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How do plants regulate the function, community structure, and diversity of mycorrhizal fungi?

David Johnson¹⁺, Marleen IJdo¹,², David R. Genny², Ian C. Anderson² and Ian J. Alexander¹

¹ School of Biological Sciences, Cruickshank Building, University of Aberdeen, Aberdeen AB31 5TR, UK
² The Macaulay Institute, Cragiebuckler, Aberdeen AB15 8QH, UK

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Abstract

In many semi-natural and natural ecosystems, mycorrhizal fungi are the most abundant and functionally important group of soil micro-organisms. They are almost wholly dependent on their host plants to supply them with photosynthate in return for which they enable the plant to access greater quantities of nutrients. Thus, there is considerable potential for plant communities to regulate the structure and function of mycorrhizal communities. This paper reviews some of the key recent developments that have enabled the influence of plant species richness, composition, and age on mycorrhizal communities in boreal forests and temperate grassland to be determined. It discusses the emerging evidence that, in some situations, plant species richness is related to mycorrhizal species richness, in contrast to previous thinking. The paper also includes some preliminary data on the effect of host stand age on root-associated basidiomycete communities. It concludes by highlighting some of the new methodological advances that promise to unravel the linkages between mycorrhizal diversity and their function in situ.

Key words: Arbuscular mycorrhiza, ¹³C, ¹⁴C, carbon cycling, ectomycorrhiza, plant species diversity, stable isotope probing.

Introduction

In recent years, there has been a growing awareness amongst plant ecologists and soil microbial ecologists that understanding the connectivity between their study organisms is of utmost importance. The interactions between plants and soil micro-organisms are particularly important because plants represent the main pathway through which carbon, the element that severely limits microbial growth, enters soil. This is achieved in a number of ways including litter fall, root turnover, passive exudation of carbon in the rhizosphere in the form of simple organic compounds, and active transfer of carbon to organisms colonizing plant roots. From the reciprocal viewpoint, micro-organisms have essential roles in the mineralization of nutrients into forms available for plant uptake, as well as a number of other advantages.

One of the main drivers of this joined-up thinking is the importance now placed on understanding global biodiversity. This has occurred not just because of the alarming loss of species that has occurred in the last thousand years or so, but also because the pressures that are now placed on the terrestrial environment have the potential to cause further widespread loss of species (Thomas et al., 2004). From the plant perspective, species richness (here used interchangeably with diversity) of ecosystems has received considerable attention in recent years and has generated an equally large amount of controversy. On the one hand, increasing plant species diversity has been shown to lead to a progressive increase in ecosystem productivity (Hector et al., 1999; Tilman et al., 2001), while on the other hand, productivity has been shown to respond non-linearly to increasing plant species diversity, producing a ‘hump-backed’ response (Grime, 1973). However, there is a risk that these studies generate debates based on an entirely phytocentric view of ‘ecosystem function’. It could be argued, for example, that for many semi-natural grasslands, which are often subjected to various degrees of grazing pressure, plant productivity is not a particularly useful or important measure of ecosystem function. Very few studies have attempted to take a more holistic view by determining how plant species diversity may affect wider and more relevant indicators of ecosystem function.

* To whom correspondence should be addressed. Fax: +44 (0)1224 272703. E-mail: D.Johnson@Abdn.ac.uk

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In many semi-natural and natural ecosystems, mycorrhizal fungi are the most abundant and functionally important groups of soil microorganisms (Smith and Read, 1997). Virtually all the plants in temperate grassland form mutualistic associations with arbuscular mycorrhizal (AM) fungi (Read et al., 1976). AM fungi provide many benefits to plants, most notably the ability to access substantially greater amounts of nutrients. In limestone and calcareous grassland, the key nutrient involved is phosphorus, because calcium phosphate is relatively unavailable to non-mycorrhizal plants. The energy required by AM fungi to exploit the heterogeneous soil environment so effectively is supplied by the host plant in the form of carbohydrates. The carbon demand that this places on plants can be quite high; laboratory studies have shown that this can be as much as 20% of the total mount of carbon fixed by the plant (Pearson and Jakobsen, 1993), although it should be stressed that these values are derived from highly mycrotrophic species such as cucumber, and the range most commonly seen is 5–10% (Johnson et al., 2005). Nevertheless, there can be no doubt that AM fungi are important conduits of recent plant photosynthate to soil. In addition, this pathway is a very rapid one. Field studies suggest that most carbon is allocated to AM fungi within 24 h of fixation (Johnson et al., 2002a, b) and the turnover of fine hyphae itself is likely to be in the order of a few days (Staddon et al., 2003).

