Networks of power and influence: the role of mycorrhizal mycelium in controlling plant communities and agroecosystem functioning[1]

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Abstract: Extraradical mycelia of mycorrhizal fungi are normally the "hidden half" of the symbiosis, but they are powerful underground influences upon biogeochemical cycling, the composition of plant communities, and agroecosystem functioning. Mycorrhizal mycelial networks are the most dynamic and functionally diverse components of the symbiosis, and recent estimates suggest they are empowered by receiving as much as 10% or more of the net photosynthate of their host plants. They often constitute 20%–30% of total soil microbial biomass yet are undetected by standard measures of biomass used by soil scientists and agromomists. Mycorrhizal mycelia provide extensive pathways for carbon and nutrient fluxes through soil, often exceeding tens of metres per gram of soil. We consider the amounts of photosynthate "power" allocated to these mycelial networks and how this is used in fungal respiration, biomass, and growth and in influencing soil, plant, and ecosystem processes. The costs and functional "benefits" to plants linking to these networks are fungal specific and, because of variations in physiology and host specificity, are not shared equally; some plants even depend exclusively on these networks for carbon. We briefly assess the potential contribution of extraradical mycorrhizal mycelium to sustainable agriculture and maintenance of biodiversity and highlight technologies that promise new vistas and improved fine-scale resolution of the dynamic spatial and temporal functioning of these networks in soil.

Key words: arbuscular mycorrhiza, ectomycorrhiza, extraradical mycelium, hyphal networks.

Résumé : Sauf exception, les mycéliums extraracinaires des champignons mycorhiziens constituent la face cachée de la symbiose, mais ils exercent de puissantes influences sur le cyclage biogéochimique, la composition des communautés végétales et le fonctionnement des écosystèmes. Les réseaux mycéliens des mycorhizes constituent les composantes les plus dynamiques et les plus fonctionnellement diverses de la symbiose, et les estimations récentes suggèrent que leur puissance vient du fait qu'ils reçoivent jusqu'à 10 % ou plus du produit net de la photosynthèse des plantes hôtes. Ils constituent souvent de 20 % à 30 % de la biomasse microbienne totale du sol, mais demeurent tout de même non détectés par les mesures standards de biomasse utilisées par les pédologues et les agronomes. Les mycéliums mycorhiziens fournissent des sentiers extensifs permettant les flux de carbone et de nutriments à travers le sol, dépassant souvent des dizaines de kilomètres par gramme de sol. Les auteurs considèrent les quantités de « puissance » photosynthétique allouées à ces réseaux mycéliens, comment elles sont utilisées pour leur respiration, leur biomasse et leur croissance, et comment elles influencent les processus à l'échelle de la plante et des écosystèmes. Le coût et les « bénéfices » fonctionnels pour les plantes reliées à ces réseaux sont spécifiques au champignon et, dû aux variations physiologiques et de spécificité de l'hôte, ne sont par répartis également; certaines plantes dépendent même exclusivement de ces réseaux pour leur carbone. Les auteurs évaluent brièvement la contribution potentielle des mycéliums mycorhiziens extraracinaires pour l'agriculture durable et le maintien de la biodiversité, et mettent en lumière les technologies prometteuses pour le développement de nouveaux concepts et l'amélioration de la fine résolution de la dynamique spatiale et du fonctionnement temporel de ces réseaux du sols.

Mots clés : mycorhizes arbusculaires, ectomycorhizes, mycélium extraracinaire, réseaux mycéliens.

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Introduction

Mycorrhizal mycelial networks: the hidden half of the symbiosis

The extraradical mycorrhizal mycelium (ERMM) is the part of the symbiosis most intimately connected to the soil and most directly involved in uptaking nutrients and influencing soil properties. These mycelial networks provide pathways for reciprocal transfer of carbon (C) received from host-plant roots and nutrients taken up from the soil. They also transfer nutrients and C between plants interlinked by the same mycelial network (Simard et al. 2002). ERMM are complex and dynamic components of the symbiosis but are highly sensitive to disturbance and to alterations in soil properties, such as pH and nutrient status, brought about by pollution, conventional agricultural, and forest management (Wallenda and Kottke 1998; Brunner 2001; Erland and Taylor 2002; McGonigle and Miller 2000; Kabir and Koide 2002). Whilst the ERMM is increasingly recognized as the main nutrient-absorbing interface of the plant–mycorrhiza soil system (Smith and Read 1997), it remains the most poorly understood and most difficult part of the symbiosis to study (Staddon et al. 2003). Nonetheless, very significant advances have recently been made in our understanding of the structure and functioning of these networks and their potential effects on major processes such as biogeochemical cycles, soil aggregation, the composition and functioning of plant communities, and agroecosystem functioning.

This paper reviews some of the recent experimental and observational studies on ERMM of the two main types of mycorrhizas, arbuscular (AM) and ectomycorrrhiza (EM), and presents compelling evidence that confirms the central importance of these networks as major pathways for C flux through soil and powerful influences on plant community composition and agroecosystem functioning. As understanding of the importance of ERMM has increased, there is growing evidence of a major role for these fungal systems in both seminatural ecosystems and, increasingly, in sustainable agriculture, such as organic and reduced-tillage management systems, where mycorrhizal functioning appears to be more important than in conventional intensive agriculture.

Progress in understanding the nature, extent, functioning, and identity of mycorrhizal fungal networks has been seriously hampered by the difficulties inherent in observing and studying mycelial systems without disturbing and destroying them. These difficulties are particularly hard to overcome outside the laboratory, and our knowledge of the functioning of these networks has been strongly based on reductionist studies, the relevance of which to the field situation is often difficult to confirm. Routine studies of mycorrhizal occurrence and functioning ignore ERMM, since the standard methods of assessing mycorrhizal occurrence are based on fungal colonization of roots (e.g., Dalpé 1993; Horton and Bruns 2001), or in the case of AM fungi, sometimes based on the occurrence of soilborne spores (Bever et al. 2001). Although ERMM are often the most important sources of inoculum in established plant communities with AM (Merryweather and Fitter 1998), spore counts remain the most widely used method of quantifying AM inoculum, even though spore viability is often very low (e.g., McGonigle and Miller 1996). As a consequence, the external mycelium, which is the fungal structure of mycorrhiza that is most intimately associated with the soil and furthest from the roots, and by implication the most critical for nutrient uptake, is normally overlooked and has been rarely recorded. Only in the past decade have studies started to focus specifically on the extent and functioning of ERMM in the field.

Not only are ERMM obscured by the soil matrix, but they are also intermingled with mycelium of saprotrophic fungi from which they are often not readily distinguishable. The diffuse mycelium of many EM fungi appear identical to hyphae of saprotrophs to which many of them may be closely related (Hibbett et al. 2000) and with which they share many functional attributes (Leake et al. 2002). Whereas AM mycelia have some features such as angular projections, thick walls, and infrequent septa that enable the thicker parts, albeit with some difficulty, to be visually differentiated from saprotrophs, there remain uncertainties, particularly when examining the finer distal parts of their hyphal networks.

The measurement of ERMM length, especially of AM fungi, has played a central role in establishing the importance of extraradical mycelium and its functional significance as an entity distinct from the root infecting hyphae. Whilst the aqueous membrane filtration technique for extraction and quantification of hyphal lengths is long established, in recent years it has undergone various refinements, such as agitation with sodium hexametaphosphate to disperse clays (Schweiger et al. 1999), which have improved hyphal cleaning and the increasing use of vital stains to distinguish active and dead hyphae (Kabir et al. 1998*a*). The difficulty in distinguishing EM mycelia from saprotrophs remains a major barrier to assessment of EM hyphal lengths, although a few studies have attempted these measurements in simplified systems using sand-grown plants, where fungal populations are likely to be dominated by mycorrhizal mycelium (Querejeta et al. 2003).

Developments in methods used to study mycorrhizal mycelia

As awareness of the importance of ERMM has increased, efforts to develop techniques to study it have redoubled, and some of the most important recent developments have arisen from innovative combinations of methods to address formerly intractable aspects of structure and function of ERMM (Table 1). Of particular importance has been the development of methods such as root-free hyphal compartments, combined with isotope tracers and molecular analyses, which have allowed the effects of ERMM to be distinguished from those of roots both in the laboratory, and increasingly, in the field. This has enabled most of the major functions of intact mycelial networks to be investigated: transport of C, weathering of minerals, production of extracellular enzymes, mineralization of nitrogen (N) and phosphorus (P), uptake of nutrients, transport of nutrients, and interactions with other organisms. As a result of these methodological advances, substantial progress has recently been made in our understanding of the lengths of mycorrhizal hyphae in soil, the C and nutrient fluxes through them, their contributions to the global C, P, and N cycles, and their interactions with other organisms.

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Table 1. Some methods used to study the structure and functioning of extraradical mycorrhizal mycelium.

Method	Example Reference
Detection and quantification of extraradical mycelium of AM	
Extraction and measurement of hyphae	
Aqueous membrane filtration	Boddington et al. 1999
Rotating wire frame	Vilariño et al. 1993
Buried membrane	Baláz and Vosátka 2001
In vitro observations	
Root-organ cultures	Fortin et al. 2002
Whole plant monoxenic mycorrhizal culture	Giovannetti et al. 2001
Phospholipid fatty acid analysis	Olsson et al. 2003
Glomalin immunofluorescent binding assay	Wright 2000
PCR identification of AM hyphae in soil	Hunt et al. 2004
Detection and quantification of extraradical mycelium of EM	
Thin-layer soil microcosms	Read 1992
Digital image analysis linked to autoradiography	Leake et al. 2002
Phospholipid fatty acid analysis	Nilsson and Wallander 2003
Ergosterol	Wallander et al. 2001
Hyphal ingrowth bags	Wallander et al. 2001
T-RFLP to study the spatial distribution of hyphae of different EM fungi down the soil profile	Dickie et al. 2002
Competitive PCR to quantify EM mycelia of particular species in forest soil	Guidot et al. 2002*a*
Transport of nutrients and carbon in extraradical mycelium of AM	
Root-excluding hyphal ingrowth compartments in laboratory and field studies with ^{32}P	Schweiger and Jakobsen 2000
^{31}P NMR of intact mycelia in soil	Rasmussen et al. 2000
Movement of tubular vacuole system revealed by fluorescence tracer microscopy	Uetake et al. 2002
Mobilization and uptake of nutrients by hyphae in monoxenic root-organ cultures	Hawkins et al. 2000
Root-excluding hyphal ingrowth compartments in laboratory and field studies with ^{13}C and ^{14}C	Johnson et al. 2002*a*, 2002*b*
Measurement of ^{14}C in handpicked AM hyphae after host-plant exposure to $^{14}CO_2$-free air	Staddon et al. 2003
Transport of nutrients and carbon in extraradical mycelium of EM	
^{31}P NMR of polyphosphate metabolism *Suillus bovinus* mycorrhizal with pine	Gerlitz and Gerlitz 1997
Digital image analysis linked to autoradiography of ^{32}P transport in mycelial networks	Lindahl et al. 1999
^{14}C and digital autoradiography of mycelial networks of seedlings in soil microcosms	Leake et al. 2001
Movement of tubular vacuole system revealed by fluorescence tracer microscopy	Ashford and Allaway 2002
Mobilization and uptake of nutrients by hyphae in mesh ingrowth bags in the field	Nilsson and Wallander 2003
Combined DNA identification of hyphae with analysis of their uptake or binding of elements	Wallander et al. 2003

Note: AM, arbuscular mycorrhiza; EM, ectomycorrhiza.

Biochemical markers for biomass estimation and species identification

Fluorescent antibody assays have been developed that can selectively detect AM hyphae in soil. Assays based on antibodies that bind to the hydrophobic glycoprotein glomalin, which is produced by all isolates of AM fungi that have been tested to date (Wright 2000), are not well suited to simple quantification of AM hyphae, because glomalin is often stabilized in soil after the mycorrhizal mycelium has decayed. However, antibodies with AM fungal lineage specificity have been reported (Allen et al. 1999) and may be used in soil. Indeed, apart from these, there are no suitable biochemical markers for estimating the biomass of mixed species of AM fungi in the field. Other approaches, such as relating root-length colonization by AM to ergosterol concentrations (e.g., Hart and Reader 2002*a*, 2002*b*), have been called into question since ergosterol appears to be absent from all AM fungi tested to date both in axenic culture and in spores and hyphae from soil (Olsson et al. 2003). The other widely used AM marker, the phospholipid fatty acid 16:1ω5, must be calibrated to biomass for each species (Olsson et al. 2003), so in natural mixed communities of AM hyphae it provides only a very crude estimate of biomass. This marker is almost certainly not specific to AM, although it does appear to be especially abundant in them.

A combination of biochemical markers has been used to determine EM biomass, including ergosterol (Ek 1997; Wallander et al. 2001) and the phospholipid fatty acid 18:2ω6,9 (Wallander, et al. 2001; Nilsson and Wallander 2003), but neither of these is EM specific. It was only by growing plants in the laboratory in a sandy soil of very low organic matter content and by having nonmycorrhizal control plants for comparison that Ek (1997) was able to subtract the ergosterol content of saprotrophic fungi and convert ergosterol measurements to EM biomass. Both Wallander et al. (2001) and Nilsson and Wallander (2003) have extended this approach to forest soils in the field by comparing the concentration of ergosterol and phospholipid biochemical markers

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in trenched plots, from which mycorrhizal roots were excluded, and adjacent untrenched plots.

The application of DNA-based identification methods for mycorrhizal mycelium in soil has lagged far behind the use of this technology for the characterization of mycorrhizal communities on ectomycorrhizal root tips (Horton and Bruns 2001; Tedersoo et al. 2003) or in roots of AM plant communities (Husband et al. 2002; Vandenkoornhuyse et al. 2002). To date there are only a few records of molecular identification of mycorrhizal mycelium in the soil, but effective methods now exist for both AM (Jacquot et al. 2000; Hunt et al. 2004) and EM fungi (Dickie et al. 2002).

Molecular identification of mycorrhizal hyphae has opened a new window on the diversity and spatial structuring of ERMM in soil. Terminal restriction fragment length polymorphism (T-RFLP) has allowed the spatial distribution of hyphae of different EM fungi down the soil profile under pine forests to be determined (Dickie et al. 2002; Landeweert et al. 2003). By combining PCR identification and elemental analysis of EM mycelia colonizing nutrient patches of wood ash, Wallander et al. (2003) have been able to identify the specific fungi foraging in these patches and the main elements that they are accumulating in their mycelia. These kinds of approaches will be invaluable to establish functional differences between mycorrhizal fungi in the field. A further exciting development is the ability to quantify amounts of mycelia of specific EM fungi in natural soil by competitive PCR (Guidot et al. 2002*a*). This technique holds exceptional promise for future studies on the importance of mycorrhizal mycelia in the field, opening the way for population and community functioning studies of mycorrhizal mycelial networks in situ.

AM hyphal networks: observation in vitro and in soil

AM hyphal lengths are determined by painstaking visual discrimination from nonmycorrhizal hyphae on gridded membranes or by comparing total hyphal length counts in soil from mycorrhiza-inoculated and nonmycorrhizal control plants grown in sterilized soil. An inevitable limitation of hyphal extraction methods is that they fragment the network in the cleaning process. Although it may be possible to distinguish live and dead AM hyphae using vital stains (Kabir et al. 1998*a*), the part of the network that is likely to be most active in uptake of nutrients, the distal portion that is most finely branched and intimately attached to the soil, is not readily recovered and is rarely observed. As a consequence, new in vitro techniques such as mycorrhizal root-organ cultures (Fortin et al. 2002) have been important in allowing the occurrence, development, growth, and properties of extremely delicate and relatively short-lived mycorrhizal mycelia to be observed nondestructively in the laboratory. Such approaches have highlighted the potential importance of branched absorbing structures (BAS) described by Bago et al. (1998). The main findings from mycorrhizal root-organ cultures have been comprehensively reviewed by Fortin et al. (2002), who reported that three species of *Acaulospora*, four of *Gigaspora*, three of *Scutellospora*, one of *Sclerocystis*, and 16 of *Glomus* have now been grown monoxenically by this method. This approach has enabled detailed studies of AM nutrient transport, their abilities to assimilate certain organic nutrients (Koide and Kabir 2000; Hawkins et al. 2000), C metabolism and transport (Bago et al. 2002), and various aspects of their growth, spore production, and physiology (Fortin et al. 2002). Such studies have been conducted monoxenically and in some cases in dual cultures with other soil microorganisms to enable interactions between AM mycelia and mycoparasites, saprotrophs, and nutrient-solubilizing bacteria to be investigated (Fortin et al. 2002).

