Viewpoints

In situ mycorrhizal function – knowledge gaps and future directions

Summary

We know a lot about the *potential* functions of mycorrhizas, but whether or not these are *realized* in the field where plants simultaneously experience a range of biotic interactions and fluctuating abiotic conditions is more or less unknown. In this Viewpoint, we present findings from a literature survey of papers on mycorrhizal function published in *New Phytologist* during the past 30 years. This survey showed that most functional studies are still conducted under controlled conditions, target mostly arbuscular and ectomycorrhizas, and focus on nutrient and carbon dynamics of the symbiosis. We also share discussions from a workshop, '*In situ* mycorrhizal function: how do we get relevant data from a messy world?', held at the 9th International Conference on Mycorrhiza (ICOM9) in August 2017. In this workshop, we examined possibilities and limitations of old and new techniques for field research, and participants expressed the need to learn more about fungal traits and how they may relate to function. We argue that moving mycorrhizal experiments into the field will allow us not only to quantify realized functions, but also to revisit old paradigms and possibly discover new functions.

Background

Since research on mycorrhizas began, we have learned a great deal about the taxonomic identity and richness of the symbiotic partners, as well as their form and potential function (Smith & Read, 2008). Most functional studies have targeted nutritional benefits to the host of the symbioses, and groundbreaking work has shown that fungal symbionts can provide the majority of nutrients required by plants (Smith *et al.*, 2003) and access to nutrient sources that are otherwise unavailable to them (Read & Perez-Moreno, 2003). Persistent debates remain about functional diversity and complementarity, evolution and selection of function, and host–fungus interactions operating along the mutualism to parasitism continuum to name a few. The most striking gap in our opinion, however, is the weak knowledge of the functional properties of mycorrhizas in field settings. In this Viewpoint, we highlight the current state of research on mycorrhizal function, identify obstacles to field research, and outline critical questions and approaches we believe can move the field beyond *potential* to *realized* mycorrhizal function. These ideas are partly based on outcomes of the workshop, '*In situ* mycorrhizal function: how do we get relevant data from a messy world?' that we held at the 9th International Conference on Mycorrhiza (ICOM9) in August 2017. Moving mycorrhizal research into the field involves many challenges, but will allow us not only to quantify realized functions, but to revisit and examine old paradigms, and potentially discover new functions.

Current state of research on mycorrhizal function

To get a better idea of the nature of functional mycorrhizal research, we searched Web of Science on October 15, 2017, using the option 'All Databases' and the search terms 'Mycorrhiza* and function' restricted to *New Phytologist* papers published between 1987 and 2017. We chose to focus on *New Phytologist* publications due to the journal's long history of publishing high-quality, mechanistic mycorrhizal research. This returned 212 publications, but 145 of those were rejected because they were reviews, commentaries, or clearly addressed a separate topic. For the remaining 67 papers, we collected information regarding mycorrhizal type studied, the function targeted, whether individual fungi or communities were included, how the symbiosis was manipulated, what measurements were collected, and whether the study was conducted in the greenhouse, field or involved axenic cultures (see Supporting Information Table S1 for specific papers included and information extracted, and Table S2 for a summary). This was by no means meant to be exhaustive, and there clearly are well-known functional papers that were excluded because they were published elsewhere or were not targeted by our search terms. However, we argue that these publications include a relevant subset of high-quality mycorrhizal studies published during the past 30 years that illustrate specific functions and symbioses researched, as well as approaches chosen.