In temperate and boreal forest communities, most trees associate with ectomycorrhizal (EcM) fungi. As with AM fungi, the significance of ectomycorrhizas for the major elemental cycles is enormous. In microcosms 20–30% of current assimilate from seedling hosts is allocated to EcM fungi (Söderström, 2002). Over 50% of CO₂ released from boreal forest soils is accounted for by the respiration of tree roots and their associated EcM fungi (Högberg et al., 2001) and at least 30% of the microbial biomass in boreal forest soils is the extraradical hyphae of EcM fungi (Högberg and Högberg, 2002). EcM fungi are, therefore, significant sinks for plant photosynthate, the carbon being used to fuel the growth of extensive mycelial networks producing the so-called ‘wood-wide web’. In return, EcM fungi have the ability to short-circuit conventional nutrient-cycling pathways by taking up organic forms of nitrogen and phosphorus. Given the widely acknowledged fact that mycorrhizal fungi form ecologically important connections between plants and soils, it seems unusual therefore that they are rarely considered in studies investigating how plant communities can impact on ecosystems functioning.

While the response of ecosystems to changing plant diversity is clearly important, environmental perturbations or land use change may result in situations where replacement of a given plant species may occur but plant diversity remains similar. Because plants are the main pathway through which carbon enters the energy-impoverished soil environment, it seems reasonable to ask the question how the species composition of plant communities, not just their diversity or richness, may influence ecosystem and especially microbial functioning. Expanding this argument more widely, it may be desirable to determine how functional groups of plants or indeed the age of plants within a given community affect ecosystem processes. The latter point may be particularly applicable to plant communities created artificially and that have existed for relatively short time scales, such as forestry plantations.

The advent of several new molecular and stable isotope techniques (such as stable isotope probing) in recent years and their application to the natural environment has significantly increased the understanding of microbial diversity and functioning, especially in relation to carbon cycling. The aim of this paper is therefore to review the progress that has been made in understanding how the three principal factors of (i) diversity, (ii) composition, and (iii) age of plant communities in temperate and boreal biomes affect mycorrhizal community composition and function, and how these new studies fit hypotheses derived from previous studies. These three factors are likely to have the greatest impact on mycorrhizal communities, but it is acknowledged that there are undoubtedly other related processes not considered here, such as positive or negative plant interactions.

Species composition of plant communities

Of the three factors discussed here, the importance of the composition of a given plant community in influencing mycorrhizal communities is likely to stand out as the most obvious. Indeed, the importance of plant community composition of forests as determinants of EcM community composition is now well established largely because of the observations that many EcM fungal species show some level of host specificity or preference. Much of the pioneering work demonstrating that certain EcM fungal species associate with specific plants, while others have broad host ranges, was undertaken in the coniferous forests of the Pacific Northwest (Trappe, 1962; Molina and Trappe, 1982). The observations by Trappe (1962) were based largely on distributions of sporocarps; the assumption being that the production of sporocarps was indicative of mycorrhizal association. This assumption has been challenged based on an analysis of mycorrhizal morphotypes on root tips (Taylor and Alexander, 1990) and, more recently, by molecular methods that enable their fungal endophytes to be identified more rigorously (Gardes and Bruns, 1996; Jonsson et al., 1999). Needless to say, the relationship between above- and below-ground species is, more often than not, poor. Molina and Trappe (1982) undertook a more rigorous approach to screening mycorrhizal specificity by testing the abilities of 27 EcM isolates to form mycorrhizas with seven conifer host plant species in pure culture...
microcosms. This work demonstrated a wide range of ‘EcM host potentials’ ranging from, for example, the Alnus-specific symbiont Alpova diplophloeus, through the intermediate specificity of Cortinarius pistorius on Pseudotsuga menziesii, Larix occidentalis, Tsuga heterophylla, and Picea sitchensis, to the generalist symbiont Paxillus involutus. Molecular tools have meant that host specificity and preference of EcM fungi can now be investigated in the field (Horton and Bruns, 1998; Horton et al., 1999; Cullings et al., 2000; Kennedy et al., 2003). Generally, these studies show that the most abundant fungi in the community are not host specific, but that a minority of species show marked host specificity or preference (greater relative abundance on certain hosts).

