Viewpoints

Exploring the role of ectomycorrhizal fungi in soil carbon dynamics

Summary

The extent to which ectomycorrhizal (ECM) fungi enable plants to access organic nitrogen (N) bound in soil organic matter (SOM) and transfer this growth-limiting nutrient to their plant host, has important implications for our understanding of plant–fungal interactions, and the cycling and storage of carbon (C) and N in terrestrial ecosystems. Empirical evidence currently supports a range of perspectives, suggesting that ECM vary in their ability to provide their host with N bound in SOM, and that this capacity can both positively and negatively influence soil C storage. To help resolve the multiplicity of observations, we gathered a group of researchers to explore the role of ECM fungi in soil C dynamics, and propose new directions that hold promise to resolve competing hypotheses and contrasting observations. In this Viewpoint, we summarize these deliberations and identify areas of inquiry that hold promise for increasing our understanding of these fundamental and widespread plant symbionts and their role in ecosystem-level biogeochemistry.

Introduction

The potential for ectomycorrhizal (ECM) fungi to participate in soil organic matter (SOM) dynamics represents one of the most active areas of ecological research (Talbot *et al.*, 2008; Averill *et al.*, 2014; Lindahl & Tunlid, 2015; Sterkenburg *et al.*, 2018). This topic has widespread importance for our understanding of plant growth (Terrer *et al.*, 2016, 2017) and soil carbon (C) storage, which has been suggested to both increase (Orwin *et al.*, 2011; Averill *et al.*, 2014; Averill & Hawkes, 2016) and decrease (Phillips *et al.*, 2014) due to the activity of these widespread plant symbionts. Recent laboratory evidence suggests that ECM fungi may obtain nitrogen (N) from organic compounds composing SOM, potentially allowing plants to access growth-limiting N beyond inorganic sources (Nicolás *et al.*, 2019). The potential for widespread access to organic N compounds in forest ecosystems would fundamentally alter our understanding of NPP and plant nutrition (Näsholm *et al.*, 2009).

Research investigating the role of ECM fungi in SOM dynamics and plant N acquisition is important from several perspectives, ranging from our basic understanding of SOM dynamics (Averill *et al.*, 2014) to the importance and accurate representation of mycorrhizas in global biogeochemical models (Clemmensen *et al.*, 2013; Franklin *et al.*, 2014; Terrer *et al.*, 2016, 2017; Brzostek *et al.*, 2017). Empirical evidence, including the work of Gadgil & Gadgil (1971, 1975), has provided support for an array of mechanistic perspectives (Fernandez & Kennedy, 2015), and several high-profile papers have recently reignited the importance of resolving the multiplicity of viewpoints surrounding this fundamental ecological dynamic (Talbot *et al.*, 2008; Averill *et al.*, 2014; Bödeker *et al.*, 2016; Terrer *et al.*, 2016; Norby *et al.*, 2017). Moreover, if we can identify the underlying processes through which mycorrhizas influence soil C storage, then we may be able to harness physiological variation among them to better manage forests and sequester greater amounts of anthropogenic CO_2 into SOM.

Toward that end, we held a workshop to gather a small group of researchers working on this issue from multiple perspectives. Our two-day workshop at the University of Michigan on 21–22 May 2018 consisted of presentations and discussions that focused thought on resolving the processes that may underlie the seemingly disparate empirical evidence that currently exists regarding the ability of ECM fungi to provide plants with N bound in SOM and to mediate soil C dynamics. For example, it is presently unclear whether all ECM taxa can acquire N bound in SOM for plant use; nor do we understand the net effect of ECM-facilitated access to N on ecosystem-level processes. Below, we summarize points of general agreement amongst participants, and propose new avenues of research that hold promise for resolving contrasting empirical observations.