Not surprisingly, most studies targeted ectomycorrhiza (EM, 48%) and arbuscular mycorrhiza (AM, 48%) while ericoid and orchid mycorrhizas were the focus in < 5% of studies. Likewise, nutrient (67%) and carbon (40%) movements were the main functions assessed, whereas other functions, such as water relations (7%) and pathogen protection (1%) were rarely studied. This is particularly interesting given the recent Delavaux *et al.* (2017) meta-analysis showing that services other than nutrient acquisition can be equally important in AM, and results by Bennett *et al.* (2017) highlighting the central role of pathogen protection by EM fungi. Mycorrhizas were most often manipulated using inoculation (46%), whereas ingrowth cores (10%) and fungicides (4%) were less utilized. The use of genetic manipulation (e.g. knock out or expression systems) to reveal function was restricted to EM in these publications (14%), although plant mutants have been very informative in AM research published elsewhere (e.g. Barker

et al., 1998; Javot *et al.*, 2007). Some experiments (31%) relied on other manipulations, such as CO_2 enrichments or nutrient amendments, which indirectly manipulated mycorrhizal fungal abundance or composition, and thus limited assessments of mycorrhizal function to correlation analyses between mycorrhizal responses and other variables measured.

Most studies measured biomass responses by both plants and fungi (Fig. 1; Table S1). While biomass represents the consequence of the sum of all functions, it provides limited information about the underlying mechanism(s) unless paired with specific treatments, such as nutrient additions or water manipulations. Also, biomass may not be a good measure of fitness (Varga & Kytöviita, 2010), especially when extrapolated from short-term greenhouse experiments. To understand mechanistic relationships, it is essential to distinguish between the measurement of actual function and various proxies of function. This applies to other variables as well. For instance, genomic studies need to be combined with host or fungal mutants or other experimental approaches to verify actual, not just potential, function. Likewise, solely quantifying differences in enzymatic activity between mycorrhizal and non-mycorrhizal treatments may have limited value unless it is combined with some measure of how shifts in activity influence either plant or fungus.

The most striking finding from the literature survey, however, was that – despite pleas by other researchers more than 20 years ago (Read, 1991; Johnson *et al.*, 1997) – we are still primarily conducting experiments in controlled environments, not in the field. Of the 67 studies we surveyed, only three experimentally manipulated mycorrhizal abundance in the field. These studies used rotating soil cores (Johnson *et al.*, 2001) or transplanted seedlings of varying AM colonization (McGonigle & Fitter, 1988) to quantify the role of AM fungi for P acquisition, or applied fungicides to assess the role of ericoid mycorrhizal fungi in heath (Michelsen *et al.*, 1999). None of these are recent publications, which highlights the fact that while methods to manipulate mycorrhizal fungal abundances in the field are available, their use continues to be limited.

What are the barriers to field research?

The keywords 'Experiment' and 'Greenhouse/chamber' were positively correlated and opposite to 'field' and 'survey' when relationships among the 67 *New Phytologist* publications were assessed (Fig. 1; Table S1). In other words, we tend to conduct experiments under controlled conditions, and surveys in the field. Surveys can be powerful tools to understand relationships between mycorrhizas and various processes (e.g. Read, 1991; Cheeke *et al.*, 2017), but in order to quantify function, we need to experimentally manipulate the symbiosis. This is often quite challenging to do in the field. Inoculating larger areas can be costly and impractical and relies on low natural abundances of fungi, fungicides reduce the abundance of all fungi, which complicate interpretations, and rotating soil cores take a lot of maintenance to be effective. In addition to technological challenges associated with field experiments, conditions are harder to control in the field than in the greenhouse, and plants and fungi are sometimes exposed to