Given the low number of AM fungal species thus far described (about 150), and the fact that they colonize around 200,000 plant species, conventional wisdom would suggest that species composition of broad types of plant communities, say temperate grassland, does not have much influence on the diversity or function of AM fungi. This view of low specificity has, not surprisingly, been assumed to hold true for most situations in which AM fungal associations occur, but there are exceptions to this. For example, Bidartondo et al. (2002) showed extreme host specificity by certain achlorophyllous mycoheterotrophic plants on AM fungi. Whether such extreme host specificity will ever be encountered in the wider AM plant community seems unlikely. One must also consider that specificity can occur in either direction between plant and fungus. In the mycoheterotrophic case above, for example, the fungus colonizes other species of neighbouring green plants from which the mycoheterotroph obtains its carbon. In his excellent review of the subject, Sanders (2002) was unable to draw any firm conclusions about the existence of specificity from either the plant or the fungal perspective. He also questioned the relevance of identifying AM fungi at the species level, when in fact considerable variation, and possibly therefore specificity, could be occurring at the genotype level. But does it really matter if tight host specificity is not seen universally, whether from the plant or fungal perspective? What is more important, at least to address the question of the importance of plant community composition in influencing AM fungal communities, is the existence of a gradient from zero host specificity towards the extreme cases cited above. Perhaps more important still is the need to understand the many factors that may push particular fungal species/genotypes along this gradient and the direction and magnitude of their movement.

The existence of host preference by AM fungi has been known for some time; Dhillion (1992) found that Glomus geosporum and G. fasciculatum differentially colonized three native prairie grasses. In addition, the observation that increasing diversity of AM fungi can help to maintain diverse assemblages of plants would suggest some degree of host preference (van der Heijden et al., 1998a, b). This type of study also provides support for the argument that different AM fungi produce markedly different levels of root colonization, growth rates and nutritional responses in some plant species compared to others (Streitwolf-Engel et al., 1997; van der Heijden et al., 1998a, b). The ability to determine the identity of AM species within roots of naturally colonized plants has reinforced this view. Intensive analysis of neighbouring native woodland plants that form AM symbioses revealed that the patterns of colonization of their roots were distributed in a non-random way (Helgason et al., 2002). For example, the combinations of Rubus fruticosus with Scutellospora and Acer pseudoplatanus with Glomus were significantly over-represented. Using molecular techniques, Vandenkroonenys et al. (2003) and Husband et al. (2002a) have shown clear non-random associations of AM types with the different hosts in ecosystems as divergent as chalk grassland and tropical rain forest.