Whilst these in vitro methods have made a major contribution to our understanding of AM mycelia, their artificiality requires that results be corroborated, wherever possible, by studies in soil and in association with whole plants. Because root-organ cultures lack shoots they cannot supply sugars to their fungal partners in the same way as intact plants with their shoots experiencing diurnal photoperiods. Also, since shoots are the major sink for nutrients, uptake rates may be lower in root-organ cultures than in whole plants. There are indications that the short-term exposure to normal laboratory light levels, as happens during routine maintenance of root-organ cultures, can cause unusual growth responses and stimulate the hyphal branching in *Gigaspora gigantea*, *Gigaspora rosea*, and *Glomus intraradices* (Nagahashi et al. 2000).

Whole plants with monoxenic AM cultures can also be used to study mycelial functioning, and this has the advantages of maintaining the normal pathways of photosynthate supply to AM fungi and the shoot demand for nutrients and avoids the requirement to supply exogenous sugar to roots (Giovannetti et al. 2001).

To visualize the fine structure and rates of spread of AM hyphae in soil, including in the field, the burial of membranes on which the hyphae grow reveals much about the mycelial branching patterns, hyphal anastomoses, and the finer absorptive parts of the network (Baláz and Vosátka 2001). Many of these fine structures are normally broken up or obscured when hyphae are extracted from soil by wet sieving. The buried membrane method can be used in natural soil and does not suffer restriction to a limited range of AM species that applies to root-organ culture systems.

EM hyphal networks: observation in vitro and in soil

Whilst the measurement of AM hyphal lengths in soil has become increasingly common and the methods have become well established and tested (see e.g., Boddington et al. 1999), progress in the routine quantification of EM hyphae in soil has lagged far behind. The lack of visual distinction between many saprotrophic and EM fungi is highly problematic, so reliable estimates of EM hyphal lengths can be made only in plants grown in microcosms or pots (for example, containing sand) with poorly developed saprotroph populations.

Major insights into the structure and functioning of EM mycelia in soil have been gained through a combination of careful microscopic observations of ectomycorrhizal roots with adhering soil collected from forests (Agerer 2001) and the growth, normally of selected species of mycorrhizas, in thin-layer soil microcosms (Fig. 1). In this approach, most of the extraradical mycelium grows on the soil surface and therefore can be studied nondestructively (Read 1984). When combined with radioactive tracers, isotope imaging, time-lapse photography, and image analysis, the transport of

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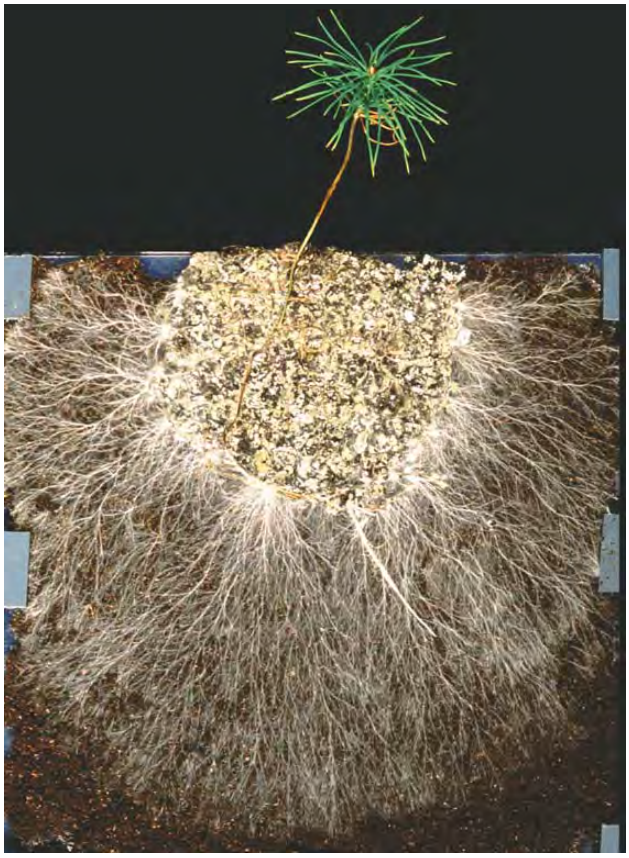
Fig. 1. The extensive and dense cover of ectomycorrhizal mycelial network of *Suillus bovinus* in association with *Pinus sylvestris* in a thin-layer (2 mm depth) microcosm of nonsterile peaty soil on a 20 cm × 20 cm sheet of Perspex. The seedling was inoculated with the mycorrhizal fungus in Petri dishes of peat–vermiculite, and this was transferred with the seedling into the upper part of the microcosm and allowed to grow for 4 weeks. (From Leake et al. 2001, reproduced with permission of Tree Physiol., Vol. 21, p. 74, © 2001 Heron Publishing.)



C and nutrients, growth, and spatial and temporal foraging activities of ERMM have been determined (see e.g., Read 1992; Bending and Read 1995; Leake et al. 2002). Real-time digital radioisotope imaging has recently enabled nondestructive multiple time-sequence quantification of ^{14}C transfer from plants to ERMM, without requiring compartmentalization of roots and hyphae (Leake et al. 2001). Whilst much has been revealed about the behaviour and physiological ecology of genera such as *Suillus*, *Rhizopogon*, *Paxillus*, *Laccaria*, *Pisolithus*, and *Cenoccoccum* (Horton and Bruns 2001), these represent a distinct subset of EM fungi that are relatively easy to culture and, in most cases, produce fairly extensive extraradical mycelium. Most EM fungi are not in culture; many genera such as *Inocybe*, *Cortinarius*, and *Russula* have proved generally intractable to routine laboratory culturing. Furthermore, the molecular analysis of EM communities on roots is revealing enormous diversity of these symbionts in established forests, and the species that are well represented in fruit bodies, which includes the majority of cultured species, are often relatively minor components of the root-tip populations (Taylor 2002; Horton and Bruns 2001).

Root-free hyphal compartmentation in the laboratory and field

Our understanding of the functioning of ERMM in soil has been greatly increased through the use of mesh barriers to provide root-free compartments into which mycorrhizal mycelium can grow (Schüepp et al. 1987; Jakobsen et al. 2001). Hyphal in-growth compartments made with root-excluding nylon mesh have been invaluable for determining the distance to which AM hyphae grow from roots (Jakobsen et al. 1992*a*) and their effective distance of nutrient foraging using radioisotope tracers (Jakobsen et al. 1992*b*; Smith et al. 2000). They have also established direct effects of AM networks on host and nonhost plants (Francis and Read 1995). Mesh bags buried in the field that contain soil mixed with radioactive P, with and without addition of fungicide, have permitted the quantification of the extent of P uptake by native populations of AM fungi (Schweiger et al. 1999; Schweiger and Jakobsen 2000). This approach was further refined by Johnson et al. (2001, 2002*a*, 2002*b*) who developed mesh-walled, soil-filled cores in which mycorrhizal mycelial development can be controlled by mechanical disruption (Fig. 2). The centre of the core is within 1 cm of the nearest roots outside, and at this distance Jakobsen et al. (1992*a*) found that for isolates of *Acaulospora*, *Scutellospora*, and *Glomus* the concentration of AM hyphal length per gram of soil was 60%–110% of values found at the root surface. By rotating the cores in soil, mycorrhizal mycelial connections to the plant roots are broken without the need for addition of fungicides or soil sterilization (Johnson et al. 2001). The hyphal in-growth cores have allowed in situ measurements of both the uptake of phosphate by ERMM and transfer of C to the mycelium from the plants by using radioactive and stable isotope tracers (Johnson et al. 2001, 2002*a*, 2002*b*).

The hyphal compartment method has also been used with EM in laboratory microcosms to study the biomass and respiration responses of ERMM supplied with nutrient patches (Ek 1997; Bidartondo et al. 2001) and in the field to estimate biomass production of EM mycelia (Wallander et al. 2001; Nilsson and Wallander 2003). The main findings of many of these studies are discussed in detail in the following sections.

Networks of power: C allocation to extraradical mycorrhizal mycelia

The hidden pathway of C flux from plant roots through mycorrhizal mycelia

Central to the functional importance of mycorrhizal mycelial networks is their energy supply from plants. Saprotrophic soil microorganisms are typically C limited, their C sources being spatially and temporally heterogeneous. In contrast, mycorrhizal fungi, by gaining direct access to plant-supplied sugars, are energized by a quality and quantity of available carbohydrate supply that is unparalleled amongst soil microbial populations. Despite the many indications that ERMM is a major component of soil biota, its contribution to soil microbial biomass is difficult to quantify (see previous section), and the pathway it provides for C movement from roots to other soil organisms is largely unrecognized and ignored. Until recently, scant attention has

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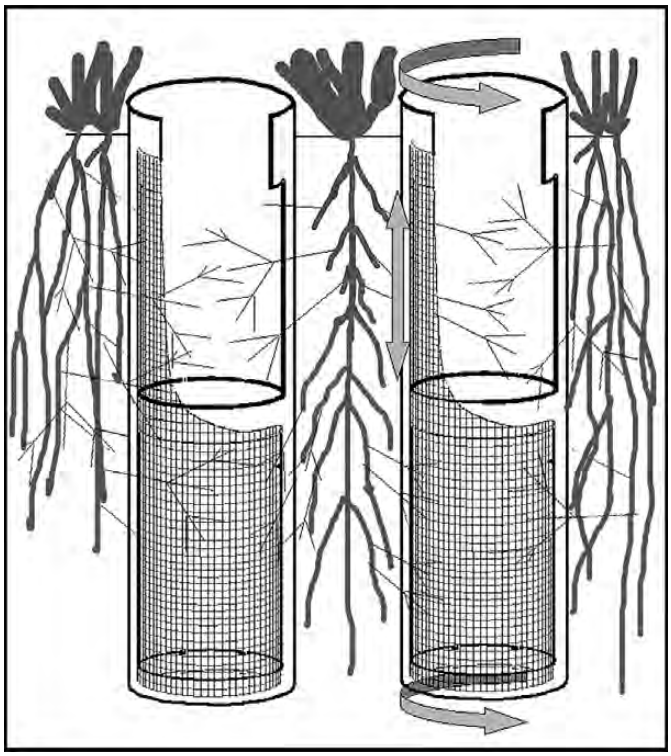
Fig. 2. Diagram of hyphal ingrowth cores developed to study mycorrhizal functioning in the field. The cores (2 cm diameter and 8 cm depth) are filled with sieved (2 mm) natural soil and inserted into grassland to allow study of the functioning of extraradical mycorrhizal mycelium in situ. Hyphae, but not roots, grow through the 10–35-mm pore-sized nylon mesh windows (mesh is shown partially cut away for clarity). Control cores (on the right) are rotated to sever the hyphal connections with roots and thus avoid the use of fungicides, which can have effects on saproptrophic as well as mycorrhizal fungal hyphae (Johnson et al. 2001).



been paid to the importance of ERMM as conduits for C movement from plants to soils. For example, De Ruiter et al. (1994) present food-web models for agroecosystems that do not recognize the existence of mycorrhizas, but place considerable emphasis on saprotrophic fungi and fungal-feeding animals. Even studies that have specifically investigated C fluxes from grassland plants to soil microbiota (e.g., Stewart and Metherell 1999; Saggar et al. 2001; Kuzyakov et al. 2001) have entirely ignored the fact that the majority of grassland plants have mycorrhizas and their mycelial systems draw directly on host photosynthate. Indeed, the most recent models of C fluxes between herbaceous plants and soil have been based upon the assumption that root exudation, sloughed cells, and dead roots provide the only significant pathways for the supply of plant-fixed C to the free-living microbial populations in soils (e.g., Toal et al. 2000; Kuzyakov et al. 2001).

This situation has arisen in part because the standard measures of soil microbial biomass discriminate against detection of mycorrhizal mycelial biomass. The widely used substrate-induced respiration technique (Anderson and Domsch 1978) is applied to sieved soil samples in which ERMM are fragmented and their vital connections to their life-supporting carbohydrate supplies from plants are destroyed. Since ERMM of AM fungi appear to be unable to assimilate exogenous sugar (Pfeffer et al. 1999), the substrate-induced respiration method discriminates against the detection of an AM contribution to microbial biomass. Even methods such as fumigation–extraction (Voroney and Winter 1993), unless applied to samples within a few hours of collection, will fail to detect the full contribution of the living mycorrhizal networks to microbial biomass and C fluxes. This very serious limitation in these techniques is rarely acknowledged. The insensitivity of these measures to intact mycorrhizal mycelial biomass raises doubts as to their suitability for assessment of microbial biomass.

However, recent estimates suggest that AM fungi comnstitute over 50% of the fungal length in some soils (Rillig et al. 2002). They can account for more than 20% of the total soil microbial biomass in pasture and prairie grasslands (Miller and Kling 2000), rising to over 30% of the microbial biomass in sandy soils (Olsson and Wilhelmsson 2000). In addition to their contribution to the live fraction, AM hyphae secrete the protein glomalin that accumulates in soil, contributing a substantial amount of the more stable soil organic C. For example in grassland, Miller and Kling (2000) suggest that as much 15% of the soil organic C pool is contributed by AM fungi, and it makes a similarly large contribution in tropical rain-forest soils where the dominant trees form AM (Rillig et al. 2001).

Recent work has shown that EM mycelia makes a similar or even larger contribution to soil C pools. By combined use of sand-filled hyphal in-growth bags inserted into forest plots and trenching to exclude living mycorrhizal roots from control plots, Wallander et al. (2001) used PLFA and $\delta^{13}C$ signatures to estimate the EM biomass in a Swedish *Pinus sylvestris* and *Picea abies* forest. They estimated that the total biomass of EM mycelia and roots was 700–900 $kg \cdot ha^{-1}$, and on the basis of previous estimates of the biomass of EM sheath on roots (Kårén and Nylund 1997), concluded that approximately 80% of the EM biomass was extraradical mycelium. A conservative estimate of the contribution of ectomycorrhizas to soil microbial biomass in forest soil is 32%, based on field observations following a large-scale girdling experiment (Högberg and Högberg 2002).

Despite the substantial biomass and associated C drain on their hosts, the actual "cost" of mycorrhiza to plants may be negligible because mycorrhizal colonization can increase the rate of photosynthesis (Wright et al. 1998), alleviate shoot N and P limitation, and cause a substantial increase in leaf area arising from improved nutrition (Read and Perez-Moreno 2003).

C allocation to mycorrhiza and extraradical mycelium

Although both AM and EM fungi clearly have an important role in the terrestrial C cycle, the quantities of C allocated to them by plants have rarely been determined. Only a handful of studies have specifically attempted to measure the C costs of ERMM.

Estimates (from pot based studies) of the net total C allocation from plants to AM range between about 2% and 20% of current assimilate (Jakobsen and Rosendahl 1990; Pearson and Jakobsen 1993; Smith and Read 1997). Estimates of the total C costs of EM, obtained from calculations

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based on field observations of mycorrhizal biomass (Vogt et al. 1982) and from laboratory studies using ^{14}C pulse labelling of tree seedlings or C budgeting studies, suggest that 7%–30% of net fixation is allocated to EM, of which 16%–71% is lost as respiration, according to laboratory studies (Finlay and Söderström 1992; Ek 1997; Bidartondo et al. 2001).