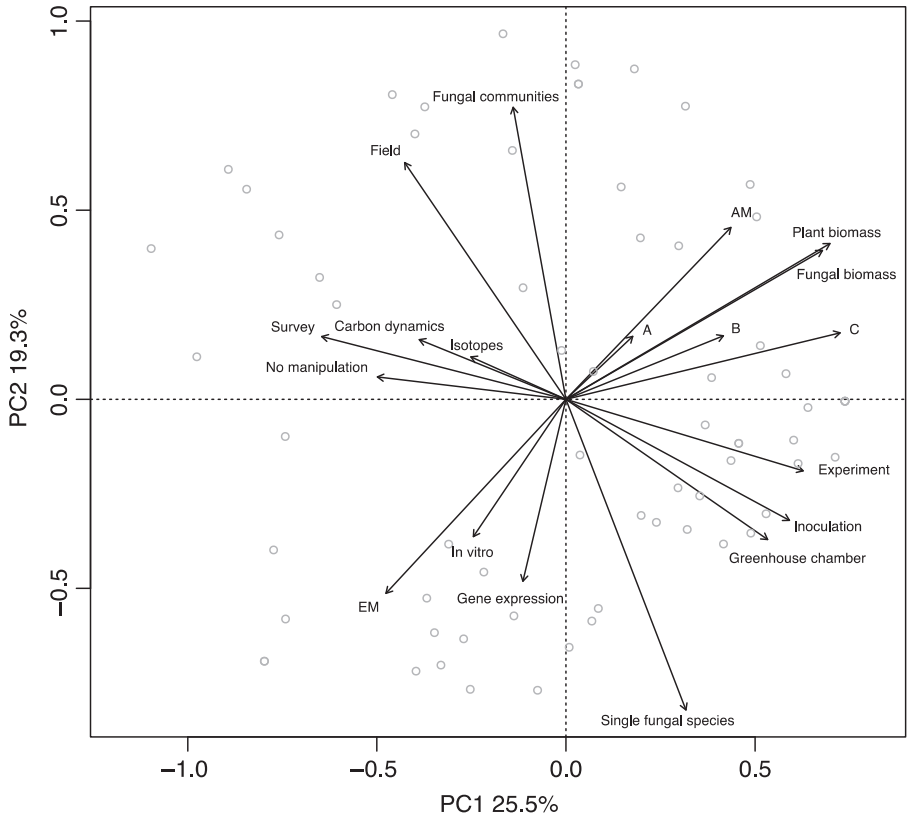

Fig. 1 Principal component analysis (PCA) biplot implemented in the VEGAN package of R, using the data in Supporting Information Table S1, excluding uninformative variables occurring 10 times or fewer. Gray points represent each paper, and arrows the strength of the variable. For visual clarity, some variables have been renamed: A, other manipulations; B, nutrient dynamics; C, plant nutrient concentration; AM, arbuscular mycorrhiza; EM, ectomycorrhiza.

multiple biotic interactions and varying abiotic conditions that can reduce the likelihood of significant responses. The logistical issues inherent in field research are compounded by the fact that access to existing long-term field experiments and the creation of new ones are sometimes challenging. This dissuades many researchers, especially early career scientists, from embarking on field experiments. Joining global research collaborations, such as Nutrient Network (www.nutnet.umt.edu), or conducting add-on studies in established Long Term Experimental Research (LTER) sites (https://lternet.edu) may be ways to circumvent obstacles and reduce cost, save time and minimize risks associated with field experiments.

Our visualization of the papers illustrated another potential barrier to progress and paradigm shifts in mycorrhizal research. In Fig. 1, AM and EM were clearly separated from each other, and so were many of the methods used, indicating that different symbioses are often researched in isolation using a specific set of tools. While this may be appropriate in cases where one symbiosis dominates, recent papers highlight how much we can learn from comparing symbioses using the same methodology (Bennett *et al.*, 2017; Cheeke *et al.*, 2017). This is not only an issue across symbioses, but also applies to different disciplines within a symbiosis. For example, many fungal community surveys implicitly assume that observed shifts have functional consequences, whereas studies assessing function often research responses using selected fungal isolates or undefined mixed inocula that lack obvious links to field systems (Table S1). Based on this, it was encouraging to see the increased crosstalk across disciplines at ICOM9 (Waller *et al.*, 2018), which we hope will reduce real or perceived divisions, enable transfer of methodology and expertise, and result in novel and creative ways of answering research questions.

What can we learn from field research and what are feasible approaches?

The field of evolution probably offers the best example of the importance of studying organisms in their natural environments. For example, how would we make sense of the shift in melanism in peppered moths were it not for the observation of concomitant changes in soot pollution combined with controlled predation experiments (summarized in Cook *et al.*, 2012)?