Johnson et al. (2004) studied AM fungal communities in grassland microcosms containing natural soil and artificial plant communities. The plants were naturally co-occurring and were obtained from the field and reconstituted to form four treatments comprising bare soil, Carex flacca only, Festuca ovina only, and a mixture 12 mainly mycorrhizal species. The diversity of AM fungi (based on T-RFLP patterns) colonizing the roots of Plantago lanceolata biomass seedlings transplanted into the microcosms significantly affected the N and P concentration in the Plantago shoots. The diversity of AM fungi was itself only influenced by the composition of the microcosms rather than other factors such as microcosm plant biomass. Interestingly, the diversity of AM fungi colonizing P. lanceolata seedlings in the bare soil treatment was greatest, while the diversity on P. lanceolata seedlings in the F. ovina monoculture was smallest. This provides further evidence of a degree of host specificity. This may have arisen through the dominance of a restricted group of fungi that were able to form an extensive and vigorous mycelial network using carbon from the established community of mature F. ovina plants. In the bare soil, such a supply of carbon would not exist, instead, most colonization of the seedling roots would have occurred from an assemblage of propagules. Because propagules in the bare soil treatment would have been exposed to these selection pressures, the loss of diversity would be avoided. Kuszala et al. (2001) demonstrated that 20 isolates representing 16 genera were able to sporulate after storage in moist soil for up to 8 months. The ability of mycorrhizal propagules to maintain their viability for relatively long-periods suggests a degree of resilience that can only be beneficial for restoring biodiversity and function. Despite the longevity of inocula, it is not yet known if mycorrhizal populations are able to reform on plant species re-introduced into a particular habitat after their loss.

One potential difficulty with many of the discussions made thus far on the topic of specificity is the assumption
that only very few species of AM fungi colonize a single plant species (Fig. 1a, b). This is not surprising given that there is a very small pool of AM fungal species thus far described, although the issue highlighted by Sanders (2002), and discussed above, regarding the usefulness of regarding AM communities only at the species level may complicate the way AM diversity on individual plants is characterized. While some plants are likely to be colonized by only a few species, there is now a growing body of evidence suggesting extensive diversity in some grassland species. For example, Vandenkoornhuyse et al. (2002) found 24 distinct phylotypes (clades of closely related sequences) in two common co-occurring grassland species Trifolium repens and Agrostis capillaris. Only three phylotypes were exclusive to T. repens and six to A. capillaris.

Thus, conceptual models of plant and AM fungal communities need to consider the possibility that a small number of host-specific species occur alongside many non-specific species (Fig. 1c). This has important consequences, because it implies some degree of functional redundancy or functional resilience in AM communities. On the other hand, loss of a particular plant species may have disproportionate impacts on the diversity of the AM fungal community. A complicating factor highlighted by Sanders (2002) is the need to relate presence with abundance, something that is currently impossible to do with any certainty for mycorrhizal fungi. For example, in the hypothetical situation in Fig. 1a, is it important that AM fungal species A colonizes only 20% of the root length of plant X compared with 90% in plant Y? In other words, the true diversity, as opposed to species-richness, of AM fungi within plant roots needs to be assessed. In reality, this argument needs to be taken one step even further, so that some ‘real’ measure of function is quantified. It does not necessarily follow that percentage root length colonized equates with greater functionality.

One clear challenge that lies ahead is the need to relate the diversity of AM fungi in the bulk soil to that in the plant roots. After all, it is the external mycelium that exploits the patchy soil environment. In the example in Fig. 1c, loss of AM species F will have greater impacts on plant species X compared with Y. The fact that mycorrhizal fungi can form shared mycelial networks adds a further complicating factor. If AM fungus species F is shared between plant X and Y, then loss of plant X may have knock-on effects for plant Y depending on the functions provided to the plant from AM fungus F (Fig. 1c). A striking recent example of this type of effect for EcM fungi has been described by Haskins and Gehring (2004). They showed that trenching to prevent the intermingling of pinyon pine and juniper roots increased EcM abundance on the pinyon pine and altered the pinyon EcM community composition.