Precise terminology can inform experimental design and interpretation

Precise definitions of the physiology of ECM fungi clarify the possible roles of ECM fungi in SOM dynamics. Foremost, we define SOM as an heterogeneous group of chemically and physically stabilized plant and microbial detritus (*sensu* Lehmann & Kleber, 2015) containing organic N in various forms (Rillig *et al.*, 2007; Nannipieri & Eldor, 2009). The proliferation of research investigating ECM fungi and SOM dynamics has resulted in widespread usage of the terms ‘saprotrophy’, ‘decomposition’, ‘decay’ and ‘modify’, to describe ECM physiology. We strongly suggest that these terms are not interchangeable and have specific meanings that need to be differentiated. Foremost, there was widespread agreement that ECM fungi are unlikely to engage in the saprotrophic metabolism of SOM, in which ECM directly obtain energy from organic compounds contained therein (Lindahl & Tunlid, 2015). This consensus is widely consistent with older mycorrhizal literature (Harley, 1969), although recent evidence

suggests that free-living saprotrophic fungi can form mycorrhizal-like associations with plant roots (Smith *et al.*, 2017). For clarity, we focus on unequivocal and well-known mycorrhizal lineages in our discussions throughout.

The decay of SOM by ECM fungi is defined as the sustained and integrated modification of chemical bonds present in SOM. This definition does not specify *which* bonds are modified, nor does it specify the *extent* of overall decay. ECM decay of SOM includes the transformation of organic matter into smaller molecules, as well as changes in functional groups (i.e. primary amines, carboxyl groups) and physical structure. These modifications may lead to the release of soluble compounds; additionally, these transformations may render remaining SOM chemically or physically resistant to further modification (Lindahl & Tunlid, 2015; Wang *et al.*, 2017). Our definition of decay is delineated from the widespread use of 'decomposition' or 'saprotrophism', which we define as the sequential and temporally integrated process in which different organic compounds contained in plant and microbial detritus are metabolized to obtain metabolic energy *and* nutrients. We note that our definition and distinction of the terms decay and decomposition are conceptually consistent with previous work (Lindahl & Tunlid, 2015), but avoid semantic irregularities that may propagate between fields of study. Indeed, referring to ECM fungi as decomposers can be misleading if taken to mean that ECM fungi are metabolically similar to free-living saprotrophs which obtain energy by metabolizing plant and microbial detritus.

Our definition of SOM decay by ECM fungi emphasizes the interaction of specific oxidative and hydrolytic extracellular ECM activities (enzymes and hydroxyl radicals) in relation to the range of chemical bonds present in SOM. Furthermore, this mechanistic decay perspective focuses attention on the fundamental elements that are thought to be critical to understanding the net effect of ECM fungi on SOM dynamics, particularly the production and conformation of enzymes and hydroxyl radicals that are needed to liberate organic N-bearing compounds, as well as the uptake of these compounds by ECM hyphae (Orwin *et al.*, 2011; Terrer *et al.*, 2017; Pellitier & Zak, 2018). Focusing on the enzymatic or radical catalysts that ECM fungi use to modify SOM requires a mechanistic 'match' in the parametrization of SOM compounds. For example, the C : N ratios of SOM can be helpful in certain situations to describe the chemical 'lability' of SOM to decay; however, interpretation of data may be complicated if the extracellular activities of ECM fungi, which act on certain sets of bonds present in SOM (Sinsabaugh, 2010) are studied in relation to the C : N of a target substrate. Models that represent ECM fungi and SOM stocks have variously parameterized the biochemical 'lability' of SOM (Orwin *et al.*, 2011; Moore *et al.*, 2015; Baskaran *et al.*, 2017; Terrer *et al.*, 2017), leading to a range of conclusions regarding the specific ECM extracellular catalysts that decay SOM, as well as their relative importance. As a result, some authors have argued that proteases and laccases – which are widespread in ECM fungi (Kohler *et al.*, 2015; Martin *et al.*, 2016) – are used by ECM fungi to decay SOM (Rineau *et al.*, 2015), whereas others have suggested that class II fungal peroxidases are of greater relative importance for ECM decay because they can degrade more 'recalcitrant' compounds present within SOM pools (Baskaran *et al.*, 2017; Kyaschenko *et al.*, 2017). Recent models that include both hydrolysable and oxidizable 'fractions' of SOM (Baskaran *et al.*, 2017) incorporate a more mechanistic view of ECM decay that can inform experimentation and articulation of the relative importance of different enzymatic or radical-based decay pathways.