What are the unanswered questions in terms of mycorrhizal function, and how would our perception of the symbiosis change if we were better able to capture function in the field? Are mycorrhizal fungi parasitic in nature and if so, to what extent, under what conditions and for what duration? Participants at the ICOM9 workshop were asked to identify unanswered questions related to mycorrhizal function, and we captured responses as word clouds (Fig. 2a). The key knowledge gaps centered around mycelial traits, highlighting how the fungal mycelium remains a critically important but poorly understood component of the symbioses that we rarely measure (Fig. 2a). Mycelial growth and colonization patterns differ among fungal taxa, both within AM and EM (Agerer, 2001; Hart & Reader, 2002; Koide *et al.*, 2007, and references cited therein), and these differences may translate into different functions (Courty *et al.*, 2005; Maherali & Klironomos, 2007). For example, AM fungi that predominately colonize inside the root may be better pathogen protectors than those that grow an extensive extraradical mycelium, which aids in nutrient uptake (Maherali & Klironomos, 2007). The former group appears to replace the latter as soil fertility increases, and this coincides with less beneficial plant responses in greenhouse experiments (Johnson, 1993). While parasitic relationships are certainly possible (Johnson *et al.*, 1997), it is also conceivable that AM fungi from fertilized soils provide a different function, such as pathogen protection, which is seldom assessed in greenhouse experiments. We could test this along natural or imposed fertility gradients by combining genomic approaches that target genes associated with nutrient uptake and defense with treatments specifically designed to quantify nutrient acquisition and pathogen protection by AM fungi. These studies could be combined with fungal community analyses and manipulations of fungal abundance and possibly reciprocal transplant experiments (Johnson *et al.*, 2010). While daunting, there is an

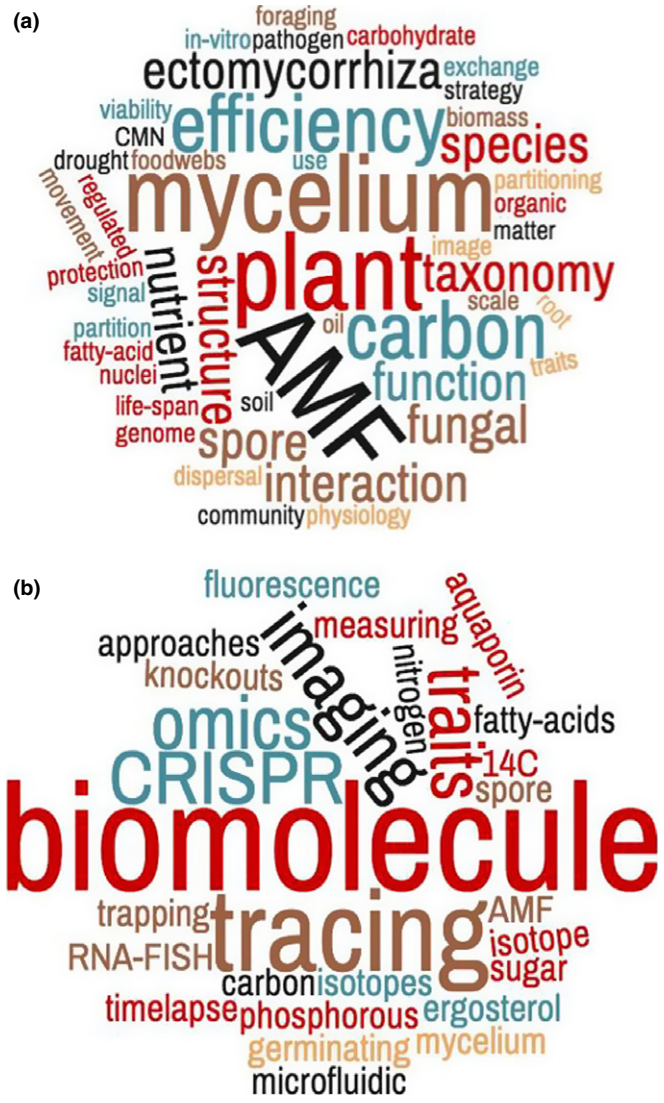

Fig. 2 Word cloud describing results from workshop participants answering the question (a) what is the most important mycorrhizal function to research, and (b) how can this be done?

urgent need to determine to what extent functions differ among taxa across all symbioses, and which plant and fungal traits, if any, predict these differences (Sikes *et al.*, 2009). We also need to quantify the relative importance of fungal abundance vs community composition, and whether different functions and communities can be predicted based on biotic and abiotic conditions. If we can make these predictions, then community analyses could finally become a proxy for function, but we have a long way to go before that is the case.