The study by Vandenkoornhuys et al. (2002) also showed that AM fungal community composition differed according to the time of sampling, indicating that the community structure of AM fungi on the two species investigated was very dynamic. This finding raises fascinating questions about the functional significance of these fungi. Are the different fungal communities at different time points reflecting different carbon or nutrient use strategies? The utilization of host-derived carbon is a fundamental component of all mycorrhizal symbioses that has the potential to be investigated in great detail by sophisticated molecular-based techniques such as stable isotope probing (SIP; Radajewski et al., 2000). SIP utilizes conventional $^{13}$CO$_2$ pulse-labelling in order to enrich the DNA of micro-organisms that receive recent photosynthate.
from plants. The labelled microbial DNA can be separated from non-labelled DNA by ultra-centrifugation and the diversity and identity of the two fractions determined using conventional PCR-based approaches, such as T-RFLP or DGGE. To date, in situ SIP has only been applied to bacterial communities in grassland systems (Griffiths et al., 2004) and bioreactors (Manefield et al., 2002). There are enormous technical hurdles to be overcome for applying SIP to AM fungal communities, but the challenge is there and the first experiments demonstrating its application to mycorrhizas are eagerly awaited. The functional significance of AM community composition for phosphorus acquisition could also be tackled by isotope probing methods. For example, given that a major component of DNA is phosphorus, the greater mass of the radionuclide $^{31}$P in relation to the stable nuclide $^{32}$P could be exploited in similar ways to $^{13}$C/$^{12}$C separation using ultra-centrifugation. While this would not have the flexibility of $^{13}$C in terms of individual species/phylotypes in intact natural communities.

Diversity of plant communities

Grassland

The majority of studies that have attempted to understand the importance of plant species diversity to productivity have focused on grasslands of one sort or another. Temperate grasslands can range from being floristically very rich (in temperate terms; i.e. $>$20 species m$^{-2}$) to floristically very poor. Differences in species richness can be seen in very close proximity in some situations, for example, the limestone grassland dales of the Peak National Park in Derbyshire, England. Despite the ubiquity and importance of AM fungi for elemental cycling, few studies have attempted to determine how plant diversity can impact on AM fungi. This is partly because the community composition, biomass, and abundance of AM fungi is difficult to measure. The lack of a reliable index of AM fungal biomass contrasts with the array of methods available to determine the biomass of soil microbial communities in general. In the last decade, determination of specific phospholipid fatty acids (typically 16:1o5) has been seen as the most useful measure, because it has high specificity to AM fungi and is found in abundance in AM mycelium (Olsson et al., 1995). Although criticisms can be levelled at the general utility and specificity of the technique, particularly in organic soils, it can nevertheless provide a useful comparative measure of AM fungal biomass. Hedlund et al. (2003) applied this technique to a pan-European experiment in which three levels of plant richness were imposed on grassland plots for 3 years. At one of their sites they found a strong positive linear relationship between plant species richness and AM biomass ($R^2$=0.6; $P<0.01$) and a negative relationship between plant biomass and AM biomass ($R^2$=0.8; $P<0.01$). It seems remarkable, given the strength and clarity of the relationship, that a similar situation was not apparent at the other four sites. Complementary measures of colonization of plant roots by AM fungi may have indicated whether the observed differences in the amounts of the AM signature fatty acid extracted from soil were reflected in planta. Nevertheless, this apparent positive relationship is supported by other work in which the production of AM fungal spores was correlated with plant species richness. For example, Chen et al. (2004) constructed a range of plots comprising 0, 1, 2, 4, 8, and 12 species of weeds from a range of plant families including Leguminosae, Ranunculaceae, and Gramineae. The number of AM fungal spores extracted increased progressively with each increment of plant diversity. A similar situation was also observed at the famous Cedar Creek biodiversity experiment utilizing a similar plant diversity gradient (1, 2, 8, and 16 species; Burrows and Pfleger, 2002). Here, AM fungi in the 16-species plots produced from 30–150% more spores and from 40–70% greater spore volumes than AM fungi in 1-species plots, although it should be noted that soil nitrate concentration was also a significant predictor of AM sporulation in parallel with plant species richness.

Evidence from signature fatty acids and spore abundance certainly suggests that plant species richness does impact on AM fungi. To be really useful, however, the identity of the AM fungi concerned is essential. Do some species of AM fungi respond more readily to plant richness than others? Does plant richness actually stimulate AM fungal species richness (or vice versa), or is the relationship with AM fungal sporulation? This latter question is of considerable importance given the wealth of evidence that has accumulated showing how diversity of AM fungi can feedback positively and stimulate host plant richness, productivity, and nutrient concentrations (van der Heijden et al., 1998a, b). The ability to identify individual species of AM fungi accurately is, therefore, of utmost importance.

