

REVIEW ARTICLE

The molecular revolution in ectomycorrhizal ecology: peeking into the black-box

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Abstract

Molecular tools have now been applied for the past 5 years to dissect ectomycorrhizal (EM) community structure, and they have propelled a resurgence in interest in the field. Results from these studies have revealed that: (i) EM communities are impressively diverse and are patchily distributed at a fine scale below ground; (ii) there is a poor correspondence between fungi that appear dominant as sporocarps vs. those that appear dominant on roots; (iii) members of Russulaceae, Thelephoraceae, and/or non-thelephoroid resupinates are among the most abundant EM taxa in ecosystems sampled to date; (iv) dissimilar plants are associated with many of the same EM species when their roots intermingle — this occurs on a small enough spatial scale that fungal individuals are likely to be shared by dissimilar plants; and (v) mycoheterotrophic plants have highly specific fungal associations. Although, these results have been impressive, they have been tempered by sampling difficulties and limited by the taxonomic resolution of restriction fragment length polymorphism methods. Minor modifications of the sampling schemes, and more use of direct sequencing, has the potential to solve these problems. Use of additional methods, such as *in situ* hybridization to ribosomal RNA or hybridization coupled to microarrays, are necessary to open up the analysis of the mycelial component of community structure.

Keywords: community ecology, ectomycorrhizae, fungi, PCR

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Introduction

Ectomycorrhizal (EM) symbioses are important on a global scale because the dominant trees in most of the world's temperate and boreal forests and in large areas of tropical and subtropical forests are ectomycorrhizal (Allen 1991; Read 1991). For host trees these fungi serve as the primary nutrient gathering interface, scavenging nitrogen, phosphorus, and trace elements from both inorganic and organic pools in the soil; this is done in exchange for fixed carbon from the tree. For many trees, such as those in the Pinaceae, Fagaceae, Betulaceae, and Dipterocarpaceae, it is clear that normal growth and survival is dependent on colonization by EM fungi (Smith & Read 1997). EM fungi are also important because they include high value

edible fungi such as truffles (*Tuber* spp.), matsutake (*Tricholoma* spp.), chanterelles (*Cantharellus* spp.), and *Boletus edulis*.

Fungi that form ectomycorrhizae are not a monophyletic group; traditional classification and recent molecular systematic studies demonstrate that this trait has been independently derived many times and perhaps convergently lost as well (Gargas *et al.* 1995; Hibbett *et al.* 1997, 2000; Bruns *et al.* 1998). Over 5000 species of EM fungi have been described (Molina *et al.* 1992). The symbionts span all of the phyla of true fungi (Zygomycota, Ascomycota, and Basidiomycota), and occur in at least 15 families within the Basidiomycota and Ascomycota. However, from molecular clock estimates and the fossil records of their plant hosts, it appears that most of the diversity arose relatively recently, perhaps initially in the Cretaceous with later extensive radiation around 25–60 Ma (LePage *et al.* 1997; Bruns *et al.* 1998). This pattern is in contrast to that of the arbuscular mycorrhizal fungi that fall within a single

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monophyletic group, the Glomales, and appear to have arisen with the land plants 400–500 Ma (Simon *et al.* 1993; Pirozynski & Dalpé 1989).

EM fungus communities are species rich, and many basic questions about these communities remain unanswered. These include: (i) what is the structure of these communities in terms of numbers and abundance of species; (ii) how is structure maintained and affected by factors such as host diversity, soil types, organic inputs, disturbance, and succession; (iii) does structure have significant effects on ecosystem functions (e.g. nutrient cycling and retention), or on plant community structure; and (iv) what is the natural history or autecology of the dominant species. Tackling these questions in a quantitative manner presents several challenges unique to mycorrhizal fungi.

The first problem is that EM species composition is not easily manipulated in natural or laboratory settings. This is because a large percentage of EM fungi either grow poorly or do not grow at all in culture, and because addition of spores or mycelial inoculum rarely results in colonization under nonsterile conditions. Seedlings that are inoculated under sterile or semi-sterile conditions can be out-planted, but replacement by indigenous fungi is a common result (e.g. Bledsoe *et al.* 1982; Danielson & Visser 1989), except perhaps when the host is planted outside its natural range (Selosse *et al.* 1998a). Much has been learned about behaviour and physiological ecology of individual species, particularly of *Suillus*, *Rhizopogon*, *Paxillus*, *Laccaria*, *Pisolithus*, and *Cenococcum*, in laboratory microcosms, but the overwhelming majority of EM fungi have not been amenable to such manipulations.