Linking functional traits and composition is challenging for many reasons (van der Heijden & Scheublin, 2007), but studies that have combined these approaches have been informative. For example, Helgason *et al.* (2002) showed that the most abundant isolate of AM fungus colonizing *Acer pseudoplatanus* in the field was also the most beneficial when inoculated onto *A. pseudoplatanus* under controlled conditions. This suggests that the nonrandom distribution of AM fungi in that system is driven by selective carbon allocation to the better mutualist (*sensu* Kiers *et al.*, 2011) rather than competitive interactions among fungi where the more competitive fungus (that may also be the poorer mutualist, Bever *et al.*, 2009) wins. The Helgason *et al.* study also highlights the benefit of combining surveys and controlled experiments. Other examples of papers that have linked function and composition include Walker *et al.* (2014), which measured enzyme activities of individual EM root tips coupled with molecular analyses to identify the fungus involved, and Clemmensen *et al.* (2015), which combined high throughput sequencing and observations of EM fungal growth forms and decomposability to help explain shifts in carbon and nitrogen sequestrations along a boreal chronosequence. These studies are noteworthy because they not only quantified the extent of functional differences among fungal species, but also linked those differences to specific traits and distribution patterns. These studies indicate that much can be learned about mycorrhizas in field settings without having to manipulate fungal abundances when this proves to be too challenging. Another approach that requires no manipulations of fungal abundance is reciprocal transplant experiments where local adaptation in the mycorrhizal symbioses can be assessed (Johnson *et al.*, 2010). Overall, in order to better understand mycorrhizal function, we need to use a consortia of methods and approaches, including surveys, reciprocal transplants, and experiments that manipulate fungal abundances.

The utility of existing and emerging technologies

During the ICOM9 workshop, we discussed current and emerging technologies available to quantify mycorrhizal function in more natural settings. Organizers and invited speakers highlighted ways to manipulate mycorrhizal fungal abundances in the field using fungicides and ingrowth cores (Ylva Lekberg), as well as the potential use of mutants (Thomas Irving), radioactive isotopes (Katie Field), enzymes (Björn Lindahl), and quantum dots (Victor Caldas), to study mycorrhizal function, nutrient transformation, uptake and movement. Thorunn Helgason outlined the potential use of genomic approaches, and Edith Hammer described a new technology that mimics the complexity of soils to increase realism in laboratory studies (Aleklett *et al.*, 2018). Finally, Gaby Deckmyn reminded us all that in order to make full use of modeling, we need to collect more and better metadata (particularly environmental) during our surveys and experiments (Deckmyn *et al.*, 2014).

Following these presentations, participants were asked what methods to use to address unanswered questions regarding mycorrhizal function highlighted earlier. Two themes emerged: (1) the application, in the field, of techniques typically confined to the laboratory, such as biomolecule analyses including various -omics approaches (genomics, transcriptomics and proteomics), and (2) the development of ways to capture the behavior of the mycorrhizal symbiosis directly. Most commonly highlighted were ways to image the symbiosis in the field and to develop and increase the range of biomarkers used to trace function (Fig. 2b). This could be done using cutting-edge technology, such as the 'lab on a chip' microfluidics and quantum dots (Aleklett *et al.*, 2018), as well as established methods, such as minirhizotrons (Hendrick & Pregitzer, 1996) and signature fatty acids (Olsson, 1999). The latter two can be combined with carbon-isotopes to inform on carbon allocation specifically to mycorrhizal fungi (Bending & Read, 1995; Olsson & Johnson, 2005).