Traditionally, this has been achieved by the painstaking analysis of AM fungal sporule communities. Recently, Landis et al. (2004) applied this approach to try and unravel the linkages between plant diversity and AM fungal diversity. The authors made use of a natural gradient in plant species richness through an oak woodland and found strong positive correlations between AM fungal, and plant, species richness. However, as with all correlative studies, the authors correctly point out that it is impossible to determine cause and effect when using such natural gradients. Of course, the reliability of using the morphological features of spores for the determination of AM species identity is highly contentious (Sanders, 2004). The very fact that only about 150 AM fungal species have thus far been identified and yet they colonize tens of thousands of plant species and show host specificity, suggests that spor morphology is a poor discriminator. Revisiting
the experimental gradient used by Landis et al. (2004) with the appropriate molecular tools would be an obvious next step. Nevertheless, despite its limitations, the study by Landis et al. (2004) appears to contradict the views of Allen et al. (1995) who state that ‘the diversity of mycorrhizal fungi does not follow patterns of plant diversity’.

Important insights into AM species identification have recently been attained using molecular methods targeting the small sub-unit (SSU) of rRNA. The development of primers to identify AM fungi colonizing plant roots (Helgason et al., 1998; Vandenkoornhuyse et al., 2002) has made it possible to study the diversity of AM fungi with a greater degree of certainty and precision than was hitherto possible. These studies (Helgason et al., 1998, 1999; Daniell et al., 2001; Husband et al., 2002a) are revealing a greater diversity of AM fungi in roots than could be recognized by morphometric studies. They have also resulted in the discovery of previously unrecorded species (Helgason et al., 1998, 1999; Daniell et al., 2001; Husband et al., 2002b; Vandenkoornhuyse et al., 2002, 2003) and evidence of hitherto hidden host specificity (Bidartondo et al., 2002; Vandenkoornhuyse et al., 2002, 2003). Furthermore, such studies have revealed that both the composition of AM fungal communities in roots (Helgason et al., 2002; Vandenkoornhuyse et al., 2002, 2003; Johnson et al., 2004) and the production of their spores (Bever, 2002) can be influenced by host plants. One important factor is that, as with any PCR-based assay, the results are prone to a degree of bias. For example, it is virtually impossible at present to quantify the abundance of an AM fungal genotype based on T-RFLP, and so it is not possible to determine even the extent of root colonization. On its own, diversity has little value and so complementary information on abundance, biomass, and function should be obtained where possible.

Boreal forests

Approximately 6000 species of EcM fungi have been described, considerably more than AM fungi. This has led to the assumption that EcM fungi have greater host specificity than AM fungi (Allen et al., 1995). Most EcM fungi are basidiomycetes and many produce distinctive sporocarps although perhaps 5% are ascomycetes, the identification of which is more challenging. The influence of plant species richness on ectomycorrhizal fungi is rather less clear than for AM fungi, primarily because the required experiments have yet to be done (which is not surprising given the obvious difficulties involved), but also because the diversity of EcM fungi contrasts markedly with their host plant communities. It seems unusual, therefore, that a general consensus seems to have appeared that suggests EcM richness is not in any way related to plant richness (Allen et al., 1995). These views may have arisen by taking a viewpoint at the scale of the biome, at which it is difficult to see detailed patterns emerging against other variables that undoubtedly affect mycorrhizal community structure. Certainly, there is information detailing how both edaphic and climatic variables affect EcM diversity. Despite this consensus, there is, nevertheless, some evidence to suggest that plant species richness may be important. For example, simply manipulating a plant community to contain either a monoculture or a two-species mixture can have consequences for EcM community structure. Jones et al. (1997) found that EcM communities had greater species evenness (relative abundance) when paper birch and Douglas fir were grown in combination compared to when they were grown in monoculture, although this finding did not hold for EcM richness or diversity.