Second, vegetative structures of these fungi (i.e. mycorrhizae and mycelium in the soil) occur largely below ground and are difficult to track and identify. Attaching species names to below ground structures has been problematic because the taxonomy of these organisms is based on their sexual states (e.g. mushrooms, truffles, etc.), and the vegetative states are much smaller and more difficult to distinguish. For these reasons most of the earlier literature on EM communities was based on collecting and quantifying fruiting structures, or, if based on mycorrhizae, left large numbers of species unidentified or lumped into nebulous types (e.g. 'brown type'). Thus, the below ground dynamics and roles of the fungi were largely relegated to a single functional guild, if not the so-called 'black-box' (Allen 1991).

A marriage of molecules and morphology to the rescue

The most important methodological advance in the study of EM communities has been the application of the polymerase chain reaction (PCR) for identification (Mullis

& Faloona 1987; Gardes *et al.* 1991; Henrion *et al.* 1992; Lanfranco *et al.* 1998). The primary amplification targets have been ribosomal genes and spacers; these regions combine the advantages of high copy number, highly conserved sequence tracks that can serve as sites for primer design, and variable regions between the priming sites. Both universal, and fungal or plant specific primers, have been designed (White *et al.* 1990; Cullings 1992; Gardes & Bruns 1993; Egger 1995; Vrålstad *et al.* 2000), the former have been designed for various taxonomic levels.

The use of PCR and ribosomal genes is a familiar theme in microbial ecology. The field of EM community ecology differs from other areas of microbial ecology in that these methods are used almost strictly for identification, rather than for both identification and quantification, as is common in prokaryotic communities. This is a tremendous advantage, because it avoids most of the sticky issues and additional effort involved with PCR quantification. The reason molecular quantification is generally unnecessary is that ectomycorrhizae are small macroscopic packages (e.g. colonized root tips) that can be counted or weighed. In contrast to the situation in arbuscular mycorrhizae, EM colonization is usually obvious from the external appearance of the root tips (Fig. 1). A few taxa of fungi can be more cryptic in their colonization, such as the so-called dark septate fungi (*Phialophora*, *Chloridium* and *Pialocephala*) or other ascomycetous fungi such as *Wilcoxina* spp., but even these can be recognized with a little effort.

In addition to the advantage of independent quantification, morphological differences among ectomycorrhizae can be used to sort species. Some differences such as shape and colour are obvious even to the untrained eye and can be sorted into discrete groups rapidly but are insufficient to differentiate among closely related species (Fig. 1). Other morphological characters, such as those that involve hyphal types and arrangements, require significant experience to recognize and more careful treatment of the specimens, but can yield identifications directly without molecular analyses in systems that are well characterized (Agerer 1987–96; Ingleby *et al.* 1990; Goodman *et al.* 1996–98). There are trade-offs involved here, however. The more thorough the morphological characterization, the fewer the samples that can be sorted in a given time; therefore, the large numbers of samples necessary for community-level studies often makes a strictly morphological approach impractical for large scale questions. In addition, the DNA in samples degrades rapidly, so the longer the pre-extraction processing, the higher the risk that they will not amplify. Conversely, the cruder the sorting the more likely it is that a morphotype will contain multiple species, and thus defeat the purpose of the sorting. This raises the question of how much to morphotype, but we defer our discussion of this question until other sampling issues are addressed.