Carefully defining ECM decay requires that both the biochemical composition and the physical accessibility of SOM are considered. Several decades of experimentation suggest that ECM fungi can decay simple organic substrates in culture (reviewed in Smith & Read, 2008); these studies also show that one of the key factors controlling the extent to which ECM fungi can modify SOM is the biochemical conformation of the SOM itself. For example, although a range of ECM fungi can incorporate organic N into their biomass when grown on simple protein (e.g. bovine serum albumin), when this substrate is complexed with tannins, ECM growth and N-uptake are reduced substantially (Bending & Read, 1996). By extension, the degree to which N-bearing organic molecules present in SOM are complexed with phenolic moieties may mediate the accessibility of N in SOM to ECM fungi. Recent efforts to introduce authentic field-derived SOM substrates into culture-based studies is an important step in developing our understanding of the physiological capacity of ECM fungi to decay SOM (Nicolás *et al.*, 2019).

Alongside studies investigating the biochemical composition of SOM, a growing body of research highlights how physiochemical binding of SOM to minerals, as well as aggregate formation, determines the fate of SOM throughout decay processes (Torn *et al.*, 1997; Schmidt *et al.*, 2011; Lehmann & Kleber, 2015). Indeed, some studies suggest that the adsorption of SOM to clay micelles determines decay trajectories to a greater degree than does the biochemical composition of SOM alone (Lehmann & Kleber, 2015; Newcomb *et al.*, 2017). Most work on SOM stabilization and persistence focuses on a broadly conceived microbial pool and rarely considers the specific activity and physiology of ECM fungi (but see Wang *et al.*, 2017). However, given that ECM hyphae are morphologically similar to their saprotrophic ancestors, elements of current research investigating free-living saprotroph attack of mineral- and aggregate-bound SOM can inform our understanding of ECM decay and SOM accessibility (Wang *et al.*, 2017).

Substantial physiological variation across ECM lineages in their capacity to modify SOM

One of the most significant points of consensus was that the capacity for ECM fungi to modify SOM is likely to vary substantially across evolutionary lineages. ECM fungi have independently evolved > 85 times (Tedersoo & Smith, 2013), primarily from free-living Dikaryotic saprotrophs. Available genomic surveys of ECM fungi reveal substantial variation in the copy number of genes putatively involved in the modification of SOM (Kohler *et al.*, 2015; Shah *et al.*, 2016; Pellitier & Zak, 2018). For example, the copy number of class II peroxidase genes, as well as the number of lytic polysaccharide monooxygenase (LPMO) genes, spans an order of magnitude amongst independent lineages of ECM fungi (Kohler *et al.*, 2015). Conference participants also agreed that many lineages of ECM fungi have the genetic potential to produce

some combination of hydroxyl radicals, glycoside hydrolases and laccases, suggesting that ECM can decay SOM to a degree (Pritsch & Garbaye, 2011; Phillips *et al.*, 2013; Terrer *et al.*, 2017; Op De Beeck *et al.*, 2018). However, when such findings are placed in relation to other fungal guilds (i.e. white rot and brown rot saprotrophs, as well as ericoid mycorrhiza), the abundance of genes potentially involved in SOM modification is substantially lower in nearly all lineages of ECM fungi (Martino *et al.*, 2018), suggesting that ECM are not likely to mediate the modification of SOM to the same extent as free-living saprotrophs.

Context-dependence of ECM and SOM modification

An additional point of consensus was the recognition that the modification of SOM by ECM fungi is likely to be strongly context-dependent. Such a consensus is notable because it will propel future research investigating whether or not ECM fungi function in similar ways across distinct forest ecosystems (Read, 1991; Phillips *et al.*, 2013; Averill *et al.*, 2014; Terrer *et al.*, 2017; Steidinger *et al.*, 2018). Conference participants engaged in lengthy deliberations regarding the soil conditions in which ECM fungi are most likely to participate in the substantial decay of SOM. Through these discussions, soil N availability consistently emerged as a plausible factor governing ECM decay. This consensus mirrors some current models that parameterize ECM decay as a function of soil fertility (Orwin *et al.*, 2011; Franklin *et al.*, 2014; Baskaran *et al.*, 2017). At this time, however, relatively limited direct evidence exists to test these hypotheses under field settings (but see Lilleskov *et al.*, 2002; Sterkenburg *et al.*, 2015; Kyaschenko *et al.*, 2017). Intriguingly, Bödeker *et al.* (2014) observed that class II peroxidase expression by ECM fungi in the genus *Cortinarius* was downregulated in boreal forests amended with inorganic N. Similarly, other studies have found significant variation in the transcriptomic profiles of laboratory grown ECM fungi (Shah *et al.*, 2016) and laboratory soil environments (Doré *et al.*, 2015).