C allocation to AM extraradical mycelium

It was estimated (using mesh-compartmentalized pots) that 0.7%–0.8% of net C fixation by cucumber was allocated to the ERMM of its AM partner (out of a total of 20% allocation to mycorrhiza) in 4 d following ^{14}C pulse-labelling of the plants (Jakobsen and Rosendahl 1990; Pearson and Jakobsen 1993). These pioneering measurements were a major advance, but the values appear to have underestimated the true C cost of AM mycelia, in part because maximum C allocation to external mycelium occurs within 24 h after pulse labelling of shoots (Johnson et al. 2002*a*; Staddon et al. 2003). The amounts of C allocated to support ERMM in the field has now been estimated for the first time, over a shorter time course, in a permanent grassland using hyphal in-growth cores and $^{13}CO_2$ pulse labelling of the surrounding turf with CO_2 supplied at atmospheric CO_2 concentrations (Johnson et al. 2002*a*, 2002*b*). Control cores were briefly twisted immediately prior to commencing labelling to sever AM hyphae passing through the mesh. Respiratory fluxes of C from the AM networks within 21 h of pulse labelling accounted for 3.9%–6.2% of the net fixation, with peak release of $^{13}CO_2$ from the hyphal compartments occurring only 9–14 h after the C was fixed in the shoots, whereas maximum ^{13}C allocation to fine roots peaked later, after 21 h (Johnson et al. 2002*a*).

In the studies of Johnson et al. (2002*a*), the amount of C allocated to biomass of ERMM could not be determined by the ^{13}C pulse-labelling approach because the high organic matter content of the soil diluted the ^{13}C tracer. However, in a parallel analysis in which blocks of turf were removed from the same field site and in which identical hyphal in-growth cores were then established, $^{14}CO_2$ pulse labelling of the shoots was used to quantify C allocation to AM biomass and AM-derived soil C (Johnson et al. 2002*b*). This revealed that AM mycelia in the unrotated hyphal compartments accounted for about 3% of net fixation 72 h after pulse labelling. There was no evidence that the chosen 72-h harvest interval coincided with the maximum ^{14}C content in the hyphal compartment, so the 3% allocation value should be regarded as a conservative estimate. Taken together with the estimated respiratory fluxes measured through the AM mycelia, the total C allocation to ERMM in the grassland in early autumn was 9% of net C fixation. These figures are remarkably similar to the results of Domanski et al. (2001), who found that 11% of the ^{14}C fixed by pot-grown *Lolium perenne* was in the soil 6 h later. They found that about 10% of the total C fixed was respired from below ground and 7% remained in the soil, but in this study the contribution of ERMM to these components was not considered.

The C allocation to ERMM in the grassland study (Johnson et al. 2002*a*, 2002*b*) is about an order of magnitude higher than estimated net fixation allocated to ERMM in the pot studies of cucumber (Jakobsen and Rosendhal 1990; Pearson and Jakobsen 1993). However, the latter did not include respiratory fluxes through mycorrhiza, although they noted that the peak in $^{14}CO_2$ respiration from roots plus mycelium occurred during the 16-h labelling period. Their measurements of ^{14}C allocation to hyphae required extraction of the fungal material from soil by a sieving procedure that may have had incomplete recovery, and fragmentation of the mycelia may have caused leakage and loss of labelled C. Their measurements on C allocation to hyphal compartments were taken 70–96 h after labelling, which is likely to be considerably past the peak ^{14}C concentration in the hyphae.

While the respiratory release of labelled C from AM hyphae is now known to be maximal from 12 to 16 h after pulse labelling of the plants, the optimal time for sampling ERMM for total labelled C remains uncertain, but appears to be soon after labelling, probably within the first 24 h (see Staddon et al. 2003). It will be governed by the balance between the rates of C transfer, the rates of respiration, and the rates of C incorporation into hyphal C storage and growth. Many pulse-labelling studies have either used relatively long fixation periods, which make it difficult to establish the chronology of C fluxes, or have taken the first samples many days after labelling (e.g., Stewart and Metherell 1999), and so may miss the importance of rapid C transfers and fluxes that happen sooner than this, such as those through AM and roots (Domanski et al. 2001). A fuller knowledge of the rates, routes, and fate of C fluxes through the ERMM of AM is required to avoid these difficulties in future.

For the first few hours after the hyphal in-growth cores were removed from the soil in the field and the hyphal connections to plant roots severed, $^{13}CO_2$ release from the cores remained fairly constant (Johnson et al. 2002*a*), but within 24 h it rapidly declined (Fig. 3), suggesting that the contribution of AM mycelia to soil respiration is quickly lost when all connections to host roots are severed. These observations corroborate the conclusions of laboratory studies that have indicated that AM primarily use current plant assimilate (Wright et al. 1998) and that most of the C flux from plants to their mycorrhizal associates occurs rapidly, often within 24 h of fixation. The dependence on recent assimilate has also been shown in respiration from EM mycelia, which decreased by 60%–95% within 24 h of detachment from host roots (Söderström and Read 1987).

Pulse labelling of turf with $^{13}CO_2$ combined with root-excluding hyphal in-growth cores (Johnson et al. 2002*a*, 2002*b*)is a powerful tool for the study of in situ AM functioning. Further studies of these kinds are now clearly required to establish the typical quantities of C allocated to ERMM in a range of grassland, arable fields, and woodlands using pulse-labelling studies of appropriate duration and sampling frequencies. It is now established that the amounts of C allocated from plants to AM vary depending upon both the plant and fungal species (Lerat et al. 2003), and it is to be expected that as AM fungi differ in the extent and biomass of ERMM, such differences will also occur in C allocation to the extraradical mycelium.

C allocation to EM extraradical mycelium

As with AM, there are very few estimates of the proportion of plant C fixation that is allocated to extraradical EM mycelium. In mycorrhizal *Pinus ponderosa* seedlings, extra-

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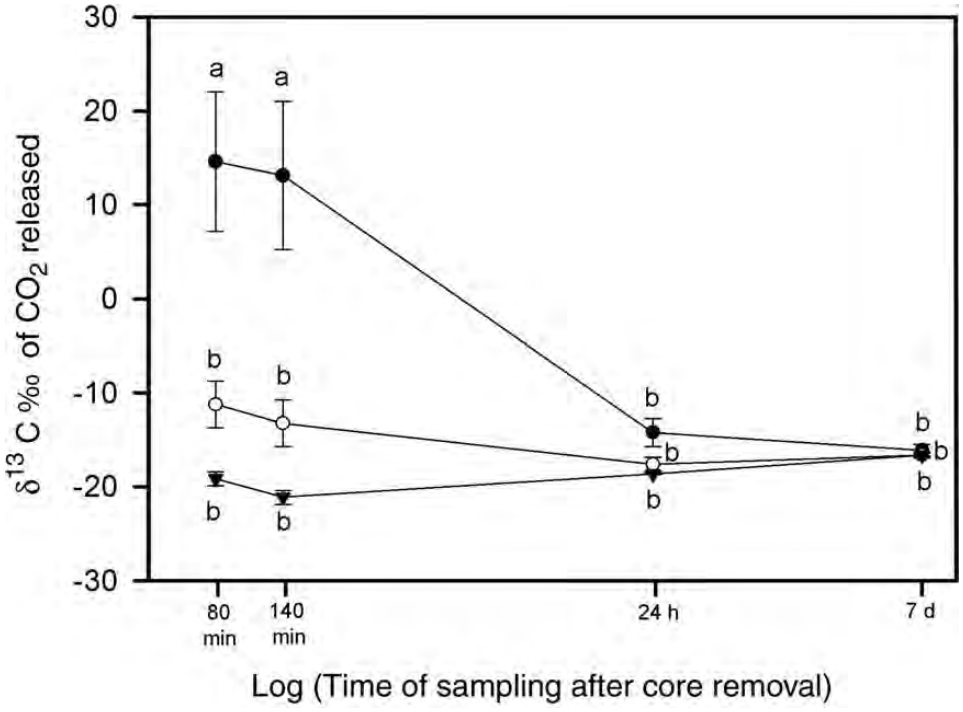
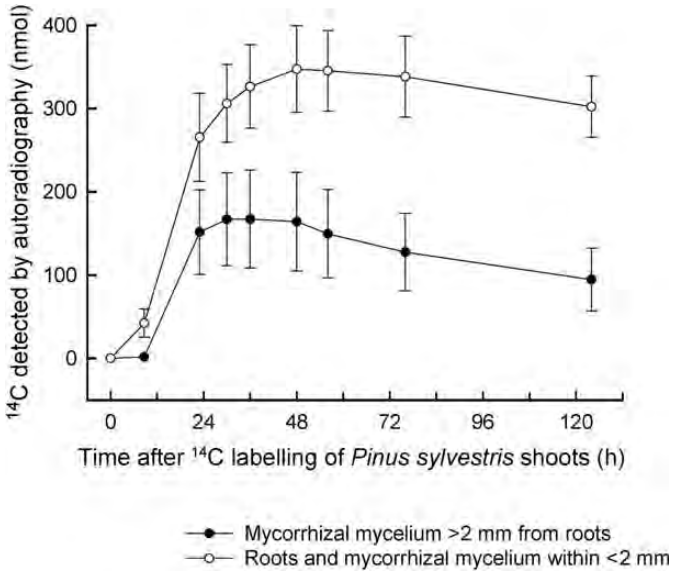
Fig. 3. Isotopic signature of ($\delta^{13}C‰$) of CO_2 release from static (solid circles) and rotated (open circles) mycorrhizal hyphal ingrowth cores in field plots in which the turf was pulse labelled for 5 h with $^{13}CO_2$ as well as from pooled static and rotated cores from unlabelled plots (triangles). The cores were all removed from the ground 9 h after the pulse labelling, and the release of $^{13}CO_2$ was monitored 80 min, 140 min, 24 h, and 7 d later. Bars indicate SE of the mean (n = 4). Data are replotted from Johnson et al. (2002*a*).



radical hyphae contributed 4% of the total respiration (Rygiewicz and Anderson 1994), while 8%–19% of net fixation of *Betula pendula* in ectomycorrhizal association with *Paxillus involutus* in a sandy forest soil was respired by ERMM in a root-free hyphal compartment, and these mycelia received 20%–29% of the net fixation (Ek 1997). In these studies, respiration from compartments containing both roots and extraradical mycelium were not included in the estimates of C flux through ERMM, since root and mycelial respiration cannot be distinguished in these compartments. Consequently, the total C allocation and respiration from mycorrhizal mycelium will have been even higher than the reported values. Using a similar microcosm system, in which half the soil volume was occupied by roots and mycorrhiza and the other half was a root-free hyphal compartment, the proportion of net fixation by *Pinus muricata* that was allocated to ERMM in the hyphal compartments ranged from 2.8%–10% with three species of *Rhizopogon*, 8.4% with *Suillus pungens*, and 21% with *Paxillus involutus* (Bidartondo et al. 2001). Since ERMM in the root compartments ranged from 18%–120% of the biomass in the hyphal compartments, these remain very conservative estimates of the total C allocation to extraradical mycelium.

In soil-based microcosms the transfer of ^{14}C label from $^{14}CO_2$ fixed by shoots of *Pinus sylvestris* seedlings to *Suillus bovinus* mycelium was first detected by digital autoradiography within 8 h after photosynthesis and reached maximum concentration in the mycelium by 30 h (Fig. 4), whereas the ^{14}C activity in the roots peaked 18 h later (Leake et al. 2001). These results confirm the importance of recent fixed photosynthate allocated to EM mycorrhiza and are corroborated by field studies showing that daily variations in the δ^{13} C isotopic signature of recently fixed C in trees is closely followed, within a few days, by the isotope signature of root and rhizosphere respiration, which must include EM mycelium (Ekblad and Högberg 2001). Measuring

Fig. 4. The mean amount of supplied, pulse-labelled ^{14}C detected by digital autoradiography in surface roots and mycorrhizal mycelium up to 2 mm from roots (open circles) and in external mycorrhizal mycelium >2 mm from roots (solid circles) over 0–124 h after exposure of *Pinus sylvestris* shoots to $^{14}CO_2$ for 3 h. Bars indicate SE of the mean (n = 4 replicate microcosms).



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total ^{14}C allocation into the ERMM of the EM fungus in the microcosms revealed that it contained 9% of the total ^{14}C in the plants (including ectomycorrhizal roots) 5 d after labelling (Leake et al. 2001). At its peak concentration in the extraradical mycelium, 30 h after labelling, the ^{14}C percent allocation is estimated to be approximately 14% of net fixation. These estimates are close to the 17% net ^{14}C pulse-label photosynthate allocated to extraradical EM mycelia of an unknown fungus associated with *Pinus densiflora* seedlings grown on black cotton cloth overlain onto soil, using a similar digital autoradiographic method (Wu et al. 2002). Most of this ^{14}C transfer to ERMM also occurred rapidly, within 2–3 d after pulse labelling (Wu et al. 2002).

C pools and C turnover in mycorrhizal networks

In both the studies by Leake et al. (2001) and Wu et al. (2002) there was a steady decline in ^{14}C concentration in mycorrhizal mycelium once the peak was reached (Fig. 4). This decline was most marked just behind the young, actively growing margins of the network. These temporal variations in ^{14}C content suggest that there are two distinct fates of C within ERMM. A major part is rapidly translocated throughout the network and quickly respired, whereas some of the C enters more stable, longer term pools, for example, in hyphal walls and structural components of mycelium. The portion that has short residence time in the hyphae will include some C that is transferred back to host plants, for example, as amino acids generated from the hyphal uptake of inorganic N. The amounts of C returning to the plant by this route will be equivalent to 5 C atoms for each atom of NH_4^+-N assimilated, but not all N assimilated requires return of recent host assimilate, since considerable amounts of N may be acquired by direct uptake of amino acids from soil. The energy demands for mineral nutrient assimilation and transport to the host appear to be quite considerable, for example, respiration from ectomycorrhizal ERMM growing in root-free soil compartments increased within 24 h after addition of ammonium or NO_3^--N and continued to increase for several days afterwards (Ek 1997). The mean respiration rate of ERMM increased by 54%–180% over the 4 d after addition of mineral N, and the mycelial respiratory output was equivalent to 4–13 atoms of C for each atom of N gained by the plants.

Evidence for two distinct C pools in ERMM of AM, one fast and one slow turnover, has also been provided by Staddon et al. (2003), who have used a novel isotope tracer technique to study C flux through mycorrhizal mycelia. They supplied *Plantago lanceolata* for 5 h during the day with CO_2 derived from fossil fuel that was free of ^{14}C and then extracted and analysed the ^{14}C concentration in extraradical AM mycelia over a period of 1 month. Maximum depletion of ^{14}C concentration in AM mycelia occurred within a day or so of C fixation; up to 16% of the C content of the mycelia was replaced by new assimilate from the 5-h labelling. This confirms the extremely rapid transfer of current assimilates from plants to mycorrhizal mycelium. Over a subsequent period of 6 d the ^{14}C concentration in the mycelia showed a linear increase with time following the return to fixation of atmospheric $^{14}CO_2$ by the plants. From 6 to 30 d, the ^{14}C concentration in ERMM changed very little with time, but remained about 2.5% depleted.

The fast-turnover C pool in the ERMM, revealed by the magnitude and rate of ^{14}C concentration increase in the first 6 d following ^{14}C depletion, was interpreted by Staddon et al. (2003) as evidence that most of the hyphae have a lifespan of 5–6 d. However, this incorrectly presumes that the C allocated to the mycelium is exclusively used in biomass production, whereas it is more likely that the fast-turnover C pool is the main source of the C used in respiration. The studies by Johnson et al. (2002*a*, 2002*b*) suggest that respiration from ERMM of AM fungi may account for more than two thirds of the C transferred to them from plants in the first 3 d after pulse labelling. In addition, they found that the $^{13}CO_2$ release from the hyphal compartments fell rapidly soon after dawn on the day after labelling and remained low during the day, but rose again at night. This suggests that much of the hyphal respiration is of C fixed in the few hours immediately preceding, except at night when it must depend on sugars mobilized from stored plant carbohydrate pools such as starch. The longer term C pool in the hyphae, represented by the 2.5% ^{14}C depletion value found by Staddon et al. (2003) from 6–30 d postlabelling, is likely to more closely reflect biomass production and hyphal turnover.