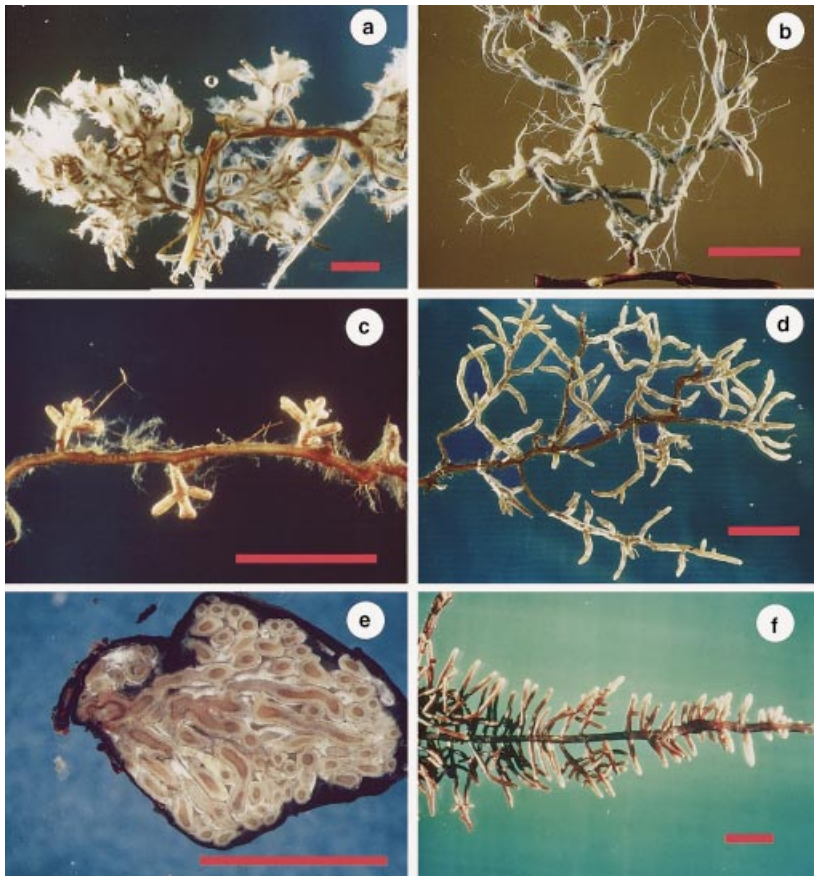


Fig. 1 Variation in EM root tip morphology. (a) *Hebeloma crustuliniforme* and Douglas-fir by R. Molina. (b) *Cortinarius* sp. and Douglas-fir by B. Zak. (c) *Melanogaster intermedius* and *Arbutus menziesii* by R. Molina. (d) *Amanita muscaria* and *Picea sitchensis* by R. Molina. (e) *Rhizopogon vinicolor* and Douglas-fir, cross section of a tubercle by D. Luoma. (f) *Lactarius rubrilacteus* and Douglas-fir by B. Zak. Scale bars equal 5 mm.

RFLP matching analysis is king, but the monarchy is insufficient

Fungus identification with ITS

Most of the molecular ecology on EM fungi has involved restriction analyses of the internal transcribed spacer (ITS) region. This nuclear region, which is well known to the fields of molecular ecology and fungal systematics, lies between the small subunit (SSU) and the large subunit (LSU) ribosomal RNA (rRNA) genes and contains two noncoding spacer regions separated by the 5.8S rRNA gene. In fungi it is typically about 650–900 bp in size, including the 5.8S gene. It is usually amplified by either the universal primer pair (ITS1 and ITS4; White *et al.* 1990; Gardes *et al.* 1991), or a fungal specific, or basidiomycete specific pair (ITS1f and ITS4 or ITS1f and ITS4b, respectively; Gardes & Bruns 1993). There has been a mistaken impression in the literature that the ITS1 and ITS4 primers are fungal specific rather than universal; this idea has been reinforced by the observation that they do not amplify the ITS of the Pinaceae very well. Nevertheless, ITS1 and ITS4 were designed with plant sequences in mind and in some hosts (e.g. members of the Monotropoideae) these sequences are co-amplified with those of the target

fungi. Many other taxon-specific ITS primers have been developed. However, primers designed to show specificity for a particular group should be used with caution as a lack of amplification with the primer pair can be misinterpreted. For instance, primers intended for a specific group (e.g. Basidiomycete or Ascomycete) may not amplify DNA from every species within the intended group.

Restriction fragment length polymorphism (RFLP) analysis of the ITS region has been popular because it separates many species quickly and relatively cheaply with minimal technical requirements. Typically two or three restriction enzyme digests are enough to distinguish most species (Nylund *et al.* 1995; Gardes & Bruns 1996a; Dahlberg *et al.* 1997; Kårén *et al.* 1997; Pritsch *et al.* 1997, 2000; Gehring *et al.* 1998; Jonsson *et al.* 1999a,b; Mahmood *et al.* 1999; Eberhardt *et al.* 2000; Methvyn *et al.* 2000). The reason so few digests are needed is that sequence differences between taxa are usually the result of indels, insertion or deletions of nucleotides that cause length variations. Thus, it is not necessary for enzyme recognition sites to change in order to create a unique RFLP pattern; enzymes that cleave the region into small fragments will reveal small size differences. For these reasons side-by-side comparisons of patterns is an extremely simple and sensitive method for determining identity or near identity at the species level,

and should be employed to check putative matches observed from different gels.

The main problem with the ITS–RFLP approach is that when it is used alone, the number of unidentified types typically remains quite high. This is true even at locations from which extensive ITS–RFLP databases are available from sporocarp samples (Kårén & Nylund 1997). This is partially caused by the fact that RFLP databases tend to be composed primarily of species that make large or obvious sporocarps, and as discussed below, these species are often not the most common ones encountered on colonized roots. However, even when a species is represented in an RFLP database, matching an unknown to it presents additional problems. Exact matches based on database searches are uncommon, because size estimates for fragments vary and intraspecific variation exists across large geographical scales (Kårén *et al.* 1997; Farmer & Sylvia 1998; Snelson *et al.* 1998a; Methvyn *et al.* 2000). Furthermore, the meaning of near matches cannot be satisfactorily quantified, because the information content of an RFLP pattern is very limited. For example, when ITS–RFLP patterns are used to build phylogenetic trees, different species within a genus are frequently dispersed across the tree, and branch support metrics, such as bootstrap values, are either not employed or are below levels that one would desire for confident placements. Switching to acrylamide gels, automated sequencers, and GENESCAN technology solves many of the accuracy and comparison problems (Peter *et al.* 2001), but increases the cost/sample substantially. An additional problem with RFLP databases is that they are not standardized in terms of the primers used, the enzymes used, or the way the information is stored and retrieved. They are also problematic in that there is no standard location to find such data, although some web sites are under construction. We suggest that RFLP databases work best when they are focused on specific ecosystems, which will keep the fungal diversity low enough to minimize intraspecific variation in ITS sequences, thereby increasing the chances of matches and reducing the potential for over estimating diversity. A regional focus will also keep the fungal diversity low enough to allow one to master the morphological variation of EM. However, the central problem of unknown fungal RFLP patterns is not easily overcome by RFLP approaches alone and direct sequence analysis is required for the identification of many unidentified RFLP types (see below).

