely Chaetothyriales) were only observed, using transmission electron microscopy, in the cellular compartment of the stem tissues. In their study, Gegenbauer *et al.* (2023) found two strikingly distinct types of hyphae in ant-inhabited pseudobulbs: a thick septate, melanized type assigned to 'black fungi' from the orders Chaetothyriales, Cladosporiales and Mycosphaerellales in the ant wastes and a thin, septate endophytic type belonging to the order Hypocreales. Hyphae of the second

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type of fungi were observed, with transverse section and scanning electron microscopy, along the cell walls, several layers deep into the living cells and into cells of the vascular bundles. Remarkably, both exo- and endophytic fungi co-occurred in the layers of dead cells and 1–3 layers in the living tissue, a zone called by the authors the 'interface zone' (fig. 2 in Gegenbauer *et al*., 2023).

Whether the fungi inside plant tissues play indirect (i.e. degrade complex molecules into simpler ones that can be incorporated by the plant through absorptive tissues) or direct roles in transferring nutrients to the plant tissues is not straightforward to ascertain. Traditional isotope-ratio mass spectrometry is based on bulk plant samples and cannot provide information on plant–hyphae cellular and subcellular nitrogen dynamics. An innovative solution to this problem comes from new advances in secondary-ion mass spectrometry imaging techniques (SIMS) used since the 2000s for various biological studies (e.g. Bougoure *et al*., 2014; Bonnin & Rizzoli, 2020). When coupled with isotope labelling and other correlative microscopy techniques, SIMS analysis represents a stimulating new avenue for the examination and quantification of *in situ* incorporation and transfer of isotopic labelling at the subcellular spatial scale. Leroy *et al*. (2017), first to use nanoscale secondary ion mass spectrometry (NanoSIMS) technology in ant–plant–fungi interactions, found that the endophytic hyphae actively transfer nitrogen from a ^{15}N-labelling solution to the plant's stem tissues, suggesting that it may occur for nutrient-derived ant wastes. In their study, Gegenbauer *et al*. (2023) employed both time-of-flight secondary ion mass spectrometry (ToF-SIMS) and NanoSIMS. They found a ^{15}N enrichment of fungal hyphae and evidence of the transport of the ^{15}N-labelling solution along cell walls (i.e. apoplast), indicating that fungal hyphae are particularly efficient structures for absorbing ^{15}N from the ant waste site. Based on their results, the authors propose the existence of two simultaneous uptake pathways. The first one is a two-way nitrogen transfer involving both Chaetothyriales and Hypocreales fungi sequentially in two steps. First, the black fungi take-up the ^{15}N label from ant wastes and process it via their intermediary metabolism. Amino acids are then released back into the apoplast of cells of the interface zone. Second, endophytic Hypocreales could absorb the ^{15}N-labelled amino acids and transfer them to the vascular bundles. The second pathway is a direct one-way nitrogen transfer via endophytic Hypocreales hyphae that have grown into the ant wastes. The interface zone in *C. bilamellatum* pseudobulb tissue is thus an important interface at a fungus–fungus interaction level with a synergistic effect for nutrient transfer. Gegenbauer *et al*. (2023) provide us with a first insight into how the interaction of two unrelated fungi enhance a rapid transfer of nutrients from ant wastes to the plant tissues.

Given the nutrient-poor nature of the tropics and specifically in the canopy habitats, such cryptic associations may be an important component of plant nutrient relations. Gegenbauer *et al*. (2023) show that the epiphytic orchid *C. bilamellatum* sacrifices *c*. 50% of the volume of water storage tissues in the pseudobulbs for ants housing, indicating that the access to nutrients may be more important than water storage. Efficient nutrient uptake by the plants would be beneficial for both plant and ant mutualistic partners, as it would allow for the coupling of plant and ant colony growth. Whether the interaction between endophytic fungi and the host plant is mutualistic is still unknown. Endophytic fungi find protection in the above-ground plant tissues and access to ant wastes, and may be functionally equivalent to mycorrhizal mycelia in roots (Leroy *et al*., 2017; Gegenbauer *et al*., 2023). However, neither of these studies provided evidence that carbon from plant photosynthates is reciprocally transferred into the hyphae. Yet, the natural abundances of carbon stable isotopes provided some insights into the possible use of *H. physophora* tissues as a carbon source by the fungus (Orivel *et al*., 2017). Regardless of the exact nature of the plant–fungi interaction, the nutritional benefits provided by the fungi to the plant could positively affect the evolutionary persistence of the ant–plant interaction, especially since these fungi are strictly associated with the presence of the ants. The study by Gegenbauer *et al*. (2023) informs us that ant–plant interactions can be more complex than previously thought due to cryptic associations and that we still have much to learn about plant strategies for acquiring nutrients.