Another recent study that attempted to calculate the longevity and turnover time of ERMM in grassland concluded that only one quarter to one third of the hyphal network is turned over annually (Miller and Kling 2000). Such estimates are clearly prone to many sources of error, and it is difficult to reconcile these calculated turnover rates with laboratory observations of ERMM. Observations on AM networks in undisturbed monoxenic cultures have shown that the very narrow thin-walled hyphae that form terminal branches have a functional lifespan similar to that of arbuscules: they develop in 7 d, and by 5 weeks the cells are empty and have septa separating them from the living mycelia (Bago 2000). In addition to natural senescence in the field, disturbance of the hyphal networks by various soil animals, such as worms and fungivores, by seasonal extremes of temperature and water availability, and by fungal pathogens is likely to ensure higher rates of turnover than normally seen in the laboratory.

Knowledge of the C pools in ERMM of AM fungi and the nature of the substances transported from the intraradical to extraradical mycelium has advanced considerably. Bago et al. (2002) indicate that lipid and glycogen are the main C compounds transported into the extraradical mycelium, and they suggest that N is transported back to the plants as amino acids, possibly associated with polyphosphate. The incorporation of mineral N into amino acids by the fungus and their transfer to the plant would return some of the C skeletons originally supplied by the plant. These postulated metabolic and transport pathways match those that are known to occur in EM mycelium (Martin et al. 1998), and interdependence between N, P, K, and Mg transport has been shown in EM mycelia (Jentschke et al. 2001).

Clearly, more work needs to be done to establish the turnover rates of C in different ERMM networks and the nature of their internal C pools. Undoubtedly, isotope tracer tools have the potential to unlock the secrets of mycorrhizal functioning in laboratory and the field, and the use of labelling with fossil ^{14}C-free CO_2 in the field has enormous potential,

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avoiding the high cost of both ^{13}C and ^{14}C and the restrictions on use of the latter radioisotope.

Networks of power: the lengths, absorptive areas, growth rates, and interconnections of extraradical mycorrhizal mycelia

Lengths of mycorrhizal hyphae

In an increasing number of studies, the lengths of ERMM have been measured over time courses in both the laboratory and the field, revealing the nature and extent of these hyphal networks and the rapidity of their production. Such studies have confirmed the importance of mycorrhizal networks as major components of soil microbial biomass and have shown the importance of the length and absorptive area of mycorrhizal networks for nutrient acquisition.

Lengths of arbuscular mycorrhizal hyphae in soil

ERMM length measurements are normally expressed per unit mass or volume of soil or per unit length of mycorrhiza-infected root (Table 2). The latter expression is affected by the length of root present and by the proportion of it that is infected by mycorrhiza. It is strongly affected by the nature of the plant roots, how coarse or fine, and it is difficult to relate values obtained from different species or from studies with very different rates of root colonization. Plants with AM have widely different root types, and this has large effects on the length of hyphae per unit length of infected root. For example, *Lolium perenne* gave 14 m AM hyphae·g soil^{-1} but only 1 m hyphae·m infected root^{-1}, since this grass has an extensive fibrous root system with moderate to high levels of mycorrhizal colonization (Tisdall and Oades 1979). In contrast, the much coarser rooted *Trifolium repens* supported hyphal lengths in soil of only 3 m·g^{-1}, but this provided 46 m hyphae·m infected root^{-1} (Table 2).

The lengths of ERMM of AM fungi are typically one to two orders of magnitude longer than the lengths of mycorrhiza-colonized roots and typically range from 3–30 m·g soil^{-1} (Table 2). Considering that the studies of hyphal lengths have been carried out on a wide range of fungi and under various laboratory and field conditions on a variety of soil types, the reported values are remarkably similar. In the pot studies (Table 2) the range of AM hyphal lengths (2–29 m·g soil^{-1}) are almost identical to those reported from root-free hyphal compartments of pots (2–36 m·g soil^{-1}) where lower hyphal densities might have been expected due to the greater distances from roots. This raises the interesting possibility that AM hyphae grow more intensively in root-free soil, possibly because of the higher availability of nutrients outside the rhizosphere depletion zones.

The highest recorded hyphal lengths in AM communities have been reported in *Acer saccharum* woodland (Table 2), but in this case AM fungi were not distinguished from saprotrophs, although the mycorrhizal mycelium is likely to make a considerable contribution to the total fungal biomass (Klironomos et al. 1993). In the absence of woodland studies, the highest densities of AM hyphal lengths have, unsurprisingly, been reported in grasslands, where there is minimal soil disturbance and permanent plant cover. Here, they ranged from 68–101 m·g soil^{-1} in a prairie and from 45–74 m·g soil^{-1} in a pasture, with maximum lengths in November and minimum in June (Miller et al. 1995, Table 2). These lengths of mycorrhizal hyphae are vast: assuming a similar density of AM mycelia throughout the top 10 cm of the soil profile alone, there would be sufficient length of AM mycelial to stretch all the way around the equator of the earth in just 4 m^2 of grassland. Further studies are required to confirm that these observations are typical. Ploughing and disturbance is known to reduce the extent of AM mycelial networks (McGonigle and Miller 2000; Kabir et al. 1998*a*, 1998*b*), and in the field, AM hyphal lengths in annual crops are typically in the range 2–8 m·g soil^{-1} and tend to be lower than in many of the pot studies. These differences are likely to be due to higher planting and rooting densities of pot-grown plants than in the field.

There remain very serious gaps in our knowledge of the amounts of ERMM produced by many plant communities. With the exception of measurements from maize grown in an 8-month-old agroforestry plot (Boddington et al. 1999), studies of AM hyphal lengths to date have focused almost exclusively on herbaceous plants, and the laboratory studies have been restricted to a very limited range of species, more than half of which appear to have included a single plant species, *Trifolium subterraneum* (Table 2). As a consequence, with the exception of records of AM hyphal lengths of outdoor-grown *Populus tremoloides* saplings in open-top chambers, where AM hyphal lengths significantly increased with elevated CO_2 (Klironomos et al. 1997), the biomass and extent of AM hyphae in temperate deciduous forests and tropical rain forests dominated by plants with AM fungi is unknown. The abundance of total hyphae in the *Acer saccharum* woodland studied by (Klironomos et al. 1993), which far exceeds the total AM and saprotophic fungi in grassland and pot-plant studies, suggests that there may be orders of magnitude longer AM hyphal lengths in woodlands with AM host trees. ERMM are expected to be particularly important in their biomass and functioning in woodland, since trees have a high C-fixing potential and depend upon efficient nutrient cycling to maintain productivity. In the future, in addition to studies of AM hyphal lengths in woodland and seminatural vegetation, a much wider range of host species should be studied, as AM hyphal production in field plots has been shown to depend upon the host-plant species (Rillig et al. 2002), and more fungi need to be studied, too, since AM fungi differ greatly in the extent of their ERMM (Jakobsen et al. 1992*b*).

Lengths of ectomycorrhizal hyphae in soil

As a consequence of lack of visual distinction between hyphae of EM and saprotrophic fungi (see Introduction), there are few estimates of lengths of hyphae produced by EM (Table 3) and enormous uncertainty about their typical densities in soil in the field. Current estimates range from 30–8000 m hyphae·m root^{-1} (Smith and Read 1997) and 3–600 m·g soil^{-1} (Table 3). Local hyphal proliferation may give much higher values than these. EM fungi differ greatly in the extent to which they produce extraradical mycelium, some species producing little mycelium, apart from the mycorrhizal mantle, whereas others produce extensive mycelial networks that extend decimetres from the roots and have considerable biomass (see Agerer 2001).

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Table 2. Length of arbuscular mycorrhizal (AM) extraradical mycorrhizal mycelium in soil, expressed per unit mass of soil or per metre of infected roots.

Culture conditions and plant species	AM fungus	Hyphal length per unit soil mass ($m \cdot g^{-1}$)	Hyphal length per metre colonized root length (m)	Reference
Pot				
Trifolium subterraneum, 42 d	*Acaulospora laevis*	29	1055	Abbott and Robson 1985
Trifolium subterraneum, 42 d	*Glomus tenue*	26	1422	Abbott and Robson 1985
Trifolium subterraneum	*Scutellospora calospora*	2–25	nd	Sanders et al. 1977
Trifolium subterraneum, 42 d	*Gigaspora calospora*	17	1232	Abbott and Robson 1985
Lolium perenne	*Glomus* sp.	8	49	Tisdall and Oades 1979
Pisum sativum, 33–57 d, mixed inoculum	*Glomus* (three species)	4–6	470–600*	Gavito et al. 2002
Trifolium subterraneum, 42 d	*Glomus fasciculatum*	5	250	Abbott and Robson 1985
Allium cepa	*Glomus mosseae*	nd	79–250	Sanders 1975
Allium cepa	*Glomus mosseae*	nd	71	Sanders et al. 1977
Allium cepa	*Glomus macrocarpon*	nd	71	Sanders et al. 1977
Allium cepa	*Glomus microcarpon*	nd	71	Sanders et al. 1977
Pot, hyphal compartment				
Linum usitatissimum	*Glomus mosseae* (two isolates)	26–36	nd	Schweiger and Jakobsen 2000
Trifolium subterraneum	*Glomus mosseae*	6–29*	22–103	Drew et al. 2003
Trifolium subterraneum	*Glomus intraradices*	4–27*	8–57	Drew et al. 2003
Cucumis sativus	*Glomus fasciculatum*	27	113	Jakobsen and Rosendahl 1991
Trifolium subterraneum	*Acaulospora laevis*	15–25	nd	Jakobsen et al. 1992*b*
Trifolium subterraneum	*Glomus* sp.	10–25	nd	Jakobsen et al. 1992*b*
Linum usitatissimum	*Glomus intraradices*	19	nd	Schweiger and Jakobsen 2000
Linum usitatissimum	*Glomus caledonium*	16	nd	Schweiger and Jakobsen 2000
Trifolium subterraneum	*Scutellospora calospora*	2–15	nd	Jakobsen et al. 1992*b*
Trifolium subterraneum	Native populations	4–11	nd	Schweiger et al. 1999
Linum usitatissimum, 32 d	Native populations	10	nd	Thingstrup et al. 2000
Linum usitatissimum	*Glomus claroideum*	5	nd	Schweiger and Jakobsen 2000
Linum usitatissimum	*Glomus geosporum*	5	nd	Schweiger and Jakobsen 2000
Trifolium repens	*Glomus mosseae*	2–5*	nd	Li et al. 1991
Pisum sativum, 33–57 d, mixed inoculum	*Glomus* (three species)	2–3	210–220*	Gavito et al. 2002
Linum usitatissimum	*Scutellospora callospora*	2	nd	Schweiger and Jakobsen 2000
Field				
Acer saccharum forest, length of all fungi	Native populations	(640–4200)	nd	Klironomos et al. 1993
Prairie, 11 years old	Native populations	68–101†	800–1030	Miller et al. 1995
Ungrazed pasture, 17 years old	Native populations	45–74†	440–1240	Miller et al. 1995
Zea mays, preceded by fallow or cover crop	Native populations	10–35	nd	Kabir and Koide 2002
Sown *Lolium perenne*	Native populations	14	1	Tisdall and Oades 1979
Populus tremuloides saplings in soil–sand, 14 months	Native populations	2–8	nd	Klironomos et al. 1997

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Table 2. (*concluded*).

Culture conditions and plant species	AM fungus	Hyphal length per unit soil mass ($m \cdot g^{-1}$)	Hyphal length per metre colonized root length (m)	Reference
Wheat–rape–barley rotation	Native populations	2–8	nd	Boddington et al.1999
Native grassland, Serengeti	Native populations	0–7	nd	McNaughton and Oestenheld 1990
Avena barbata, 2 years	Native populations	6	nd	Rillig et al. 2002
Taeniatherum caput-medusae, 2 years	Native populations	5	nd	Rillig et al. 2002
Aegilops triuncialis, 2 years	Native populations	4	nd	Rillig et al. 2002
Trifolium microcephalum, 2 years	Native populations	4	nd	Rillig et al. 2002
Amsinckia douglasiana 2 years	Native populations	3	nd	Rillig et al. 2002
Tropical legume tree and *Zea mays*	Native populations	3–4	nd	Boddington et al.1999
Zea mays, conventional, reduced, and zero tillage	Native populations	2–4*	nd	Kabir et al. 1998*a*
Trifolium repens	Native populations	3	46	Tisdall and Oades 1979

Note: Values are rounded to the nearest whole number. nd, no data.
*Where original values were expressed per cubic centimetre of soil, these were expressed per gram of soil, assuming a typical bulk density of 1.32 $g \cdot cm^{-3}$ for cultivated mineral soils (Brady 1984).
†Where original values were expressed per cubic centimetre of soil, these were expressed per gram of soil, assuming a typical bulk density of 1.10 $g \cdot cm^{-3}$ for permanent grassland (Brady 1984).

To date, most of the estimates of EM hyphal lengths have been carried out with seedlings inoculated with single EM species in laboratory studies, and the extent of mycelial production is difficult to extrapolate to natural forests and plantations with established trees and diverse assemblages of mycorrhizal symbionts. The only attempt to determine EM hyphal lengths in forest soil is based on an indirect approach in which regression relationships between mycelial respiration and biomass were used to estimate mycorrhizal mycelia length in a Swedish pine forest (Finlay and Söderström 1989), and this gave values of 200 $m \cdot g\ soil^{-1}$ (Table 3). Given that both the quantity and proportion of net C fixation allocated to mycorrhiza is likely to normally be higher in forests than in grassland, this seems a reasonable estimate and not dissimilar to those indicated by recent soil microcosm studies with tree seedlings (cf. Tables 2 and 3).

In the species that produce the most extensive mycelial networks, these are often highly differentiated structures with hyphal cords or rhizomorphs comprising hydrophobic hyphal aggregates, used for long-distance transport, whereas the distal absorptive mycelium is typically hydrophilic to enable the uptake of nutrients from soil solution (Olsson et al. 2002). Some species, such as *Hydnellum*, *Hysterangium*, *Suillus*, *Paxillus*, *Amanita*, and *Boletus*, produce extensive hydrophobic conducting mycelium, whereas others, such as *Laccaria*, *Hebeloma*, and *Thelephora*, produce mainly hydrophilic mycelial systems that can be sparse or extensive depending on the individual species (Olsson et al. 2002). Whilst many species produce extensive extraradical mycelium, some species such as *Lactarius subdulcis* are almost completely confined to the sheath (Agerer 2001). These differences must strongly influence the nature of the nutrient pools that different EM fungi use.

Absorptive area of extraradical mycorrhizal mycelium

The finest absorptive AM hyphae in soil are typically 2 μm in diameter, compared with root-hair diameters of 10–20 μm and fine-root diameters of 100–500 μm. Read (1999) noted that assuming an equal dry mass per unit volume of these different structures, in geometric terms the C cost per unit absorptive area of fine mycorrhizal hyphae is approximately 10 times more efficient than that of root hairs and about 100 times more efficient than that of roots. Because of the enormous length of mycorrhizal hyphae in soil they can provide a surface area for absorption that is similar to, or even greater than, that of roots. If we assume, for the sake of argument, that mycorrhizal hyphae are 3 μm in diameter and that the average root diameter is 500 μm, it would require 170 m of hyphae to provide the same surface area for absorption as 1 m of root lacking root hairs.