Plant identification with nuclear LSU

Some researchers may wish to identify the plant species from mycorrhizal root extracts. 28KJ is a plant specific primer that, in combination with a universal primer 28C, will amplify a small portion of the 28S rRNA gene of plants even when fungal DNA is present (e.g. an extract obtained from an EM root) (Cullings 1992). The PCR product is then

subjected to RFLP analysis, and compared to patterns from unknown samples to those generated from identified leaf extracts (Horton & Bruns 1998; Horton *et al.* 1999; Stendell *et al.* 1999; Cullings *et al.* 2000). We have had good success separating conifers at the genus level (*Pinus*, *Pseudotsuga*, *Tsuga*). Angiosperms pose a more difficult situation in that, in our experience, some are difficult to amplify with this primer pair (*Quercus*/Fagaceae, *Adenostoma*/Rosaceae, *Pickeringia*/Fabaceae). Developing plant specific primers for the ITS region or another target that allows specific amplification of a wider variety of plant species and allows separation of species is desirable.

The addition of sequence analysis to get beyond 'unknowns'

An important step to take for at least the dominant RFLP types identified in a given study is direct sequence analysis, but relatively few researchers have done so. The additional effort or expense required to generate sequence data has lessened to the point that obtaining sequence data is as routine as obtaining RFLP data. While there are an increasing number of loci or genetic regions available for sequence analysis, currently several options are particularly useful.

For most microbial ecologists the molecule of choice would typically be the SSU rRNA gene. For EM fungi this gene has not been a target, partially because many of the critical taxa have not been sequenced and more importantly because the resolution is low relative to the amount of sequencing effort required (Bruns *et al.* 1992). However, 18S sequences have now been determined for some of the important ectomycorrhizal groups such as the Pezizales, Gomphaceae, and Cantharellaceae (Hibbett *et al.* 1997; Landvik *et al.* 1997; O'Donnell *et al.* 1997; Norman & Egger 1999; Pine *et al.* 1999), which should increase the value of this gene for identification of EM fungi. A very quick way to search for SSU sequence matches is via the Ribosomal Database project, which provides web-based search and treeing algorithms (<http://www.cme.msu.edu/RDP/html/index.html>; Olsen *et al.* 1993).

The 5' end of LSU rRNA gene is another possible target for sequence identification. It is a more variable, and therefore a more informative, target than the SSU, but so far it appears to have been used only twice for identification of unknown EM ascomycetes. Here it allowed placement at the generic level or above (Baar *et al.* 1999; Taylor & Bruns 1999). It has now become much more useful as the large number of new sequences from the Agaricales and Boletales have recently been added (Moncalvo *et al.* 2000).

A small piece of the mitochondrial large subunit rRNA gene (mtLSU) has been used extensively by our laboratory groups for identification of EM basidiomycetes (Bruns *et al.* 1998; Table 1). This region provides unambiguous

Table 1 Proportion of ectomycorrhizal root tip biomass identified

	Species match	Family ID	Unknown RFLP	Unknown morphology
Horton & Bruns 1998	0.762	0.174	0.064	0
Horton <i>et al.</i> 1999	0.225	0.600	0.122	0.052
Stendell <i>et al.</i> 1999	0.252	0.555	0.186	0.007
Bidartondo <i>et al.</i> 2000*	0.164	0.659	0.116	0.061
Taylor & Bruns 1999†	0.840	0.154	0.015	0
Hemlock/Douglas-fir‡	0.360	0.383	0.004	0.217
Mean ± 1 S.D. of biomass	0.442 ± 0.27	0.415 ± 0.20	0.084 ± 0.06	0.055 ± 0.08

*Without ECM directly associated with *Sarcodes* root ball (Bidartondo, personal communication).

†Proportion of ECM in samples from soils samples taken in the forest.

‡Horton, unpublished data.

placements of unknown sequences into family sized groups such as Russulaceae, Thelephoraceae, Amanitaceae, *Suillus* and allies, *Boletus* and allies, *Gomphus* and allies, Hygrophoraceae, and Cantharellaceae. However, it provides little or no resolution within these groups. Initially, placements within Cortinariaceae and Tricholomataceae, two important EM fungal families, were difficult to interpret, but we have recently added a number of taxa from *Tricholoma*, *Inocybe* and *Cortinarius* and are confident that unknown sequences from these genera are now placed within separate clades. One important problem with this database is that the mitochondrial genome in fungi can contain introns, which make it difficult to amplify the region. The introns can be present in some, but not all collections of a species and although usually rare, they are common in a few genera (Bruns *et al.* 1998). Several primers have been developed that allow amplification around the introns, but these primers have not worked across all genera (e.g. *Albatrellus*).