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References

Bazile V, Moran JA, Le Moguédec G, Marshall DJ, Gaume L. 2012. A carnivorous plant fed by its ant symbiont: a unique multi-faceted nutritional mutualism. *PLoS ONE* 7: e361.

Blatrix R, Debaud S, Salax-Lopez A, Born C, Benoit L, McKey D, Attéké C, Djiéto-Lordon C. 2013. Repeated evolution of fungal cultivar specificity in independently evolved ant–plant–fungus symbioses. *PLoS ONE* 8: e68101.

Bonnin EA, Rizzoli SO. 2020. Novel secondary ion mass spectrometry methods for the examination of metabolic effects at the cellular and subcellular levels. *Frontiers in Behavioral Neuroscience* 14: 124.

Bougoure J, Ludwig M, Brundrett M, Cliff J, Clode P, Kilburn MR, Grierson P. 2014. High-resolution secondary ion mass spectrometry analysis of carbon dynamics in mycorrhizas formed by an obligately myco-heterotrophic orchid. *Plant, Cell & Environment* 37: 1223–1230.

Chomicki G, Renner SS. 2019. Farming by ants remodels nutrient uptake in epiphytes. *New Phytologist* 223: 2011–2023.

Defossez E, Djiéto-Lordon C, McKey D, Selosse M-A, Blatrix R. 2011. Plant–ants feed their host plant, but above all a fungal symbiont to recycle nitrogen. *Proceedings of the Royal Society of London B: Biological Sciences* **278**: 1419–1426.

Dejean A, Petitclerc F, Roux O, Orivel J, Leroy C. 2012. Does exogenic food benefit both partners in an ant–plant mutualism? The case of *Cecropia obtusa* and its guest *Azteca* plant–ants. *Comptes Rendus Biologies* **335**: 214–219.

Gegenbauer C, Bellaire A, Schintlmeister A, Schmid MC, Kubicek M, Voglmayr H, Zotz G, Richter A, Mayer VE. 2023. Exo- and endophytic fungi enable rapid transfer of nutrients from ant waste to orchid tissue. *New Phytologist* **238**: 2210–2223.

Gegenbauer C, Mayer VE, Zotz G, Richter A. 2012. Uptake of ant-derived nitrogen in the myrmecophytic orchid *Caularthron bilamellatum. Annals of Botany* **110**: 757–766.

Leroy C, Jauneau A, Martinez Y, Cabin-Flaman A, Gibouin D, Orivel J, Séjalon-Delmas N. 2017. Exploring fungus–plant N transfer in a tripartite ant–plant–fungus mutualism. *Annals of Botany* **120**: 417–426.

Leroy C, Sejalon-Delmas N, Jauneau A, Ruiz-Gonzalez M-X, Gryta H, Jargeat P, Corbara B, Dejean A, Orivel J. 2011. Trophic mediation by a fungus in a tripartite ant–plant mutualism. *Journal of Ecology* **99**: 583–590.

Lucas JM, Clay NA, Kaspari M. 2018. Nutrient transfer supports a beneficial relationship between the canopy ant, *Azteca trigona*, and its host tree. *Ecological Entomology* **43**: 621–628.

Nepel M, Voglmayr H, Blatrix R, Longino JT, Fiedler K, Schönenberger J, Mayer VE. 2016. Ant-cultivated Chaetothyriales in hollow stems of myrmecophytic *Cecropia* sp. trees – diversity and patterns. *Fungal Ecology* **23**: 131–140.

Orivel J, Malé P-JG, Lauth J, Roux O, Petitclerc F, Dejean A, Leroy C. 2017. Trade-offs in ant–plant–fungus mutualism. *Proceedings of the Royal Society of London B: Biological Sciences* **284**: 20161679.

Ruiz-González M-X, Malé P-JG, Leroy C, Dejean A, Gryta H, Jargeat P, Quilichini A, Orivel J. 2011. Specific, non-nutritional association between an Ascomycete fungus and *Allomerus* plant–ants. *Biology Letters* **7**: 475–479.

Treseder KK, Davidson DW, Ehleringer JR. 1995. Absorption of ant-provided carbon dioxide and nitrogen by a tropical epiphyte. *Nature* **375**: 137–139.

Voglmayr H, Mayer V, Maschwitz U, Moog J, Djieto-Lordon C, Blatrix R. 2011. The diversity of ant-associated black yeasts: insights into a newly discovered world of symbiotic interactions. *Fungal Biology* **115**: 1077–1091.

Watkins JE, Cardelus CL, Mack MC. 2008. Ants mediate nitrogen relations of an epiphytic fern. *New Phytologist* **150**: 5–8.

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