The typical lengths of AM hyphae of between 3 and 35 $m \cdot g\ soil^{-1}$, reported in Table 2, have an external surface area of 0.3–2.9 $cm^2 \cdot g\ soil^{-1}$, if the mean hyphal diameter is 2.6 μm (as found by Jakobsen and Rosendhal (1990)), or 0.4–4.5 $cm^2 \cdot g\ soil^{-1}$, if the mean hyphal diameter is 4 μm (as found by Miller et al. (1995)). However, external surface area may vary even more widely than this, as AM hyphal diameters ranged between 1.2 and 18 μm (mean 3.7–7.9 μm) in five species from three genera (*Acaulospora*, *Glomus*, and *Scutellospora*) (Dodd et al. 2000). In a typical grassland soil with bulk density close to 1 $g \cdot cm^{-3}$, the absorptive area of

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Table 3. Length of ectomycorrhizal (EM) extraradical mycorrhizal mycelium in soil, expressed per unit mass of soil or per metre of infected roots.

Culture conditions and plant species	Ectomycorrhizal fungus	Hyphal length per unit soil mass ($m \cdot g^{-1}$)	Hyphal length per metre root length (m)	Reference
Pot or soil microcosm				
Pinus sylvestris in nonsterile peat		nd	1000–8000	Read and Boyd 1986
Pinus taeda in pots of fertilized sand	*Pisolithus tinctorius*	7	504	Rousseau et al. 1994
Pinus taeda in pots of fertilized sand	*Cenococcum geophilum*	3	nd	Rousseau et al. 1994
Salix viminalis in pots of fertilized sand	*Thelephora terrestris*	nd	308	Jones et al. 1990
Salix viminalis in pots of fertilized sand	*Laccaria proxima*	nd	289	Jones et al. 1990
Pinus sylvestris and *Alnus incana* grown together in pots of sand	*Paxillus involutus*	0.8	nd	Ekblad et al. 1995
Betula pendula in sandy forest soil, EM colonized part of hyphal compartment	*Paxillus involutus*	115–607*	nd	Ek 1997
Betula pendula in sandy forest soil–root compartment	*Paxillus involutus*	14–29*	nd	Ek 1997
Pinus muricata in sandy forest soil, root-free hyphal compartment	*Rhizopogon* (three spp.)	150–343*	nd	Bidartondo et al. 2001
Pinus muricata in sandy forest soil, root-free hyphal compartment	*Suillus pungens*	383*	nd	Bidartondo et al. 2001
Pinus muricata in sandy forest soil, root-free hyphal compartment	*Paxillus involutus*	570*	nd	Bidartondo et al. 2001
Quercus agrifolia in sandy soil mixture, root-free hyphal compartment	*Cortinarius collinitus*	1.5	nd	Querejeta et al. 2003
Quercus agrifolia in sandy soil mixture, root-free hyphal compartment	*Cenococcum geophilum*	2.5	nd	Querejeta et al. 2003
Field				
Swedish *Pinus sylvestris* forest		200	nd	Finlay and Söderström 1989

*Indicates values that are converted from biomass estimates, assuming that there are 0.83 m of hyphae per milligram of fungal biomass, which is the average of the values for *Pisolithus tintorius* and *Cenoccoccum geophilum*, given by Rousseau et al. (1994). This will overestimate hyphal lengths of species that produce appreciable amounts of multicellular hyphal cords or rhizomorphs.

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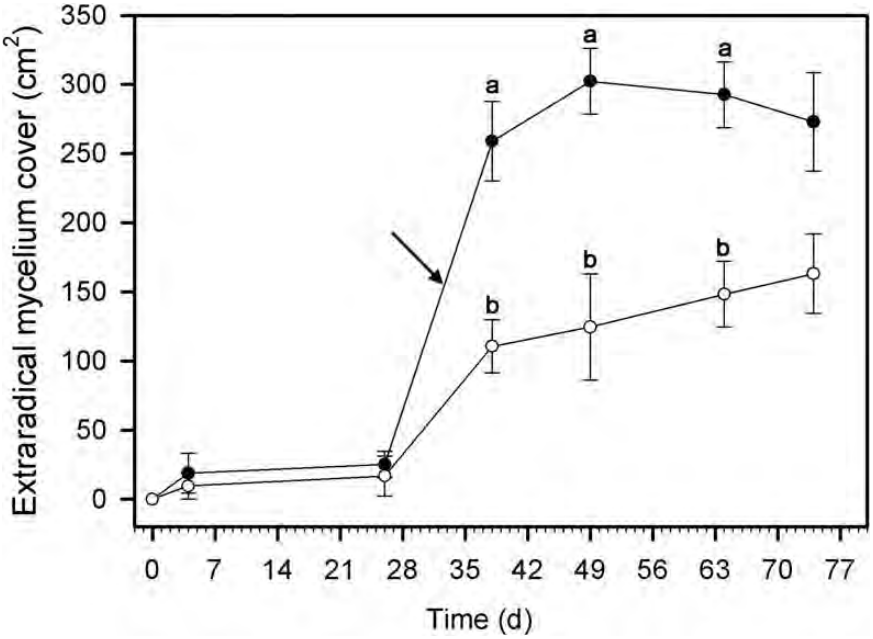
Fig. 5. The mean (±SE) hyphal cover of *Paxillus involutus* in mycorrhizal association with *Pinus sylvestris* in peat microcosms (see Fig. 1), which received additions of 0.5 g of air-dried, partially decayed *Pinus* litter from a forest floor (solid symbols) or (open symbols) received no litter (n = 3 replicate microcosms). The litter was added 32 d after the mycorrhizal seedlings were transplanted into the peat. An arrow indicates the time of litter addition. Letters indicate significant differences between mean values for treatments with and without litter ($P < 0.05$) for comparisons on the same date. Note the doubling of hyphal cover in less than 10 d.



mycorrhizal hyphae in the top 10 cm of the profile could therefore be expected to range from 3 to 90 $m^2 \cdot (m^2 \text{ turf})^{-1}$.

The ratio of absorptive area of ERMM to root area is typically much higher in EM than in AM plants, not only because EM plants have relatively short and course roots and generally lack root hairs, but because the mycelial networks of their mycorrhizas are normally much more extensive. With sand-grown *Pinus taeda* inoculated with *Pisolithus tinctorius*, the ectomycorrhizal mycelium constituted 75% of the absorptive area, only 25% being provided by roots, but the EM mycelium was only 5% of the biomass of root plus mycorrhiza (Rousseau et al. 1994). The potential absorptive area of EM hyphae per gram of soil in this study was only 0.47 $cm^2 \cdot g^{-1}$ with *P. tinctorius* and 0.28 $cm^2 \cdot g^{-1}$ when the trees were grown with *Cenococcum geophilum*. Much higher values than these would be expected in forest soils, as growth and biomass production of EM fungi, and C allocation to them, is greatly stimulated in the presence of forest floor litter (Bending and Read 1995; Leake et al. 2001; Read and Perez-Moreno 2003). Indeed, the mean area of soil covered by hyphae of *Paxillus involutus* in association with *Pinus sylvestris* in 20 cm × 20 cm peat microcosms increased from 110 cm^2 in control microcosms to over 260 cm^2 within 10 d of adding a patch of 0.5 g of air-dried, partially decayed pine litter (Fig. 5; Donnelly et al. 2003). In this 10-d period the ERMM increased its occupancy of the available soil surface area from 15% to 36% in response to the single litter patch. Observations of these kinds have demonstrated the exceptional speed, precision, intensity, and effectiveness of nutrient foraging by mycorrhizal mycelium.

Growth rates of extraradical mycorrhizal mycelium

The production of ERMM is very dynamic, and the lengths can increase very quickly when plants are establishing in bare soil. For example, Thingstrup et al. (2000) have shown that over a 32-d growth period the length of AM hyphae in pots of flax increased in the final 9 d from less than 2 $m \cdot g\ soil^{-1}$ to over 10 $m \cdot g\ soil^{-1}$, and there was no indication that it had stopped increasing at that point. With *Trifolium subterraneum* grown in pots inoculated with either *Glomus fasciculatum* or *Gigaspora calospora*, AM hyphal lengths increased rapidly from 10 $m \cdot g\ soil^{-1}$ at 4 weeks, to 20 $m \cdot g\ soil^{-1}$ at 5 weeks, and 25 $m \cdot g\ soil^{-1}$ at 7 weeks (Abbott and Robson 1985). At the same time the length of hyphae per unit root length infected also changed markedly, but in this case peak values were attained at 4 or 5 weeks, and the values fell sharply by week 7, as the length of infected root increased more rapidly than hyphal lengths during the last 2 weeks. Consequently, in most pot studies the duration of the experiment will have a critical effect on hyphal length estimates, and where only single harvests are taken the reported values may not be representative of mature plants. Similarly, with annual field crops, large seasonal variations in AM hyphal lengths are to be expected and have recently been confirmed for maize (Kabir et al. 1998*a*), in which the maximum AM hyphal densities increased up to flowering but declined thereafter.

AM hyphal extension rates have frequently been reported in the range 0.3–3.3 $mm \cdot d^{-1}$ (Jakobsen et al. 1992*a*), but much higher rates occur in some EM fungi. In peat microcosms, two strains of *Paxillus involutus* and two of *Suillus bovinus*, all grown in association with *Pinus sylvestris*, had radial extension rates of 7–8 and 5–6 $mm \cdot d^{-1}$, respectively (Donnelly et al. 2003). At a forest ecosystem scale, Wallander et al. (2001) have estimated that the equivalent of between 125–200 kg $ERMM \cdot ha^{-1} \cdot year^{-1}$ grew into sand-

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filled mesh bags buried in Swedish pine–spruce forest soil, and most of this biomass production occurred in a relatively short time from July to September. Assuming a mean hyphal length to mass ratio of 0.83 $m\mu g^{-1}$, (Rousseau et al. 1994; see Table 3) the ERMM in the forest annually produces an absorptive area of 70–112 $m^2 \cdot (m^2$ of forest floor$)^{-1}$, and the total length of new mycorrhizal hyphae produced in 2.5 m^2 of forest floor would be long enough to stretch from pole to pole around the world each year.

Vegetative spread of ectomycorrhizal networks and spatial distribution of genets

The extensive growth and biomass of ectomycorrhizal mycelial systems allows many of these fungi to spread vegetatively from root to root below ground and for a single genotype to colonize extensive areas. By somatic compatibility testing, Dahlberg and Stenlid (1990) showed that the sizes of clones of the ectomycorrhizal fungus *Suillus bovinus* in a Swedish pine forest increased with age of the forest. More recently, DNA sequence studies (Sawyer et al. 2003; Guidot et al. 2004) or microsatellite markers (Dunham et al. 2003; Kretzer et al. 2004) have been used to study the extent of genets of a range of ectomycorrhizal fungi. Such methods avoid the tedious requirement to isolate and culture the fungus from each sample location to test its genet identity. The DNA-based approaches have greatly facilitated studies of genet sizes, including in species that are not readily culturable. Genet sizes vary widely between species and may be strongly affected by the age of the forest and by factors such as disturbance (Guidot et al. 2002*b*). Individual genets may extend over large contiguous areas, with a number of reports showing individual genets occupying over 50 m^2 (Anderson et al. 2001; Sawyer, et al. 2003), but it is most unlikely that the mycelial network remains completely interlinked over this scale. The development of large genets confirms long-term genet persistence of representatives of some genera like *Suillus*, but in other cases, like *Hebeloma cylindrosporum*, it has been suggested that most genets turn over rapidly and may be replaced annually through new spore germination (Guidot et al. 2004).

Interconnections of mycorrhizal networks

Amongst the key features of mycorrhizal mycelial networks that make them functionally distinctive from roots is their ability to form unique pathways for nutrient and C transport interconnecting plants below ground (Simard et al. 2002). Further, EM mycelia, particularly in those species that produce extensive mycelia, are often topologically complex, with frequent anastomoses that provide multiple alternative transport pathways between foraging mycelium and their C sources, the roots. In the older portions of EM networks produced by species that develop multicellular hyphal cords, such as *Suillus bovinus*, a tangentially interconnected net-like structure is often well developed.

The structural complexity of internal transport pathways within mycelial networks has only recently been discovered. Tubular vacuole systems that allow simultaneous bidirectional transport to be maintained in the hyphae have been observed in both EM (Ashford and Allaway 2002) and AM (Uetake et al. 2002) fungi. In view of their continuity over long distances, the tubular vacuole system in the largely aseptate arterial hyphae of AM fungi (Uetake et al. 2002) and in septate EM mycelia (Allaway and Ashford 2001) provide the most likely means of simultaneous rapid transport in opposite directions of C obtained from the host and nutrients acquired from the soil. Motile pleomorphic tubule–vacuole systems have been shown to move in opposite directions in the same cell, but experimental proof of nutrient and C transport are required (Ashford and Allaway 2002). Regulation and control of the internal transport pathways is likely to be complex, since the directions of C and nutrient flows may alter when parts of the network colonize new roots or access new nutrient sources.

The extent of mycelial interconnection between plants must be controlled by host–fungus specificity. At an ecosystem level, plants that share one particular type of mycorrhiza, for example, EM or AM, form guilds (Perry 1998) within which plants may join common mycelial networks. The low global diversity of AM fungi, which may number a few hundred species in contrast with the high diversity of plants (approx. 250 000 species; Wilson 1992), most of which have this type of mycorrhiza, has engendered low host specificity. However, molecular studies have revealed diverse assemblages of 20–30 AM fungal species in established plant communities (Vandenkoornhuyse et al. 2002; Husband et al. 2002) and uncovered hitherto hidden patterns of host specificity (Vandenkoornhuyse et al. 2002). Together with results from pot studies of specificity in host–fungus interactions affecting plant growth and AM spore production (Bever 2002), the established view of very low host specificity in AM (Sanders 2002) is now being seriously challenged (Sanders 2003). It seems likely that in diverse plant communities virtually all mycorrhiza-compatible plants will join common mycelial networks but that not all plant species will share the same fungal partners (species or individuals of the same genet), so that a complex community of overlapping host–fungus species interactions occurs.

Networks of influence: the effect of ERMM on biogeochemical cycles, plant community composition, and agroecosystem functioning

In parallel with the recent increasing awareness of the biomass, extent, interconnections, and C fluxes through ERMM, there has been a growing recognition of the multifunctional importance of these networks in biogeochemical cycles, plant community composition, and agroecosystem functioning.

Mycorrhizal mycelial networks: a major force in biogeochemical cycling

The empowerment of mycorrhizal networks with substantial amounts of host-derived C allows them to play central roles in major biogeochemical cycles. The ERMM of EM fungi show considerable interspecific and interstrain differences in the structure and functioning of their ERMM (Colpaert et al. 1992; Ek 1997; Leake et al. 2001; Donnelly et al. 2003).

Whilst there is less structural complexity and variation in AM hyphal networks, there are, nonetheless, clearly emerg-

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ing functional differences between AM fungi in the extent and efficiency with which they forage for P from roots (Smith et al. 2000), and in part this may be explained by differences in the lengths and extension distances of AM hyphae (Schweiger and Jakobsen 2000). There also appear to be differences between AM fungal species and strains in their abilities to assimilate organic N sources (Hawkins et al. 2000).

P and N uptake by mycorrhizal mycelium

The most important function of AM for plant growth is normally to increase uptake of P, but AM fungi, as has long been recognized for EM, are involved in the uptake of many different nutrient elements (N, Cu, Fe, K, and Zn), including some organic sources of N and P (Smith and Read 1997). It is only in the past decade that there has been strong evidence that AM mycelia play an important role in mineralization and uptake of organic P (Tarafdar and Marschner 1994), and using monoxenic cultures it has now been possible to unequivocally demonstrate this process (Koide and Kabir 2000). AM hyphal networks possess wall-bound extracellular phosphatase enzymes (Joner et al. 2000*a*), and their narrow diameters and rapid linear extension rates should enable them to place these enzymes in soil pores that are too small and too far from the root to be directly accessed by root hairs. The extent to which the potential of AM fungi to use organic P sources is realized in the field is, however, unclear (see Joner et al. 2000*a*), although recent studies suggest it may be significant in some soil types (Feng et al. 2003).