The 5.8S nuclear rRNA gene, which is included within the ITS region, has also been used for very broad level identification (Cullings & Vogler 1998). This database was designed to help researchers identify the source of their unknown ITS-PCR amplifications as fungal, plant, or animal DNA. Such a tool is useful for avoiding problems of analysing nontarget DNA from extracts (Camacho *et al.* 1997; Redecker *et al.* 1999). It can also be applied to resolve the phylum of fungi. However, many fungal sequences are not clearly placed into phyla, and those that are do not receive a high level of bootstrap support. Thus, interpretation of such analyses needs to be cautious.

The spacers within the ITS region are probably the ideal sequences to use for identification because they have the resolving power to place unknowns to the species level or at least within a species group. A fast way to take advantage of the ITS data currently deposited is to search GenBank or EMBL using only the spacer sequences from unknown samples; sequence variation within the spacers is so high that only very closely related taxa are retrieved. This approach was recently used to identify the EM

associates of *Pisonia grandis* and *Corallorhiza* as members of the Thelephora/Tomentella clade (Chambers *et al.* 1998; McKendrick *et al.* 2000). Because there are relatively few EM species that are the focus of detailed research, this approach rarely allows identification at the species level, only genus or species group, and there are many gaps in the taxonomic coverage. Therefore, unknown ITS sequences can still be relatively uninformative if they belong to under-represented groups. Nevertheless, if all researchers deposited ITS sequences from at least the major species found in their studies, this would greatly increase chances that these species would eventually be identified, and it would also increase the comparability of species lists across studies.

Species level identifications with ITS sequences require greater prior taxon sampling and more thorough phylogenetic analysis. Thus, this approach is limited to genera in which an ITS-based molecular systematic study has been conducted. Currently this is true for only a handful of ectomycorrhizal genera (e.g. *Wilcoxina*, *Tricharina*, *Dermocybe*, *Suillus*, *Peziza*, *Hebeloma*, *Plicaria*) (Egger 1995; Liu *et al.* 1995, 1997; Kretzer *et al.* 1996; Norman & Egger 1996, 1999; Aanen *et al.* 2000), but ITS sequence studies for other genera continue to be published. In order for these phylogenetic studies to be useful for identifications, the sequence alignments must be readily available, so that unknown sequences can be easily added without having to realign all previous sequences. Many alignments have been made available on individual web sites, but deposition on Treebase (<http://www.herbaria.harvard.edu/treebase/>), which provides a central depository for all phylogenetic studies, is becoming more common and makes it easier to find and use such data.

The current picture derived from such studies

Diversity and distribution

EM fungal communities are impressively diverse, even in stands dominated by a single plant species (Danielson

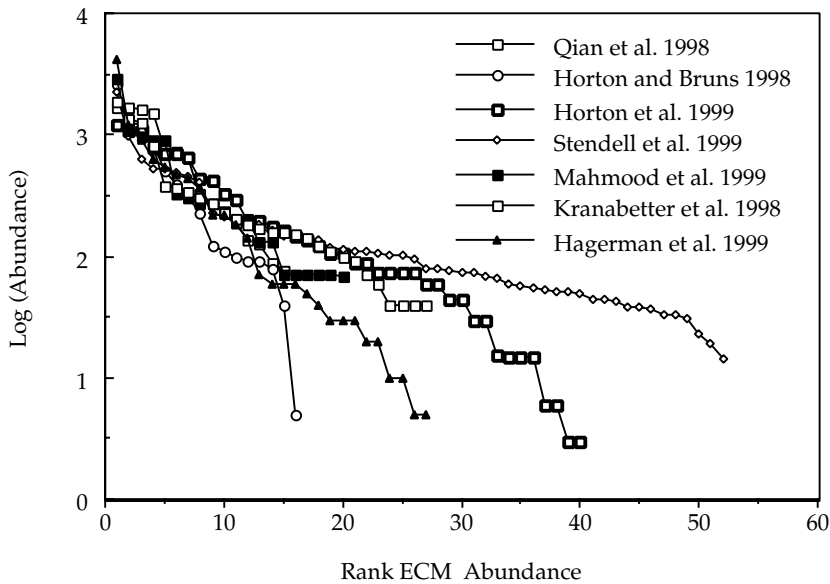


Fig. 2 Log-normal distribution of species abundance from data obtained from published sources. In all cases there is an inverse relationship between rarity and abundance for EM fungi below ground.