Finally, the fluctuating allocation of host photosynthate to ECM mutualists (Högberg *et al.*, 2010) potentially mediating decay emerged as a vibrant area of discussion. Host-derived organic compounds fuel the metabolism of ECM fungi; if ECM fungi decay SOM using energetically expensive enzymes or Fenton chemistry (Rineau *et al.*, 2012; Lindahl & Tunlid, 2015; Op De Beeck *et al.*, 2018), then the nutritional status of the plant host has implications for ECM decay (Baskaran *et al.*, 2017; Terrer *et al.*, 2017). Indeed, experiments in pure culture systems have revealed that the oxidation of SOM and the expression of associated enzymes and hydroxyl radicals are triggered by the addition of glucose, suggesting that such dynamics may be regulated by the supply of photosynthate from the plant host (Rineau *et al.*, 2013; Nicolás *et al.*, 2019). These considerations are particularly relevant given the high model sensitivity of photosynthate allocation to ECM mutualists in recent models of plant growth under elevated atmospheric CO_2 (Terrer *et al.*, 2016, 2017). These models rely on the assumption that plants allocating more photosynthate to ECM obtain greater quantities of growth-limiting N derived from SOM (Terrer *et al.*, 2016, 2017). This, however, remains an important and outstanding question to test empirically, because even if ECM fungi decay SOM to obtain N, much of that N may become immobilized in fungal biomass (Franklin *et al.*, 2014; Koide & Fernandez, 2018). Finally, recent studies suggest that ECM fungi alter SOM stocks differentially across soil horizons (Craig *et al.*, 2018), which may be related to differences in the surrounding biotic communities (Lindahl *et al.*, 2007). This remains a largely unexplored ecological feedback that could have important implications for plant growth and the cycling of C and N in soil.

ECM directly and indirectly modify the biochemical composition and amount of SOM

Current evidence suggests that ECM fungi play a key role in the accumulation and turnover of SOM in temperate and boreal forests, doing so at multiple scales and through a variety of mechanisms. Below, we have clarified and summarized several of the mechanisms that have been widely proposed.

Together, the ability for certain ECM taxa to produce a range of oxidative and hydrolytic enzymes, as well as Fenton-based oxidation of SOM, constitute one of the most widely invoked mechanisms whereby ECM fungi could alter the accumulation and turnover of SOM (Orwin *et al.*, 2011; Averill *et al.*, 2014; Shah *et al.*, 2016; Kyaschenko *et al.*, 2017; Op De Beeck *et al.*, 2018). Rather than metabolizing organic molecules in SOM, ECM may modulate SOM storage by removing organic N – the so called 'N-mining' hypothesis. This hypothesis suggests that ECM fungi oxidize SOM to varying degrees to obtain small organic N-bearing molecules (peptides) while leaving relatively C-rich substrates behind (Orwin *et al.*, 2011; Phillips *et al.*, 2013; Averill *et al.*, 2014). This has been hypothesized to result in nutrient limitation for the remainder of the free-living saprotrophic community, the so-called Gadgil effect, thereby reducing overall decomposition of SOM by saprotrophs and increasing soil C (Orwin *et al.*, 2011; Fernandez & Kennedy, 2015; Averill & Hawkes, 2016; Sterkenburg *et al.*, 2018). A frequently overlooked consideration is that the enzymatic or Fenton based chemistry needed for ECM to 'mine' organic N, would necessarily modify the molecular structure of the remaining SOM (Nicolás *et al.*, 2019); importantly these modifications may result in mix of compounds that are more *or* less chemically accessible to subsequent enzymatic attack. Here is an example where the C : N ratios of SOM may not provide sufficient detail to explore the interaction of ECM fungi and free-living saprotrophic communities. Although the N-mining hypothesis has grown in popularity, direct empirical evidence from the field that ECM oxidize organic matter and thereby release N, leading to enhanced plant nutrition, is still missing. Accordingly, broad generalizations about the decay capacity of ECM across phylogenetic lineages and ecosystems should be avoided until additional data is collected. However, we agree that such physiology is plausible (Shah *et al.*, 2016; Nicolás *et al.*, 2019) and may be of major importance in some ecosystems (Averill *et al.*, 2014; Clemmensen *et al.*, 2015; Sterkenburg *et al.*, 2018). Quantitative field-based estimates of organic N-uptake by ECM fungi would represent a major advance in our understanding of the potential magnitude of this phenomenon.