It is well established that many EM fungi are active producers of phytase and phosphatase enzymes (Leake and Read 1997), and some can obtain both P and N from a range of organic sources, including partially decayed tree litter, pollen, and nematodes (Read and Perez-Moreno 2003). In soil microcosms, between 35% and 40% of the total P content of partially decayed tree litter was removed by colonizing EM mycelium, the majority of this P being mobilized from organic compounds. In the absence of EM mycelium, moist and nonsterile, partially decayed tree litter only very slowly releases inorganic P (Bending and Read 1995). Taking the efficiency of nutrient recovery from pollen and nematodes by mycorrhizal tree seedlings in soil microcosms and estimates of the annual production of these nutrient sources in boreal forests, Read and Perez-Moreno (2003) suggest that 15% of P and 12% of N supplied to trees in these forest ecosystems may come just from nutrient uptake from these sources by EM mycelium. Furthermore, there is evidence that some EM fungi are toxic to fungal-feeding microarthropods such as collembola and that significant amounts of N can be obtained by mycorrhizal fungi digesting dead collembola (Klironomos and Hart 2001).

In addition to their roles in P nutrition, both AM and especially EM fungi play a major role in the uptake of N by plants. Evidence of the involvement of AM mycelium in the uptake of N by plants has also been strengthened by studies of monoxenic fungal cultures that have demonstrated uptake of ammonium, nitrate, glycine, and glutamine. Uptake of ^{15}N-labelled amino acids by AM hyphae and transport of N into roots has been measured for the first time (Hawkins et al. 2000). This lends support to the observation that some AM fungi increase decomposition and subsequent capture of inorganic N from complex organic materials such as plant litter and proliferate their hyphae in organic-resource patches (Hodge et al. 2001). These kinds of responses have been considered characteristic of EM but not AM fungi, and the mechanisms involved in N mobilization by AM are unclear, since there appears to be little evidence that AM fungi produce the range of macromolecule hydrolysing and oxidizing enzymes of the former (Leake and Read 1997). However, few studies have examined N use by AM fungi specifically isolated from organic-rich soils, and it is notable that the AM fungi that were most effective at taking up organic N came from an environment with low nutrient input (Hawkins et al. 2000).

In pot studies, particularly in relatively dry soil where mass flow of nutrients is restricted, AM hyphae have been found to provide an effective uptake pathway for 7%–49% of the plant N uptake from nitrate and ammonium sources (Hawkins et al. 2000; Johansen et al. 1994). Whilst the contribution of AM to plant N uptake is unusually high under these rather extreme water-stress conditions, the potential of these symbionts to assist plant N nutrition is confirmed. Once mineral N is taken up by AM hyphae, it is most likely to be transported through the hyphae to the plant in amino acids (Bago et al. 2002), as has been shown to occur in ectomycorrhizas. The incorporation of mineral N into amino acids has high metabolic costs: in plants, 20 mol ATP are required for each mole of glutamate formed from nitrate, and 5 mol ATP are required per mole glutamate formed from ammonium (Salsac et al. 1987). Mineral N assimilation consequently has a high C cost to the mycelium, and if transported as amino acids, a significant amount of this C may be transferred back to the host.

EM mycelia are particularly effective in the uptake of ammonium from soil. Ek (1997) found that 9% of the nitrate and 18% of the ammonium supplied to the mycelia of the fungus *Paxillus involutus* was transferred to its host plant, birch, in 4 d, and the mycelium incurred a significant C cost of this nutrient uptake as its respiration increased by 55%–180%. As a consequence of such rapid and effective uptake by EM, forest soils naturally contain very low concentrations of mineral N. For example, Nilsson and Wallander (2003) found only 4.6 μg NH_4^+·g soil^{-1} and 0.2 μg NO_3·g soil^{-1} in April in a Swedish conifer forest with active mycorrhizal networks, but in plots with plastic tubes inserted a year earlier to exclude EM hyphae, this increased to 68.7 μg NH_4^+·g soil^{-1} and 0.8 μg NO_3·g soil^{-1}.

Ectomycorrhizal mycelia can short-circuit the N cycle

In addition to their effective scavenging and assimilation of inorganic N, especially ammonium, ectomycorrhizal fungi have high-affinity amino acid uptake systems (Wallenda et al. 2000), and some of these fungi also have highly developed proteolytic capabilities enabling them to directly access macromolecular N (Abuzinadah and Read 1989). This process, which is particularly important in boreal forests, has necessitated a revision of the N cycle (Fig. 6*a*) and a reappraisal of the nature of competition for N between plants in these ecosystems (Näsholm et al. 1998). Utilization of organic N by EM, which is particularly important in N-limited forests (Taylor et al. 2000), not only short-circuits the con-

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Fig. 6. (*a*) The nitrogen (N) cycle for N-limited forest ecosystems dominated by ectomycorrhizal trees. The trees gain direct access to organic N in plant and microbial litter through the activities of their fungal partners (pathway 1*a*; largest arrow), thereby short-circuiting the normal mineralization pathways (pathways 3 and 4). There can be intense competition for labile organic N between ectomycorrhizal fungi and microorganisms (pathways 1*a* and 1) and for the very small amounts of ammonium that are mineralized (pathway 3). The main microbially driven pathways are (1) microbial depolymerisation and assimilation of organic N; (2) release of microbial litter; (3) mineralization (ammonification); (4) nitrification; (6) microbial immobilization; (7) humification; and (8) N fixation. Nitrate leaching and denitrification are negligible because nitrification is absent or occurs at insignificant rates. **(*b*)** The N cycle in N-polluted forest ecosystems dominated by ectomycorrhizal trees. Note the importance of inorganic N as the main N sources used by ectomycorrhizal plants and the great reduction in the utilization of organic N by ectomycorrhiza. There are increased amounts of N in organic matter and especially in ammonium and nitrate pools. There are increased rates of mineralization, nitrification, and resultant N losses through nitrate leaching and denitrification (5). The intensity of competition for N between mycorrhizal and saproptrophs is much lower than in (*a*).

ventional N cycle and greatly reduces the energetic costs of N assimilation by the fungi, but in bypassing the normal mineralization pathway it restricts the supply of mineral N to plants and microorganisms that depend upon it (Fig. 6*a*). By gaining a monopoly on the N, ectomycorrhizal fungi may assist their host plants in suppressing other guilds of plants, such as AM herbs and some hardwood saplings, whose AM fungal partners are less effective at N capture, and may strongly select for subsets of forest plants that have at least some ability to use organic N (Nåsholm et al. 1998). The reduced flow of N through the ammonification pathway, together with effective scavenging of ammonium by EM, also serves to minimize the residence time of mineral N in soil solution. In turn, this will virtually eliminate nitrification so that losses of N from the ecosystem through ammonium leaching, nitrate leaching, and denitrification are minimized and N is conserved in the forest ecosystem.

The situation is very significantly altered under enrichment by anthropogenic N deposition (Fig. 6*b*), where the proportion of EM fungi that have well-developed proteolytic capabilities decreases (Taylor et al. 2000; Lilleskov et al. 2002) and the accumulation of ammonium in the soil enhances mineralization and nitrification, culminating with major leaching losses of nitrate together with base cations (Schulze et al. 2000), often leading to serious acidification (Brunner 2001). Enrichment with mineral N provides high N availability to all guilds of plants. Whilst these effects are clearly not driven solely by the responses of the mycorrhizal fungi, their role is especially important, as they provide the major N-absorbing system for the trees.

Laboratory studies have revealed a clear mechanistic link between C supply to EM mycelia and their abilities to use protein (Eaton and Ayres 2002). The C supply to EM from their hosts has been shown to be reduced where N supply is no longer limiting plants, and in these circumstances the capacity of the fungi to take up and to immobilize N in their own biomass and tissues is reduced. This is reflected $\delta^{15}N$ signatures in pot-grown plants (Hobbie and Colpaert 2003).

Since EM trees commonly have well over 90% of their root tips colonized by mycorrhiza, and the fungus completely ensheaths the roots, it is fair to assume that virtually all the nutrients and water taken up by the plants occurs through their fungal partners. What is more difficult to ascertain is the proportion of the nutrients and water taken up by established trees that could not have been obtained by the plants if they lacked mycorrhizas. In a study by Read and Perez-Moreno (2003), comparisons were made between N and P uptake from pollen and nematode necromass by mycorrhizal and nonmycorrhizal tree seedlings grown in non-sterile soil. From their data, the proportion of the total N and P supplied in a patch of pollen that was removed by the action of ERMM was 33% and 62%, respectively. In the case of the nematode necromass, 31% of the N removal and 41% of the P removal was attributable to foraging by EM mycelium. By comparing the nutrient uptake by the mycorrhizal and nonmycorrhizal plants the proportion of the added N and P in the pollen that was only accessible to the plants through mycorrhiza can be calculated and was found to be 17% of the N and 18% of the total P.

Nutrient mobilization and mineral weathering by mycorrhizal networks

AM hyphae appear to be able to acquire P from a range of inorganic P sources, including some calcium and aluminium phosphates that have extremely low solubility (Yao et al. 2001), but it is not known whether the fungi are directly involved in their solubilization. Uptake of insoluble P sources by AM may be facilitated by P-solubilizing bacteria, and there may be mutualistic interactions between these two groups of organisms (Villegas and Fortin 2001). EM mycelia have also been shown to obtain P from a range of sparingly soluble mineral sources such as aluminium phosphate (Cumming and Weinstein 1990), and their production of organic chelators such as citric and oxalic acids, together with hydroxamate siderophores, are implicated in major mineral weathering processes and podzolization (van Breemen et al. 2000). The sparingly soluble calcium phosphate apatite stimulates EM hyphal growth in N-fertilized forest plots (Nilsson and Wallander 2003), and the enhanced weathering of apatite-P and biotite-K by some EM fungi has been demonstrated (Wallander 2000*a*, 2000*b*; Wallander et al. 2002).

These findings are of enormous significance for biogeochemistry and processes of soil maturation. Whilst the importance of some organisms, particularly lichens, has received considerable attention in studies of the weathering of rocks and development of etched and fissured mineral surfaces (see, e.g., Birkeland 1999), until very recently the involvement of mycorrhizal fungi in these processes in soils was entirely unrecognized. However, there is simply no comparison between the biomass, C flux, organic acid production, and surface area of contact with rock-forming minerals achieved by lichens, which are restricted to a few millimetres of bare rock surfaces or litter layers in open habitats and forest floors, and by mycorrhizal mycelia (see, e.g.,

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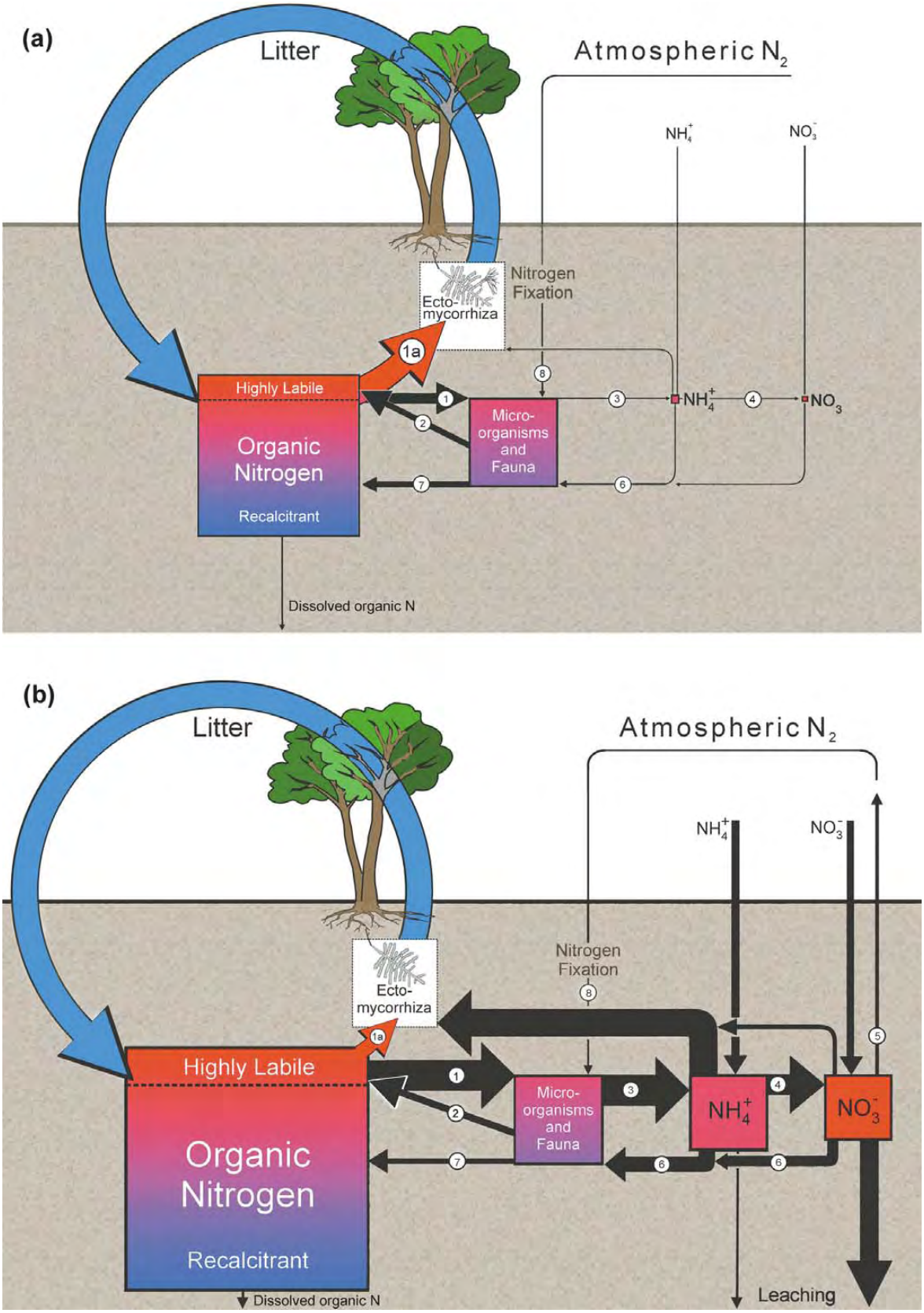

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van Breemen et al. 2000; Wallander et al. 2002). Mycorrhizal mycelium grows through decimetre depths of soil, may receive as much as 10% or more of net C fixation, can produce copious amounts of low molecular weight organic acids (Wallander et al. 2002), extends to many metres per gram of soil, and is abundant in virtually all terrestrial ecosystems.

The track etching of feldspar and other minerals with hyphal-sized tubular pores at the estimated annual rate of 15 $cm \cdot cm^3$ of soil particles$\cdot year^{-1}$ (Jongmans et al. 1997) has been attributed to EM fungi, which are now thought to be responsible for greatly accelerated mineral weathering rates in many forest soils (van Breemen et al. 2000). The high affinity of both AM and EM mycorrhizal hyphae for specific nutrient ions such as P and K results in depletion of the available pool of these ions (e.g., Li et al. 1991; Paris et al. 1995), and this, together with localized acidification caused by respiration and proton release by hyphae, particularly associated with ammonium uptake (Villegas and Fortin 2001), will tend to increase the passive dissolution of minerals containing these ions. These processes are further accelerated by organic acid chelators that are produced in abundance by many EM fungi and can form extensive crystalline deposits on their surfaces (Wallander et al. 2002).

Competition for nutrients between mycorrhizal mycelial networks and saprotrophs and effects on the C cycle

A further consequence of the highly effective nutrient mobilization and assimilation achieved by mycorrhizas is their tendency to selectively remove N, P, and K from litter, leaving behind the major C compounds and increasing the C/N, C/P, and C/K ratios of the residues (Bending 2003). As a consequence, there is intense competition, particularly for the more labile N and P sources, between mycorrhizal and saprotrophic fungi (Fig. 6*a*). This process has important implications for the decomposer communities and in part may account for the tendency of many boreal forest soils to experience very low rates of litter decomposition and to accumulate C in recalcitrant humic materials.