Table 2 Comparison of investigations of below ground EM community structure

Stand type	Age (years)	Area represented	Area sampled for sporocarps	No. of soil samples (diameter)	Actual surface area cored	No. of species from ECM data
Scots pine (birch) ¹	1–241	8 stands at 1–6 ha	200 m ² /stand	15 (2.8 cm)/stand	92 cm ² /stand	18–24/stand (135 total)
Norway spruce ²	30	2025 m ²	not given	120 (5 cm)	2352 cm ²	21
Bishop pine ³	35	0.1 ha	19 m ²	31 (10 cm)	2418 cm ²	> 20
Bishop pine/Douglas fir ⁴	35	125 m ²	not surveyed	25 (10 cm)	1963 cm ²	16
<i>Arctostaphylos</i> /Douglas fir ⁵	> 50	625 m ²	not given	12 (10 cm)	780 cm ²	> 40
Norway spruce ⁶	100	500 m ²	838 m ²	10 (1.5 × 1.5 cm)	23 cm ²	25
Ponderosa pine ⁷	100	2500 m ²	not surveyed	24 (5 cm)	942 cm ²	> 50
Scots pine ⁸	200–400	10 000 m ²	not surveyed	10 (2.8 cm)	61.5 cm ²	24
Pinyon pine ⁹	not given	3000 m ²	not given	50	not applicable	51
Bishop pine ¹⁰	34–38	625 m ²	not surveyed	15 (10 cm)	1178 cm ²	20
Bishop pine ¹¹	1	625 m ²	not surveyed	29 seedlings	not applicable	7
Douglas fir/western hemlock ¹²	90	21 000 m ²	21 000 m ²	198 (5.5 cm)	4704 cm ²	200*
Norway spruce/Scots pine ¹³	70–90	not given	1300 m ²	80 (2.8 cm)	492 cm ²	51
Red fir ¹⁴	350–400	6400 m ²	not surveyed	40 (4.6 cm)	664 cm ²	80

¹Jonsson *et al.* 1999b; ²Kårén & Nylund 1997; ³Gardes & Bruns 1996a; ⁴Horton & Bruns 1998; ⁵Horton *et al.* 1999; ⁶Dahlberg *et al.* 1997;

⁷Stendell *et al.* 1999; ⁸Jonsson *et al.* 1999a; ⁹Gehring *et al.* 1998; ¹⁰Taylor & Bruns 1999; ¹¹Baar *et al.* 1999; ¹²Luoma *et al.* 1997; ¹³Jonsson *et al.* 2000; ¹⁴Bidartondo *et al.* 2000.

*morphotypes.

1984; Visser 1995; Dahlberg *et al.* 1997; Pritsch *et al.* 1997; Gehring *et al.* 1998; Goodman & Trofymow 1998a,b; Kranabetter & Wylie 1998; Hagerman *et al.* 1999; Horton *et al.* 1999; Jonsson *et al.* 1999a,b; Stendell *et al.* 1999; Byrd *et al.* 2000). The distribution pattern of the fungi as sampled below ground leads to an inverse relationship between abundance and rarity (Fig. 2; Danielson 1984; Taylor & Alexander 1989; Visser 1995; Gardes & Bruns 1996a; Dahlberg *et al.* 1997; Kårén & Nylund 1997; Jonsson *et al.* 1999a). This distribution pattern impacts the view of species richness because of the large number of rare types.

Most studies have presented data on species richness based on 30 or fewer soil samples, often covering less than 1 ha. With these sampling efforts, 50 or fewer species of fungi were observed below ground (Table 2). In forests dominated by Douglas fir in southern Oregon (western North America), over 200 morphologically distinct EM were recorded in 198 soil samples taken over an area of about 2.1 ha (Luoma *et al.* 1997). The occurrence of the fungi at such a fine scale of patchiness below ground makes sampling a challenge. Indeed, the sampling of EM roots is typically inadequate to get a true picture of species