New Phytologist

Given the growing evidence that the dynamics of SOM are best represented as an ecosystem-level process (Lehmann & Kleber, 2015), we similarly suggest that the role of ECM fungi in SOM dynamics must be considered within a broader context of biotic and abiotic interactions (Johnson *et al.*, 2013). Accordingly, an additional mechanism whereby ECM fungi may alter SOM dynamics, but which does not involve the production of hydrolytic or oxidative enzymes (or Fenton based radicals), is via the presence of live and dead hyphae. Foremost, the 'priming-effect', that is the exudation of energy-rich low molecular weight organic compounds from metabolically active hyphae, has been proposed to alter the activity of associated soil organisms decomposing SOM (Phillips *et al.*, 2012; Sulman *et al.*, 2017). Further, ECM hyphae can contribute to the formation of mineral stabilized SOM by the secretion of mineral surface reactive metabolites (Wang *et al.*, 2017). The extent to which different ECM hyphal morphologies (Agerer, 2001) contribute to the priming and stabilization of SOM remains an important question (Hobbie & Agerer, 2010; Tedersoo *et al.*, 2012).

Similarly, the recalcitrance of dead ECM hyphae as well as their production has received considerable attention (Clemmensen *et al.*, 2013, 2015; Ekblad *et al.*, 2013; Fernandez *et al.*, 2016; Hagenbo *et al.*, 2018). For example, cord-forming rhizomorphic ECM fungi, such as those in the genus *Cortinarius*, can produce significant amounts of hyphal biomass; however, these detrital inputs may speed decay and reduce long-term accumulation of SOM (Clemmensen *et al.*, 2015). By contrast, hyphae produced by the globally widespread *Cenococcum* (Tedersoo *et al.,* 2012) can display varying degrees of melanization, which may lead to the accumulation of SOM (Fernandez & Koide, 2014). We do not yet fully understand how the necromass from hyphae with a range of morphologies and biochemical constituents influences their rate of decay and subsequently SOM formation (Certano *et al.*, 2018). Given that *c.* 20% of host NPP is allocated to belowground mutualists (Leake *et al.*, 2004; Ekblad *et al.*, 2013), the fate of ECM necromass is likely to play a large role in SOM dynamics. It is plausible that living and dead hyphae contribute differentially to SOM dynamics; therefore, disentangling specific mechanisms from the net effect of ECM activity on SOM stocks remains critical to building predictive understanding of the conditions where ECM are most likely to impact SOM stocks. To conclude, we note that the myriad mechanisms discussed above likely act in parallel, and the relative importance of each may vary with the seasonality of host photosynthate allocation belowground, the interaction of SOM with mineral surfaces, soil microbial community membership, and ecosystem type (Keller & Phillips, 2019).

Ways towards new understanding

Elucidate the chemical and physical accessibility of SOM to decay by ECM fungi

It was widely agreed that ongoing efforts to characterize and constrain the physiological capacity for ECM fungi to modify the bonds present in SOM can greatly improve model efforts that incorporate the activity of these widespread plant symbionts on SOM (Nicolás *et al.*, 2019). Similarly, estimates of organic N liberation and fungal transfer of this N to the plant host are urgently needed to inform models which suggest that sustained plants' growth under elevated CO_2 is facilitated by accessing N in SOM via ECM mutualists (Terrer *et al.*, 2017). Gathering these datasets will rest on a firm understanding of ECM decay physiology and their ability to modify the bonds present in SOM *in situ*. Research uncovering the formation and stabilization of SOM in forest ecosystems (Lehmann & Kleber, 2015) combined with detailed studies of ECM decay physiology will facilitate this effort, especially when paired with measures of plant N-uptake.