Workshop participants also highlighted the potential use of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) gene editing technology (Ran *et al.*, 2013). Mutants have provided invaluable insight into mycorrhizal functioning, and are beneficial because they circumvent the necessity to remove mycorrhizal fungi via fungicide applications or fumigations. The utility of mutants was clearly shown recently using the *rmc* tomato (*Solanum lycopersicum* L., Barker *et al.*, 1998) in a California drought experiment conducted in the field (Bowles *et al.*, 2016). However, the release of genetically modified organisms (GMOs) that incorporate genetic information from other organisms into the field poses potential risks and is strictly regulated (if permitted at all). The use of CRISPR has been raised as an alternative to GMOs in field research given that no foreign material is added, but there is currently little consensus among countries regarding gene-editing rules (*Nature*, 2017). As such, researchers should check specific regulations in their country.

While it is tempting to wait for/rely on new technologies, many of which were highlighted by workshop participants, existing ones can help us answer important questions. For example, in 1982, Chiarello *et al.* published a groundbreaking paper in *Science* that tracked phosphorus-32 (^{32}P) movements within a grassland, possibly via arbuscular mycelial networks; and Simard *et al.* (1997) used carbon-13 (^{13}C) and carbon-14 (^{14}C) to assess carbon movements among trees in the field. Both studies stimulated subsequent research on common mycorrhizal networks. More recently, however, the use of radioactive isotopes has been largely confined to very controlled conditions (e.g. Mikkelsen *et al.*, 2008). This is understandable given restrictions in many places against radioactive isotopes (^{33}P may have fewer restrictions given the lower radioactivity), but it is unfortunate given that ^{32}P and ^{33}P applied in hyphal ingrowth bags (Johnson *et al.*, 2001) could measure mycorrhizal phosphorus uptake in natural systems (Jakobsen, 1994) so long as appropriate precautions are taken to minimize risk. Combined with carbon isotopes (^{13}C or ^{14}C), we could repeat elegant cost-benefit assessments conducted in the

greenhouse more than two decades ago (Pearson & Jakobsen, 1993). Overall, the issue is not necessarily that we need new methods, but that we apply available methods *in the field* wherever possible.

Conclusions

Ernst Mayr said, 'The history of science knows scores of instances where an investigator was in possession of all the important facts for a new theory, but simply failed to ask the right question' (Mayr, 1982). Our workshop and literature survey revealed that there are a suite of 'typical' approaches that we use to study mycorrhizal function, most of them under controlled conditions, and that there are significant barriers to conducting field research. However, these obstacles are not insurmountable, and moving mycorrhizal research into the field will allow us not only to quantify realized functions, but also to revisit and examine old paradigms and potentially discover new functions. Mycorrhizas could have very important roles to play in the future, including mitigating the decreasing reserves of phosphorous fertilizers, and helping plants tolerate the increasing stress associated with more severe droughts predicted with climate change. With creativity and ambition, we can apply old and new tools to field systems, and move into the next decade carrying out transformative experiments that will begin, finally, to answer fundamental questions about what mycorrhizas *actually* do, rather than simply what they are *capable* of doing. Only then can we communicate the possibilities and limitations associated with these symbioses to people outside our field.

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References

Agerer R. 2001. Exploration types of ectomycorrhizae: a proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. *Mycorrhiza* **11**: 107–114.

Aleklett K, Kiers ET, Ohlsson P, Shimizu TS, Caldas VE, Hammer EC. 2018. Build your own soil: exploring microfluidics to create microbial habitat structures. *ISME Journal* **12**: 312–319.

Barker SJ, Stummer B, Gao L, Dispain I, O'Connor PJ, Smith SE. 1998. A mutant in *Lycopersicon esculentum* Mill. with highly reduced VA mycorrhizal colonization: isolation and preliminary characterisation. *Plant Journal* **15**: 791–797.

Bending GD, Read DJ. 1995. The structure and function of the vegetative mycelium of ectomycorrhizal plants. V. Foraging behaviour and translocation of nutrients from exploited litter. *New Phytologist* **130**: 401–409.