In particular, mycorrhizal mycelium interacts with other large fungi such as the wood decomposers, which use unusually large and long-lasting C resources in the form of coarse woody debris with very low availability of N and P. These two groups of fungi grow in decaying pieces of wood (Tedersoo et al. 2003), forage for nutrients in the same soil horizons, and share many similarities in growth and their nutrient foraging strategies (Leake et al. 2002; Boddy 2000). Many saprotrophic and ectomycorrhizal fungi are closely related, as there appears to have been evolutionary instability between them in both directions (Hibbett et al. 2000). Unsurprisingly, therefore, EM fungi retain many of the key enzyme systems of saprotrophic fungi and can deploy some of them in nutrient mobilization (Leake and Read 1997). The increasing recognition of a broad spectrum of "saprotrophic" enzyme activities in mycorrhizal fungi challenges the conventional view that mycorrhizal fungi are fundamentally different from saprotrophs and lack the ability to directly participate in decomposition processes. Soil microcosm studies (Lindahl et al. 1999; Leake et al. 2001, 2002) have revealed that mycelial systems of EM and saprotrophic wood-decomposer fungi can be antagonistic to each other, both in growth and functioning, and this antagonism is often greatest in the actively growing mycorrhizal mycelium furthest from root surfaces. This antagonism can lead to significant transfers of nutrients between the two trophic groups when they interact (Lindahl et al. 1999).

Our studies have shown that when EM mycelium encounters mycelium of saprotrophic wood- and litter-decomposing basidiomycetes in natural soil there is intense territorial conflict. Extension of mycelial cords of the aggressive saprotroph *Phanerochaete velutina* was halted by dense mycelium of *Paxillus involutus* symbiotic with *Betula pendula* (Leake et al. 2002). The mycelial cords of the saprotroph rapidly became truncated, their apices turned brown and senesced, and their growth was often deflected away from the direction of the advancing mycorrhizal mycelium, restricting their access to ectomycorrhiza-occupied regions (Fig. 7). There were, however, often marked reciprocal effects of the saprotroph on the mycorrhizal mycelium, whose growth and C allocation was directed away from the region of conflict in *Paxillus involutus* or led to a general loss of vigour and C allocation to ERMM in *Suillus bovinus* (Leake et al. 2001).

Antagonistic effects of EM on saprotrophs may serve to further slow the process of organic matter decomposition in forest soils and may facilitate the vitally important process of soil C sequestration. On the other hand, decomposition might be unaffected or accelerated (Bending 2003). Apart from effects on nutrient supplies and territorial exclusion of some saprotrophs, the drying of soil by water extraction by EM has recently been implicated in reduced litter decomposition in the presence of EM networks (Koide and Wu 2003), but there are also indications that under severe surface drought conditions, water can be supplied to mycorrhizal hyphae from plant roots, providing the trees have access to water via taproots in subsurface layers (Querejeta et al. 2003). How these counteracting processes actually affect nutrient and C cycling processes under field conditions remains unclear, but it is likely that the hydrophobicity of many EM fungal cords and rhizomorphs allows them to transport water to or from the soil, depending on the moisture regime, enabling efficient uptake of nutrients by the fine, absorptive hydrophilic hyphae, even in drying soil. The supply of water via the tree during surface drought is thought to keep the hyphae active (Querejeta et al. 2003), making them instantly available to absorb the flush of nutrients released into solution as soon as the soil rewets.

Not all interactions between mycorrhizal fungi and saprotrophs are likely to be antagonistic. Indeed, where the main N sources are highly recalcitrant tanned-protein complexes, which most EM fungi tested to date have very limited abilities to degrade, the action of saprotrophs appears to facilitate N recovery by the mycorrhizas (Wu et al. 2003).

Since AM are most common in environments in which organic matter decomposition is relatively quick and N is readily mineralized, interactions between their mycelia and those of saprotrophs is likely to be less important with respect to competition for N, but they may interact with other organisms, particularly in competition for P. In vitro studies of bacteria and fungi in the presence and absence of AM mycelial networks have revealed modest positive and negative effects (Fortin et al. 2002), but most studies in soil

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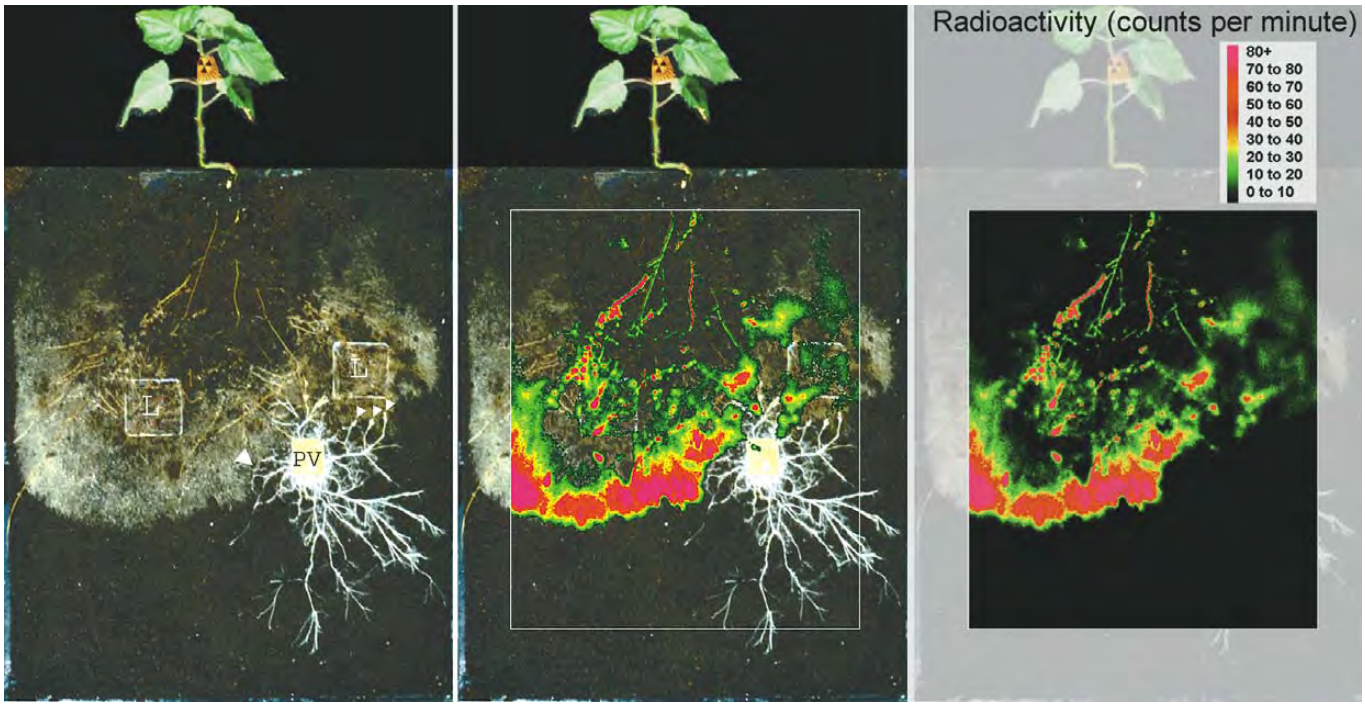
Fig. 7. The effect on carbon allocation in the extraradical mycelium of the mycorrhizal fungus *Paxillus involutus* when in contact and interaction with the wood-decay fungus *Phanerochaete velutina*, growing from a piece of wood (PV). The mycorrhizal fungus is growing in association with *Betula pendula*, which was pulse-labelled with ^{14}C, and this was quantified in a 20 cm × 24 cm belowground area by digital autoradiography. There were two patches of litter (L) in the microcosm to provide resources for the fungi. Note the truncation and browning of the *Phanerochaete* hyphal cords in contact with *Paxillus* (small arrowheads) and the deflection of the growth of the saprotroph on the right of the wood block (large arrowhead). The mycorrhizal fungus allocates the carbon it receives from the host plant away from the area of territorial combat, and its growth is locally stopped by contact with *Phanerochaete*. Modified from Leake et al. (2002).



(sandy soil) have often shown little or no effect (e.g., Olsson et al. 1996; Olsson and Wilhelmsson 2000).

Selective ion uptake and exclusion of toxic elements from plants by mycorrhiza and their external mycelium

Both AM (Joner et al. 2000*b*) and EM (Meharg and Cairney 2000) mycelial systems can immobilize and reduce plant uptake of nonessential and toxic metals such as Cd and Pb and thereby decrease the passage of these elements through the food chain. These effects are strain specific, and those isolates that are most effective at tolerating metals and excluding them from their tissues are typically found on contaminated sites (see, e.g., Malcova et al. 2003). The ability of mycorrhizal mycelium to selectively take up and pass on nutrients to their hosts whilst excluding toxic metals enables mycorrhizas to play an important role in revegetation of metal-contaminated sites. It allows some EM fungi to mobilize P from aluminosilicates and aluminium phosphate, in particular, whereby they exclude potentially phytotoxic aluminum, whilst supplying their host plants with P (Cumming and Weinstein 1990).

Protective effect of mycorrhizal mycelium against attack by root pathogens

There is evidence that both AM and EM fungi can reduce root-pathogen attack, and some of this effect may be due to interactions between external mycelium and mycelium of pathogens. Root-organ culture studies have demonstrated effects of AM mycelia on spore germination and growth of root pathogens, but the effects may be positive or negative and are different for different pathogens (see Fortin et al. 2002). In greenhouse studies beneficial effects of AM mycorrhizas have often been shown (e.g., Abdel-Fattah and Shabana 2002), but the importance of ERMM versus intraradical infection in AM is unclear.

In the case of EM, a few studies have demonstrated a protective antibiotic role of external mycelium against pathogens, in addition to the benefits of the mycorrhizal sheath. For example, *Paxillus involutus* can be antagonistic to root pathogens in the soil and suppress their growth before they have the opportunity to encounter roots (Duchesne et al. 1989). If ERMM is generally inhibitory to many root pathogens this will have important consequences for management practices in agriculture that disrupt and damage these networks (see next section).

Effects of mycorrhizal networks on plant community composition and ecosystem functioning

Plant community diversity and biomass production correlate with length of ERMM

In experimental macrocosms, AM mycorrhizal fungal diversity was found to have a strong positive effect on plant community diversity and plant productivity (van der Heijden

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et al. 1998). With increasing AM fungal diversity, hyphal length in soil rose from 2.8–6.5 $m \cdot g^{-1}$, reaching a plateau with 8–14 species. The responses to AM diversity seen in both the plant productivity and community diversity (Simpsons's diversity index) correlated almost exactly with the pattern of hyphal length increase with increasing AM diversity, and this in turn was inversely related to the quantities of P remaining in the soil. This experiment supports the hypothesis that under P-limiting conditions maximum productivity and plant biodiversity is dependent upon the amounts of external mycorrhizal mycelium produced, and diverse assemblages of AM fungi provide more external mycelium and, consequently, more P uptake than species-poor AM communities. Further work is require to test these hypotheses but there is increasing evidence that the uptake of P by AM mycorrhiza is often closely related to total AM hyphal lengths (see later section).

The role of interplant C and nutrient transport through mycorrhizal mycelial networks

A feature of mycorrhizal networks that has recently attracted enormous interest and generated the most controversy has been their transporting of nutrients and C from one plant to another through interconnecting mycelium (Simard et al. 1997, 2002). These interspecies resource transfers have far-reaching implications for evolutionary theories applied to plant communities and suggest that a radical reappraisal of conventional concepts of competition is required (Perry 1998; Wilkinson 1998). The controversy has centred on the evidence for net transport of C between plants linked by a common mycelial network, and Robinson and Fitter (1999) have gone as far as to state "it is not possible to conclude that plant–plant C transfer via a common mycorrhizal network has any significance for the composition and functioning of plant communities". However, it has been known for over 150 years that some achlorophyllous plants, called myco-heterotrophs gain all their C from fungi (Leake 1994). Over 400 species of such plants exist, and they are derived from multiple independent lineages of green plants. In recent years, using DNA-based identification, fungal partners of many of these plants have been identified for the first time, and most have proved to be EM (Taylor et al. 2002) or AM fungi (Bidartondo et al. 2002) that are coinfecting the roots of adjacent green plants. Pulse-labelling studies, and biomass measurements in experimental microcosms with and without mycelial interconnections between autotrophs and myco-heterotophs, have confirmed that C is transported via the shared EM mycelium (McKendrick et al. 2000*b*; Bidartondo et al. 2003).

The fungal partnerships of myco-heterotophs are characterized by extreme specificity (Taylor et al. 2002; Bidartondo et al. 2002; Young et al. 2002) and unusual host-plant combinations. Many orchids switch from associations with soil saprotrophs and pathogens, such as fungi in the form genus *Rhizoctonia*, to associate with EM fungi (Taylor et al. 2002; McKendrick et al. 2002) that are better able to supply large amounts of C over long periods of time. The exchanging of "normal" partnerships to gain direct access to plant-derived C has also been demonstrated for the achlorophyllous liverwort *Cryptothallus mirabilis*, whose fungal partner is a *Tulasnella*, a genus of fungi that often form orchid mycorrhizas and are normally considered saprotrophic, but in this case the fungus forms ectomycorrhizal associations with birch (Bidartondo et al. 2003).

Whilst myco-heterotrophy is recognized as an extreme "cheating" type of mycorrhiza (Bidartondo et al. 2002), it is surprisingly common and not confined to the approximately 400 species that are entirely achlorophyllous. It characterizes the early developmental stages of germination of most orchids, which number over 17 500 species, and the gametophyte stages of many club mosses and ferns. In these cases there is net C flow to the plant during the early stages of growth from seeds or spores, but the plants eventually go on to photosynthesize (Leake 1994). These "initially myco-heterotrophic" plants receive a modest C investment from their fungal partners to establish themselves as autotrophs, but providing the same fungi are retained in the adult plants, the fungi in the long term may stand to gain much more C back from these partnerships once the plants mature into autotrophs. There is increasing evidence that obligate myco-heterotrophy represents one extreme end of a continuum of C transfer from fungi to plants, and the multiple evolutionary origins of this form of nutrition is consistent with the view that a large number of plants can gain C, at least transiently, by this pathway. Myco-heterotrophs exemplify the ability of mycorrhizal networks to transport substantial quantities of C and nutrients and the potential importance of these networks for the control of species composition of natural communities. In the absence of their critical fungal partners, myco-heterotrophic plants typically fail to germinate and certainly fail to establish (McKendrick et al. 2000*a*; 2002). In these cases, mycorrhizal mycelial networks provide the ultimate control on composition of myco-heterotroph plant communities.

What remains unclear is the extent to which these kinds of interplant transfers occur routinely between interlinked green plants in the field and over what scale. Experimental evidence is fragmentary, and as Robinson and Fitter (1999) note, crucial experiments have not been conducted to exclude the possible fixation in the shoots of "receiver" plants of labelled C tracers respired from the roots and mycorrhizas of the "donor" plants supplying most of the C to the plant-interlinking mycorrhizal network in the soil. Thus, even in the most recent studies of interplant C transfers (Wu et al. 2001; Lerat et al. 2002) there remains uncertainty about the pathway of labelled C uptake by shoots, whether from refixation of C respired from below ground or through direct transfer through mycorrhizas interlinking the roots. However, there is already clear evidence that at the seedling-establishment phase many plants receive, at the very least, an indirect C subsidy from the established plants. Newly emerging seedling roots link to an established nutrient-absorbing mycelial network that has already been "paid for" by C supplied by the established plants, and the fungal colonization of their roots may also be C subsidized (Simard et al. 2002). In addition, ERMM networks provide pathways for interplant transfers of nutrients, and in some cases, depending upon the species and circumstances, substantial amounts of N, and to a lesser extent P, have been demonstrated to move through mycorrhizal mycelium interlinking plants (Simard et al. 2002). Recent studies of the ^{15}N and ^{13}C enrichment of fully myco-heterotrophic plants (Trudell

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et al. 2003) and of both green and fully myco-heterotrophic orchids (Gebauer and Meyer 2003) have shown that the myco-heterotrophs have distinctive enrichment in both ^{13}C and ^{15}N relative to co-occurring autotrophic plants. More importantly, the characteristic heavy-isotope enrichment of myco-heterotrophs has revealed that within green orchids there is a wide range of dependency upon fungal-C. Some green-leaved species previously assumed to be autotrophs are clearly gaining most of their C by myco-heterotrophy (Gebauer and Meyer 2003), confirming the view that there is a continuum between full autotrophy and full myco-heterotrophy.