Recent work at a larger scale (up to 100 m$^{-2}$) in boreal forests suggests that positive relationships between plant and EcM diversity can exist (Kernaghan et al., 2003; Ferris et al., 2000). Kernaghan et al. (2003) morphotyped fine root tips and demonstrated that around 38% of the variation in EcM diversity was attributable to overstorey plant diversity. The fact that only overstorey plant diversity was a significant factor from the range of abiotic and biotic factors tested is not only interesting but also very surprising for two reasons. Firstly, the influence of the species richness and biomass of understorey vegetation seems not to have been significant for the richness of the community as a whole. One might expect competition for resources from fungi associated with non-ectomycorrhizal plant species to be an important driver of ectomycorrhizal diversity. Secondly, no relationship was seen between the diversity of plant species determined from root samples. The implication of this is that the diversity of ectomycorrhizal communities are being driven, at least to some extent, by indirect process such as litter chemistry, rather than direct processes such as carbon allocation. Indeed, it must be seen as a priority to understand the mechanisms through which plant diversity regulates ectomycorrhizal fungal diversity (or vice versa). The hypothesis postulated above can readily be tested through the application of methods that link the function and diversity of micro-organisms, notably SIP. The application to forest systems where the soil microbial biomass is dominated by EcM fungi would seem an obvious and relatively straightforward extension of the technology, especially given the likelihood that more carbon is allocated to EcM fungi than AM fungi. Given that the molecular tools are now becoming increasingly available for soil fungi (Anderson and Cairney, 2004), the plea made by Allen et al. (1995), to explore the interactions between plant and mycorrhizal diversity through rigorous experimental design, can only be reiterated.

Influence of host plant age

The structure and function of mycorrhizal communities in response to the age of host plants is of primary concern in forestry, particularly where it is intended for timber
production. There are undoubtedly many other instances where stand age is influenced naturally, primarily by fire, and an understanding of the factors that drive changes in EcM community composition and function is of great ecological interest. However, some of the best evidence of the importance of host plant age on mycorrhizal communities has arisen from studies of AM fungi in tropical forests, and so this discussion will include some of these findings.

The notion that the age of a stand of trees could influence the community structure of EcM was first postulated in the early 1980s (Mason et al., 1982, 1983). The concept generated considerable interest because Mason and colleagues suggested that some species of EcM fungi were found only when trees were in their pioneer phase, so-called early-stage fungi and others were specific to climax vegetation, so-called late-stage fungi. This was later refined by Danielson (1984) to include a third category known as multi-stage fungi. Numerous studies have utilized forests with a gradient of stand ages to test Mason’s hypothesis with varying degrees of agreement. As a general point, it should be noted that several of these studies are compromised by lack of true replication. This essential requirement for meaningful statistical analysis is not always easy to achieve in chronosequences. Many other factors are frequently correlated with stand age and careful experimental design, field observation, and statistical analyses are required to try and separate the various factors tested.

Critics of the EcM succession hypothesis have argued that the hypothesis is only likely to hold true for pioneer plant species because the original study utilized a stand of birch (Betula pendula) that had recently colonized agricultural soil. Molina et al. (1992) go on to suggest such a simple model is wholly inadequate to represent the situation in natural forest systems because of the vast array of abiotic and biotic factors that interact to influence EcM succession. This is borne-out by some studies that have shown Douglas fir to be routinely colonized by the genus-specific fungi Rhizopogon whether they are seedlings or mature trees (Borchers and Perry, 1990). Using molecular techniques, Jonsson et al. (1999) provided evidence that the EcM fungal communities on Scots pine root tips were similar to neighbouring trees, regardless of their age, suggesting that the external fungal mycelium may be important as a determinant of EcM community structure on seedlings. This mechanism, which has been suggested for AM fungi in grassland, is not surprising, given the extent of hyphal networks in forests. The ability of a seedling to tap into an established mycelial network supported by carbon from mature overstorey plants would seem to be of considerable advantage, particularly in the comparative gloom of the forest floor. This mechanism supports the view that early-stage fungi are only likely to dominate when spores are the principal source of inoculum.