Bennett JA, Maherali H, Reinhart KO, Lekberg Y, Hart MM, Klironomos J. 2017. Plant–soil feedbacks and mycorrhizal type influence temperate forest population dynamics. *Science* **355**: 181–184.

Bever JD, Richardson SC, Lawrence BM, Holmes J, Watson M. 2009. Preferential allocation to beneficial symbiont with spatial structure maintains mycorrhizal mutualism. *Ecology Letters* **12**: 13–21.

Bowles TM, Barrios-Masias FH, Carlisle EA, Cavagnaro TR, Jackson LE. 2016. Effects of arbuscular mycorrhizae on tomato yield, nutrient uptake, water relations, and soil carbon dynamics under deficit irrigation in field conditions. *Science of the Total Environment* **566–567**: 1223–1234.

Cheeke TE, Phillips RP, Brzostek ER, Rosling A, Bever JD, Fransson P. 2017. Dominant mycorrhizal association of trees alters carbon and nutrient cycling by selecting for microbial groups with distinct enzyme function. *New Phytologist* **214**: 432–442.

Clemmensen KE, Finlay RD, Dahlberg A, Stenlid J, Wardle DA, Lindahl BD. 2015. Carbon sequestration is related to mycorrhizal fungal community shifts during long-term succession in boreal forests. *New Phytologist* **205**: 1525–1536.

Cook LM, Grant BS, Saccheri IJ, Mallet J. 2012. Selective bird predation on the peppered moth: the last experiment of Michael Majerus. *Biology Letters* **8**: 609–612.

Courty PE, Pritsch K, Schloter M, Hartmann A, Garbaye J. 2005. Activity profiling of ectomycorrhiza communities in two forest soils using multiple enzymatic tests. *New Phytologist* **167**: 309–319.

Deckmyn G, Meyer A, Smits MM, Ekblad A, Grebenc T, Komarov A, Kraigher H. 2014. Simulating ectomycorrhizal fungi and their role in carbon and nitrogen cycling in forest ecosystems. *Canadian Journal of Forest Research* **44**: 535–553.

Delavaux CS, Smith-Ramesh LM, Kuebbing SE. 2017. Beyond nutrients: a meta-analysis of the diverse effects of arbuscular mycorrhizal fungi on plants and soils. *Ecology* **98**: 2111–2119.

Hart MM, Reader RJ. 2002. Taxonomic basis for variation in the colonization strategy of arbuscular mycorrhizal fungi. *New Phytologist* **153**: 335–344.

van der Heijden MGA, Scheublin TR. 2007. Functional traits in mycorrhizal ecology: their use for predicting the impact of arbuscular mycorrhizal fungal communities on plant growth and ecosystem functioning. *New Phytologist* **174**: 244–250.

Helgason T, Merryweather JW, Denison J, Wilson P, Young JPW, Fitter AH. 2002. Selectivity and functional diversity in arbuscular mycorrhizas of co-occurring fungi and plants from a temperate deciduous woodland. *Journal of Ecology* **90**: 371–384.

Hendrick RL, Pregitzer KS. 1996. Applications of minirhizotrons to understand root function in forests and other natural ecosystems. *Plant and Soil* **185**: 293–304.

Jakobsen I. 1994. Research approaches to study the functioning of vesicular-arbuscular mycorrhizas in the field. *Plant and Soil* **159**: 141–147.

Javot H, Penmetsa RV, Terzaghi N, Cook DR, Harrison MJ. 2007. A *Medicago truncatula* phosphate transporter indispensable for the arbuscular mycorrhizal symbiosis. *Proceedings of the National Academy of Sciences, USA* **104**: 1720–1725.

Johnson D, Leake JR, Read DJ. 2001. Novel in-growth core system enables functional studies of grassland mycorrhizal mycelial networks. *New Phytologist* **152**: 555–562.

Johnson NC. 1993. Can fertilization of soil select less mutualistic mycorrhizae? *Ecological Applications* **3**: 749–757.