Effects of mycorrhizal mycelial networks on plant community composition: costs and benefits are not shared equally

It is increasingly apparent that the C costs and functional "benefits" to plants of linking to ERMM networks are fungal specific and, because of variations in physiology and host specificity, are not shared equally amongst plants (Hartnett and Wilson 2002). Grasses, for example, with their extensive fibrous root systems with long root hairs, are much less mycorrhiza dependant than coarser rooted forbs, but may contribute substantially to C supply to the ERMM (Grime et al. 1987). Whilst the former often show limited growth benefit from mycorrhiza, the latter can be highly responsive, and their response may vary greatly depending upon which fungal partners are present, so that mycorrhizal fungal species composition can have a major effect on individual plant performance and overall plant community structure (see, e.g., van der Heijden et al. 1998). The evidence of extreme specificity and fungal dependence in myco-heterotophs reinforces the need to investigate the functional importance and community consequences of host–fungus specificity that has been revealed in a number of recent studies (Vandenkoornhuyse et al. 2002; Bever 2002). In autotrophs such specificity may help to ensure that interplant C and nutrient transfers and the costs and benefits of linking to a common mycelial network are not shared too widely and preferentially benefit co-linked plants of the same species. An alternative perspective is that specialization is primarily of advantage for the specialist, not its target, since "cheats" and parasites are typically characterized by very high host specificities (Bruns et al. 2002).

Mycorrhizal mycelial networks in sustainable agriculture

Pollution and disturbance affect the extent and functioning of mycorrhizal mycelium

The environmental impact of intensive agriculture on biodiversity, soil and water pollution, and soil erosion and sustainability has become a major concern (Robinson and Sutherland 2002). As awareness of these issues has increased in recent years together with consumer concerns about food safety, such as possible health risks of pesticide and fertilizer residues, there has been significant expansion of alternative forms of agriculture, such as reduced tillage and organic and biodynamic farming. Under increasing intensification of agriculture there may be little scope for AM involvement in agricultural production (Ryan and Graham 2002), as phosphate fertilizer applications and soil disturbance have very adverse effects on AM mycelial networks (McGonigle and Miller 2000), and most AM fungal species are eliminated (Daniell et al. 2001). The loss of diversity is characterized by the virtual elimination of certain key genera such as *Acaulospora* and *Scutellospora*, both of which tend to be abundant in undisturbed communities (Daniell et al. 2001), and the dominance of communities by a single species, *Glomus mosseae*. It is suggested that the abundance of the latter may relate to its ability to form anastomoses and repair severed hyphae (see Daniell et al. 2001) and the readiness of *Glomus* sp. to form infections from hyphae and root pieces (Klironomos and Hart 2002). This contrasts with *Scutellospora*, which does not readily form anastomoses and, in the absence of intact hyphal networks, appears to depend upon spore inoculum (Klironomos and Hart 2002). There are indications that the loss of AM genera in intensive agriculture is likely to have much greater functional significance than loss of species within the same genus, since there appear to be larger functional differences at the genus level (see Hart and Klironomos 2002). This hypothesis requires more rigorous testing, as the taxonomy of Glomeromycota is currently undergoing rapid revisions with the increasing development of molecular taxonomy.

Studies of the impacts of anthropogenic pollutant N deposition on mycorrhizas have mainly focused on EM in forest ecosystems that are normally N limited and very sensitive to N enrichment. EM diversity, biomass, and fruit-body production are adversely affected by N enrichment (Erland and Taylor 2002). In short-term N addition experiments in the laboratory, EM fungi showed large increases in respiration in response to mineral N (Ek 1997; Bidartondo et al. 2001), and *Suilloid* species, which are particularly sensitive to forest N enrichment, gave up to five-fold greater increase in respiration compared with *Paxillus involutus*, which is considered relatively N tolerant. In the field, long-term (>10 years) N fertilization of forest plots reduced EM mycelial growth into sand ingrowth bags by approximately 50% (Nilsson and Wallander 2003). In highly N enriched soils the large C drain imposed on the hyphae by sustained uptake of high concentrations of mineral N is highly detrimental to the fungi, and the supply of C from the host plants to the fungi may be down-regulated when the plants are no longer N-limited (Hobbie and Colpaert 2003). However, as N supply increases, P limitation typically becomes more important. In N-fertilized forests plots, Nilsson and Wallander (2003) found that, in mesh bags of sand mixed with apatite, the growth of EM mycelium was not inhibited in the N-fertilized forest plots, where demand for P may have increased.

Whilst grasslands are typically P rather than N limited and might be considered less sensitive to pollutant or fertilizer N inputs, recent studies of effects of long-term N enrichment of species-rich grasslands have shown major effects on AM functioning (Ames 2002). Under these conditions, P becomes the key nutrient that limits plant growth, and in grasslands plant P limitation can be exacerbated by the impairment of mycorrhizal functioning through excessive N enrichment (Ames 2002). It is likely that N fertilizer is one of a number of factors that contributes to the low diversity of AM fungi in intensive agriculture, but this requires further confirmation.

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The importance of mycorrhizal mycelium in sustainable agriculture

AM hyphal lengths in soil show strong positive correlations with soil-aggregate stability (Rillig et al. 2002; Kabir and Koide 2002), P uptake efficiency (Schweiger and Jakobsen 2000), and crop-yield improvements (Kabir and Koide 2002). Interest in the development of less intensive management systems is presenting new opportunities for adapting agricultural production systems to enhance these benefits that can be gained from AM networks. Substantial improvements in "soil health" and AM functioning in field crops are gained by the doubling of lengths of AM hyphae in soil when tillage is reduced (Kabir et al. 1998*a*, 1998*b*). Similar gains are achieved by growth of AM-compatible cover crops in place of winter fallow (Kabir and Koide 2002). Frost-sensitive cover crops can be equally effective as wintergreen crops for maintaining AM inoculum potential (Kabir and Koide 2002) and are ideally suited to organic and biodynamic management systems, where herbicide use is not permitted. Improved productivity in mycorrhiza-demanding crops, for example, maize, can be achieved by substituting mycorrhizal-compatible crops (e.g., sunflower) for mycorrhiza-incompatible crops, (e.g., members of the Brassicaceae) in the preceding year of the rotation (Karasawa et al. 2002).

Reducing management intensity of farmland can increase the importance of mycorrhizal functioning in P acquisition. Using mesh-compartmented pots containing turf from winter wheat fields and the uncultivated field margins, Muckle (2003) has shown that the uptake of P from root-free mycorrhizal hyphal compartments is increased with decreasing management intensity. These effects can extend beyond the ploughed fields to include the field margins, too (Fig. 8).

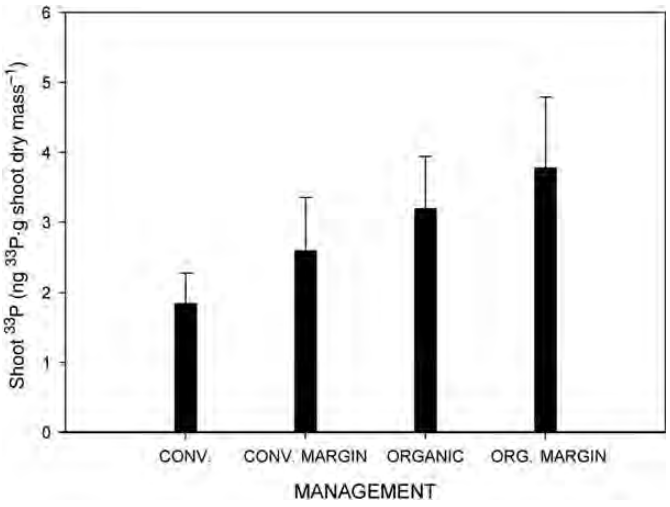
Mycorrhizal networks can contribute to sustainability by increasing nutrient-use efficiency, reducing infections by root-pathogens, and increasing soil-aggregate stability and soil physical properties. These effects provide impetus for the development of new forms of sustainable agricultural management that seek to optimize benefits gained from these symbioses.

Future directions

The progress made in our understanding of ERMM has brought the importance of these networks to the fore, but at the same time has highlighted the enormous gaps in our knowledge and uncertainties as to the networks' nature, extent, biomass, and functioning in the field. Whilst reports of AM hyphal lengths in agricultural soils are becoming increasingly common, our knowledge of them in natural ecosystems remains scant. It is particularly important that hyphal lengths and activities are now recorded in field-collected soils, particularly from under woodland, forests, and permanent grasslands, where we currently have few or no data. We also require observations from crops under different kinds of agriculture, especially in less intensive management. There is clearly a need to devise less labour-intensive methods for the counting and identification of extracted AM hyphae, and the use of image analysis systems to do this should be developed. It is also important to develop and apply methods for the identification and quantification of the biomass of different mycorrhizal species in soil and to assess their functioning in the field, for example, using isotope tracers and root-free hyphal compartments. Protocols for molecular identification of species from hyphae in soil have been successfully developed (Landeweert et al. 2003; Dickie et al. 2002) and clearly have enormous potential to reveal detailed spatial structure in communities of EM extraradical mycelia in soil, but the methods are not quantitative, and effective procedures for routine identification of AM hyphae in soil still need to be developed. The linking of mycelial location, identification, and function in soil remains a major challenge but is starting to be achieved (see, e.g., Wallander et al. 2003).

Studies of the functioning of ERMM in the field are in their infancy, but the availability of methods that enable root and hyphal activities to be distinguished should now be more widely applied to establish the quantities of nutrients and C passing through mycorrhizal mycelia in a wider range of plant communities and farmland under different types of management. It is clearly of vital importance that the quantities of C passing through ERMM are determined for a wider range of plant communities and soils to fill this major gap in our knowledge of the terrestrial C cycle. It has been suggested that mycorrhizal mycelia may comprise a third of the soil microbial biomass in forests (Högberg and Högberg 2002), and these may receive 10%–20% of net photosynthesis (Birdartondo et al. 2001). Accurate estimates of typical C allocation to mycorrhizal mycelia in the field must now be an absolute priority. Their major contribution to soil C pools, which is unknown, but thought to be substantial (Högberg and Högberg 2002), must also be quantified. The recent evi-

Fig. 8. The effect of management intensity on the mean shoot ^{33}P concentration in turfs 30 d after being provided with radiolabelled orthophosphate on anion-absorption membranes buried 1 cm inside a root-free hyphal compartment. The turfs were sown with a wildflower seed mix onto monoliths of soil removed from conventional (CONV.) or organic (ORG) managed winter wheat fields and their uncultivated margins (Margin). The monoliths came from five replicate fields from each management type and originated from North Yorkshire, two locations in Leicestershire, and one in Norfolk, all in the UK. Vertical bars indicate the SE of the means. From Muckle (2003).



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dence of the accumulation of large amounts of glomalin-C under AM plants, and its fate following land use changes (Rillig et al. 2003), highlights the need to understand more fully this important potential C store in soil and the effects of different management practices on its production and accumulation.

Some of the most exciting future prospects for studies of the functioning of mycorrhizal networks, their environmental sensing, and their physiological responses include applications of genomic and proteomic approaches. Unearthing the spatial and temporal regulation of gene expression involved in nutrient sensing, uptake and transport within mycorrhizal mycelial networks, and their localized interactions with other organisms presents a fascinating prospect. The recent analysis of expressed sequence tags in two ectomycorrhizal fungi (Peter et al. 2003) opens the door on these entirely new avenues of study. We already know that some EM fungi can sense nutrients and direct growth and C allocation into resource patches of litter (Bending and Read 1995; Leake et al. 2001; Hodge et al. 2001), mineral N (Ek 1997), or apatite (Nilsson and Wallander 2003). However, the signalling and control of resource allocation, the control of gene expression for the production of specific enzymes, secretions, and hyphal growth forms within the networks, and nutrient partitioning within the networks, including to the host plants, is currently not adequately understood. There are intriguing indications that EM growth may be tightly regulated by the specific nutrient limitations affecting the host plants: mycelial production was stimulated in mesh bags containing apatite mixed with sand but only in a forest where the trees were highly P limited (Hagerberg et al. 2003). The recent confirmation of a motile tubular vacuole system in AM (Uetake et al. 2002) as well as in EM mycelial networks (Allaway and Ashford 2001) demonstrates highly sophisticated internal structures for bidirectional transport of C and nutrients, but much has yet to be learned about the nature of the substances transported and the manner in which this is controlled and regulated.

Conclusions: looking beyond the rhizosphere into the mycorrhizosphere

The extraradical mycelium of mycorrhizal fungi is the "Cinderella" of soil microbial communities whose time has now come. The past decade of research has clearly established that, although often unacknowledged, ERMM is the hidden power behind plant community composition and ecosystem functioning through the major processes it carries out, such as nutrient uptake, weathering of minerals, soil-aggregate stability, and the way in which it alters competition between plants (van der Heijden et al. 1998). Whilst the key roles of ERMM are appreciated by increasing numbers of ecologists, soil biologists, agronomists, and foresters, its multifunctional importance needs to gain much wider recognition. This is particularly true in sustainable agriculture, where the benefits of appropriate management systems to ensure effective functioning of mycorrhizal networks are now being demonstrated in yield increases (Kabir and Koide 2002). To many, however, ERMM remain the "hidden half" of the symbiosis. The well-established concept of the rhizosphere was developed from observations on bacterial enrichment on exudates around the surfaces of roots grown without their normal mycorrhizal associations in the laboratory (e.g., Whipps and Lynch 1983). We must now recognize that mycorrhizal hyphae carry carbohydrates from plants into soil regions far beyond the conventional rhizosphere, and they release exudates, enzymes, hydrophobic glycoproteins, chelators, and dead cells and interact with other soil microorganisms (e.g., Leake et al. 2001) to create their own mycorrhizosphere with distinct microbial populations (Timonen et al. 1998; Bomberg et al. 2003).

As mycorrhizal mycelium is now known to constitute as much as 20%–30% of microbial biomass, it is necessary to critically reevaluate the value of standard measures of soil microbial biomass such as substrate-induced respiration, which selectively discriminate against detecting its major contribution to soil C pools and fluxes. The vital roles of the ERMM in soil science and plant nutrition need to be properly recognized. With few exceptions, undergraduate texts on plant physiology, plant nutrition, and agronomy pay scant attention to external mycorrhizal mycelium. Those that diagrammatically illustrate mycorrhizal mycelium typically misrepresent it as comprising highly simplistic, unbranched structures modelled on thinner versions of root-hairs, beyond which they are shown to extend, at the most, by a few millimetres. Diagrams of EM typically focus on the mantle and root-tip structure, whilst the mycorrhizal mycelium, which may account for 75% of the absorptive area (Rousseau et al. 1994), is often depicted by a few wisps of simple hyphae. The reality that external mycorrhizal mycelium form extensive and complex networks, interlink plant roots, extend far beyond the conventional "rhizosphere", and provide extremely important pathways for nutrient and C movements needs to eclipse such gross oversimplifications. Many of the multiple functions of ERMM result directly from the complex structural properties of mycelial networks, so that simplistic representations of them implicitly deny these functions. The accumulating evidence of the importance of ERMM in biogeochemical cycles, soil microbial ecology, plant communities, and agroecosystem functioning indicates that in future an even higher priority must be placed on studies of this part of the symbiosis. Conventional views on plant community functioning have been challenged by the evidence of transport of C and nutrients between some plants interlinked by a common mycelial network and by the demonstration of EM mycelia short-circuiting of the mineralization path of the N cycle. These are indeed networks of power and influence, and the time has come for us to pay due attention to them.

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