Visser (1995) studied a chronosequence of Jack pine (Pinus banksiana) that had regenerated naturally after wildfire disturbance. Her data showed that the number of EcM morphotypes increased progressively in the first 65 years before increasing at a much-reduced rate until 122 years. The communities included early-stage species such as Coltricia perennis, multi-stage species such as Suillus brevipes, and late-stage species such as Cortinarius spp. The view of the EcM community did not show the predicted decline in species richness following canopy closure (Last et al., 1987). A similar trend was seen in stands of Pinus kesiya during the initial (2–17 year) growth phase (Rao et al., 1997). Here, species richness of EcM fungi was directly proportional to the age of the stand. All of the studies cited above, however, are subject either to the vagaries of the relationship between sporocarp presence and mycorrhiza presence or from the uncertainties of EcM morphotype identification. By contrast, Husband et al. (2002b) used molecular methods to study AM fungal communities in the roots of seedlings of two co-occurring species from tropical rain forest on Barro Colorado Island. AM types that were dominant in newly germinated seedlings were replaced by other types in those seedlings which survived to the following year, and seedlings of different ages sampled at the same time point supported significantly different AM communities. Lee and Alexander (1996) obtained similar data for the EcM fungi in tropical rain forest to demonstrate that EcM community on the roots of dipterocarp seedlings changed in the 7 months following germination. As well as fungi entering the mycorrhizal community, as time progressed some fungi were lost or declined in relative abundance, clear evidence of succession. As far as is known, there have yet to be any studies using DNA-based methods to investigate the successional processes in EcM communities, despite the recognition 10 years ago that this would be a useful line of inquiry (Egger, 1995). Some preliminary data are presented here on species richness (represented by the number of dominant bands on DGGE gels) of fungi colonizing EcM roots of Scots pine in five stands ranging from 13 to 116 years old (Fig. 2). Species richness in the surface organic (L, F, and H) horizons was least in the youngest stand (13 years) and was greatest in the 59 and 116 year-old-stands. By contrast, species richness in the mineral horizon (in this case a uniform sand horizon several metres deep) did not differ between stand ages. These data suggest a rather idiosyncratic response of root-associated basidio-mycete fungal communities to host plant age.

It is clear that tree age can have impacts on EcM fungal communities, but that these may be more or less apparent in particular forest types, notably young plantations versus old growth. However now that so much painstaking observational work has been achieved, perhaps a far more important question to ask is what is actually causing the EcM communities to change in plants of different age and what are the consequences of these changes for various aspects
of functioning? There seem to be two processes occurring; changes in mycorrhizal communities on individuals with time as that individual samples the inoculum available, and also changes at the stand level associated with a range of edaphic factors. Several authors have alluded to the latter point. Visser (1995) highlighted that differences in host carbon supply could have driven the changes seen in the EcM fungal communities. This hypothesis arises from the notion that carbohydrate supply can affect EcM colonization (Björkman, 1949). The isotope tracer techniques required to determine if EcM community composition is related to host carbon supply are readily available and have been highlighted already.

Conclusions
The emerging view seems to be that host plant diversity, species composition, and age do have a role to play in regulating mycorrhizal communities. This view contrasts with previous models and assumptions and has, in the main, been possible only by the rapid development of molecular biology techniques and their application to mycorrhizal fungi. However, there is still some way to go in providing a firm experimental footing, rather than relying on correlational approaches, in order to address some of these relationships, particularly with respect to plant species diversity. The really fascinating questions about what these differences mean for various aspects of ecosystem functioning, most notably transfers of carbon, have the potential to be addressed in forthcoming months and years as a result of the integration of isotope tracers and DNA-based methods of assessing species and genotypic identity. Another future key line of inquiry, particularly for EcM fungi, relates to the importance of host plant genotypic variation, the general molecular typing methods discussed for fungi being applicable to plant communities. Variations in the formation of EcM fungi on host plant progenies have been known for some time (Marx and Bryan, 1971). Some studies also suggest that plant secondary metabolites can affect EcM colonization, and that production of these compounds is at least in part under plant genetic control.

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