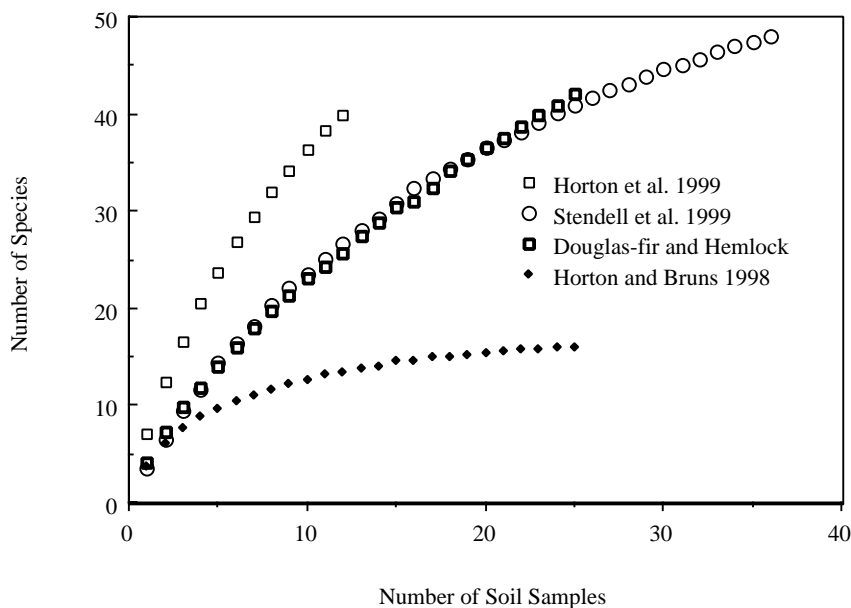


Fig. 3 Species-area curves from four studies, with the number of soil samples taken used as a substitute for the area sampled (McCune & Mefford 1999). The curve reaches the asymptote only in the Horton & Bruns (1998) study. Second-order jackknife estimates from these data support the conclusion that the observed number of taxa (o) was similar to the estimated number of taxa (e) only in Horton & Bruns (1998): Horton & Bruns (1998) $o = 16$, $e = 16.2$; Stendell *et al.* (1999) $o = 48$, $e = 71.6$; Douglas-fir and Hemlock $o = 42$, $e = 87.5$; Horton *et al.* (1999) $o = 40$, $e = 69.2$ (jackknife estimate = $S + r^1(2n - 3)/n - r^2(n - 2)$ squared/ $n(n - 1)$) where s = the observed number of species, r^1 = the number of species occurring in exactly one sampling unit, r^2 = the number of species occurring in exactly two sample units, and n = the number of sample units (Burnham & Overton 1979; Palmer 1991). This suggests that greater sampling effort was required in the other studies to better represent the diversity of EM fungi. Similar levels of diversity confound sampling efforts of plant species in tropical rain forests of Borneo (Mueller-Dombois & Ellenberg 1974).

richness (Fig. 3). If the number of soil samples has such a dramatic effect on observations of diversity, then identifying changes in richness in communities with high species richness remains somewhat ambiguous (Jonsson *et al.* 1999a,b, c; Stendell *et al.* 1999).

The distribution of mycorrhizae of many species is clustered, and most species typically occur in less than 10% of the soil samples taken (Gardes & Bruns 1996a; Flynn *et al.* 1998; (25%); Goodman & Trofymow 1998a,b; Horton *et al.* 1999; Taylor & Bruns 1999; Stendell *et al.* 1999; Bidartondo *et al.* 2000). Individual soil cores generally contain multiple species and adjacent root tips are frequently colonized by different species, although one or two species usually dominate in a core (Gardes & Bruns 1996a; Dahlberg *et al.* 1997; Pritsch *et al.* 1997; Goodman & Trofymow 1998a; Horton & Bruns 1998; Horton *et al.* 1999; Jonsson *et al.* 1999a,b; Stendell *et al.* 1999; Taylor & Bruns 1999). In several studies, the most abundant types by biomass occurred in only one or two soil cores, suggesting that shifting the sample location by a few centimetres can cause a dramatic shift in perception of species presence and abundance (Stendell *et al.* 1999; Horton *et al.* 1999; Bidartondo *et al.* 2000). For instance, notable differences were observed in both species assemblage and dominance when replicate samples were taken in consecutive

years, with year one samples taken only 25 cm from year two samples in control plots (Stendell *et al.* 1999). The degree of patchiness in the below ground occurrence of EM fungi as highlighted by these studies can be predicted, in part, by the fact that fungi initially colonize isolated points along a root system, and proliferate locally through vegetative reproduction.

Some fungi appear to be distributed at larger scales of patchiness than that indicated above. Stendell *et al.* (1999) report that a species of Russulaceae was abundant in two consecutive years of sampling, and although it occurred in six of 24 soil cores taken over the two years, it was limited to only one of their 1 m² plots. Dahlberg *et al.* (1997), reported that *Piloderma croceum* was found in only half of their study plots and then only in five neighbouring cores. Yet, *Piloderma* was so abundant in these samples that it ranked as the most abundant species overall, accounting for almost 20% of the root tips sampled. In pinyon pine stands characterized by isolated trees analogous to islands, single EM fungi dominated single trees, and the dominant fungi varied between trees (Gehring *et al.* 1998). This pattern may be influenced by the dry climate limiting airborne spore dispersal in many years, or by the stand structure of Pinyon pine, which typically has scattered trees rather than a closed forest.