Explore and incorporate physiological variation amongst ECM lineages and communities in models of SOM dynamics

Elucidating the extent to which communities distributed across distinct biomes are functionally equivalent in their capacity to modify SOM was an important future direction articulated by many workshop participants. Linked climate–ECM community models suggest large-scale taxonomic and morphological variability at the regional and continental scale (Steidinger *et al.*, 2018); plausibly, this variability suggests that widely distributed ECM communities may not be functionally equivalent (but see Talbot *et al.,* 2014). Research spanning a variety of spatial scales is recommended to uncover the environmental constraints governing the distribution of ECM fungi (Peay & Matheny, 2016), and their ability to decay SOM and transfer N to their plant host. A possible by-product of such studies is the direct refinement of the role of ECM fungi in SOM dynamics across a range of scales and biomes. Indeed, if genomic evidence is corroborated by experimental investigations revealing that not all ECM can contribute equally to the decay of SOM, then coupled plant–soil models should explore model sensitivity when variation amongst ECM lineages and communities is included. This remains a formidable, but essential, scientific challenge to address if we are to accurately portray this integral ecological interaction at an ecosystem or landscape scale.

Elucidate the consequences of ECM for SOM stocks and plant N acquisition across a range of scales

Recent research investigating the role of ECM fungi in soil biogeochemical cycles has significantly stimulated the field of mycorrhizal and plant ecology. However, the net effect of ECM activity on SOM remains relatively unknown; only a handful of studies have used modeling approaches to quantify and constrain the potential role of ECM fungi in SOM dynamics, showing that when the activities of ECM are included into models, the effect size can be quite large (Orwin *et al.*, 2011; Moore *et al.*, 2015; Baskaran *et al.*, 2017). There are significant differences in the general parameterization of these models, namely interactions with free-living saprotrophs, the proportion of organic N ECM may transfer to plant hosts, the influence of the soil environment on ECM physiology, as well as the contribution and decay of hyphal necromass. Accordingly, the role of ECM fungi in the accumulation *or* loss of SOM remains relatively undefined. One step towards

understanding the relevancy of the interaction of the 'N-mining' hypothesis and the Gadgil-effect, involves deriving quantitative estimates of the amount of organic N which different ECM lineages can liberate from SOM. In so doing, the net effect of the potential interaction of ECM fungi with free-living saprotrophs could be bounded quantitatively. Indeed, modeling approaches may ultimately provide mechanistic insight into SOM dynamics, if they explicitly probe the sensitivity of multiple plausible mechanisms of SOM modification by ECM fungi. To achieve such results, mechanistic *in vitro* studies of ECM interacting and contributing to SOM will reduce model uncertainty.

Study a broader phylogenetic range of ECM fungi under a wide range of laboratory and field conditions

There was widespread agreement that future research must embrace a wider phylogenetic range of study organisms. At present, very few ECM species are commonly used in laboratory experimental studies. Although this reflects the significant difficulty of culturing and manipulating many ECM taxa, there is an urgent need to understand the extent to which fully sequenced and commonly studied ECM species are functionally representative of their genus. Bringing experiments to the field may be the only way to close the knowledge gap arising from over-reliance on easy-to-manipulate laboratory fungi and the species that more often dominate ECM communities in the field. Finally, studying a range of ECM communities across soil fertility gradients, soil textures and forest communities provides a means by which to better understand the contexts where ECM are most likely to contribute strongly to SOM dynamics.

Closing comments

Workshop participants employ a wide range of methodological approaches, and our research encompasses broad biological and spatial scales. Here, we argue that a multi-pronged approach that leverages community-wide expertise in ecosystem-level modeling, field and laboratory experimentation, knowledge of natural history (Peay, 2014), and replicable molecular techniques (Nguyen *et al.*, 2015) will further our understanding of the physiological capacity of ECM to mediate the cycling and storage of C and N in terrestrial ecosystems. Above, we have identified important knowledge gaps in the physiological potential for ECM to modify SOM and provide host plants with additional sources of N above and beyond inorganic forms in soil solution. Until these gaps are filled, it is tenuous to assume that the manipulation of the ECM community composition is a viable means to manage terrestrial ecosystems for increased storage of anthropogenic CO_2 in soils.

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Author contributions

DRZ and PTP prepared the first draft of this paper and share in first authorship. All authors participated in the workshop, reviewed the manuscript and provided comments.

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