Johnson NC, Graham JH, Smith FA. 1997. Functioning of mycorrhizal associations along the mutualism–parasitism continuum. *New Phytologist* **135**: 575–585.

Johnson NC, Wilson GWT, 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.

Kiers ET, Duhamel M, Beesetty Y, Mensah JA, Franken O, Verbruggen E, Fellbaum CR, Kowalchuk GA, Hart MM, Bago A *et al.* 2011. Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science* **333**: 880–882.

Koide RT, Courty PE, Garbaye J. 2007. Research perspectives on functional diversity in etomycorrhizal fungi. *New Phytologist* **174**: 240–243.

Maherali H, Klironomos JN. 2007. Influence of phylogeny on fungal community assembly and ecosystem functioning. *Science* **316**: 1746–1748.

Mayr E. 1982. *The growth of biological thought: diversity, evolution, and inheritance.* London, UK: Harvard University Press.

McGonigle TP, Fitter AH. 1988. Growth and phosphorus inflows of *Trifolium repens* L. with a range of indigenous vesicular-arbuscular mycorrhizal infection levels under field conditions. *New Phytologist* **108**: 59–65.

Michelsen A, Graglia E, Schmidt IK, Jonasson S, Sleep D, Quarmby C. 1999. Differential responses of grass and a dwarf shrub to long-term changes in soil microbial biomass C, N and P following factorial addition of NPK fertilizer, fungicide and labile carbon to a heath. *New Phytologist* **143**: 523–538.

Mikkelsen BL, Rosendahl S, Jakobsen I. 2008. Underground resource allocation between individual networks of mycorrhizal fungi. *New Phytologist* **180**: 890–898.

Nature. 2017. Gene editing in legal limbo in Europe. *Nature* **542**: 392.

Olsson PA. 1999. Signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soils. *FEMS Microbiology Ecology* **29**: 303–310.

Olsson PA, Johnson NC. 2005. Tracking carbon from the atmosphere to the rhizosphere. *Ecology Letters* **8**: 1264–1270.

Pearson JN, Jakobsen I. 1993. Symbiotic exchange of carbon and phosphorus between cucumber and three arbuscular mycorrhizal fungi. *New Phytologist* **124**: 481–488.

Ran RA, Hsu PD, Wright J, Agarwala V, Scott DA, Zhang F. 2013. Genome engineering using the CRISPR-Cas9 system. *Nature Protocols* **8**: 2281–2308.

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

Read DJ, Perez-Moreno J. 2003. Mycorrhizas and nutrient cycling in ecosystems – a journey towards relevance? *New Phytologist* **157**: 475–492.

Sikes BA, Cottenie K, Klironomos JN. 2009. Plant and fungal identity determines pathogen protection of plant roots by arbuscular mycorrhizas. *Journal of Ecology* **97**: 1274–1280.

Simard SW, Perry DA, Jones MD, Myrold DD, Durall DM, Molina R. 1997. Net transfer of carbon between ectomycorrhizal tree species in the field. *Nature* **338**: 579–582.

Smith SE, Read DJ. 2008. *Mycorrhizal symbiosis.* London, UK: Academic Press.

Smith SE, Smith FA, Jakobsen I. 2003. Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. *Plant Physiology* **133**: 16–20.

Varga S, Kytöviita MM. 2010. Mycorrhizal benefit differs among the sexes in a gynodioecious species. *Ecology* **91**: 2583–2593.

Walker JKM, Cohen H, Higgins LM, Kennedy PG. 2014. Testing the link between community structure and function for ectomycorrhizal fungi involved in a global tripartite symbiosis. *New Phytologist* **202**: 287–296.

Waller LP, Felten J, Hiiesalu I, Vogt-Schilb H. 2018. Sharing resources for mutual benefit: crosstalk between disciplines deepens the understanding of mycorrhizal symbioses across scales. *New Phytologist* **217**: 29–32.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

Table S1 Information extracted from papers in our literature survey.

Table S2 Summary of information gathered in our literature survey.

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Key words: arbuscular mycorrhizas, ectomycorrhizas, field, methods, mycorrhizal function.