Some species are more uniformly distributed. In California, *Tomentella sublilacina* has been found to be present and often numerically dominant in both coastal and montane pine forests (Gardes & Bruns 1996a; Horton & Bruns 1998; Stendell *et al.* 1999; Taylor & Bruns 1999). *Tylospora fibrillosa* exhibits a similar pattern in Northern European *Picea* forests (Erland 1995; Dahlberg *et al.* 1997; Flynn *et al.* 1998; Jonsson *et al.* 1999c). *Cenococcum geophilum* was the second most abundant type reported by Dahlberg *et al.* (1997), and in contrast to *Piloderma*, it was found in every soil core. In fact, there are virtually no studies where *Cenococcum* has not been found; it has one of the most distinctive morphotypes and its distribution pattern had already been observed in pre-molecular studies.

Lack of correspondence between sporocarp and root-tip views of fungal dominance

Perhaps the most striking pattern in EM communities revealed by molecular techniques is that there is a poor correspondence between species that fruit abundantly and those that are abundant on roots (Mehmann 1995; Gardes & Bruns 1996a; Dahlberg *et al.* 1997; Kårén & Nylund 1997; Gehring *et al.* 1998; Jonsson *et al.* 1999a,b). A few species show a fairly consistent correspondence between above and below ground occurrence including *Amanita francheti* (Gardes & Bruns 1996a), several hypogeous fungi (Luoma *et al.* 1997), and species of *Lactarius* (Luoma *et al.* 1997; T. Horton, personal observation). However, as a rule, most species that fruit abundantly at a study site are not observed as abundant EM and, most species found below ground are not well represented in the sporocarp record from a site.

Could the discrepancy be exacerbated by incongruous sampling efforts above and below ground? Certainly the lack of correspondence between above and below ground species lists is at least partially caused by sampling differences. Sporocarp production for most species of fungi can be sporadic at best, and a fungus may not fruit abundantly, or at all, at a particular site during a study (Luoma 1991). It is also not surprising that many species that fruit at a site are not found in the relatively limited below ground record. As discussed above, the overwhelming majority of species are not abundant in the samples. In addition, even the most abundant species are typically found in less than 10% of the soil samples. In other words, even when sporocarps are collected, collecting the corresponding EM roots is not a trivial matter.

It is important to realise that the effort required to measure a study plot for diversity by sporocarp production is very different than that by EM root tips, especially if one is attempting to use the two measures to document the fungal community equally. Table 2 includes five studies in which a direct comparison was made between above and below ground views of EM fungi (Gardes & Bruns 1996a;

Dahlberg *et al.* 1997; Luoma *et al.* 1997; Jonsson *et al.* 1999b, 2000). In one study, approximately 0.1% of the ground sampled for sporocarps was sampled for root tips and in four of the studies the figure was less than 0.05%. This does not include the temporal aspect of sporocarp surveys being spread out over a number of years with multiple trips taken, while the root-tip collections were conducted with single sampling dates at each location. It may be that if one could sample the above and below ground occurrence of EM fungi with equal intensity, a better correspondence between species lists would be found for many fungi. However, for some fungi, abundance differences between above and below ground views are not so easily explained.

When a species fruits frequently and abundantly above ground, yet appears rare below ground, or conversely is found as a frequent and abundant root colonizer but exhibits little fruiting, sampling differences are an unlikely explanation. The best documented example of such a pattern is provided by Dahlberg *et al.* (1997), who report that the species which accounted for 70% of the annual fruiting biomass correspond to less than 30% of the colonized root tips. This pattern also extended to individual species of *Cortinarius*, which were dominant and fairly uniformly distributed fruiters, but were relatively minor components of the below ground community. Differential investment strategies, which are well known in plants (Chapin & Shaver 1985; Tilman 1994), could explain much of the observed pattern in EM fungi. For example, species that are abundant on roots but fruit rarely or in low abundance may simply invest more in vegetative growth and competition than in reproduction (Gardes & Bruns 1996a). This hypothesis would predict that those species, which fruit abundantly and are rare root colonizers, are weak vegetative competitors. However, at least one such species, *Suillus pungens*, can form large genets, which extend over hundreds of square meters; this is not what one would expect from a species that invests little in vegetative growth and competition (Bonello *et al.* 1998). The contradiction suggests that *S. pungens* may have a larger carbon budget than expected for the low number of roots it appears to colonize. Several hypotheses about how it may obtain such additional carbon have been put forth, but remain to be tested (Gardes & Bruns 1996a; Bonello *et al.* 1998).

Dominance by Russulaceae, Thelephoraceae and non-thelephoroid resupinates

A number of studies show that members of Russulaceae, Thelephoraceae, and non-thelephoroid resupinates are among the most abundant and frequent taxa on EM roots in conifer communities in both Europe and North America (Erland 1995; Gardes & Bruns 1996a; Dahlberg *et al.* 1997; Kernaghan *et al.* 1997; Luoma *et al.* 1997; Sylvia & Jarstfer 1997; Flynn *et al.* 1998; Goodman & Trofymow 1998a;

Horton & Bruns 1998; Qian *et al.* 1998; Jonsson *et al.* 1999c; Stendell *et al.* 1999; Taylor & Bruns 1999; Bidartondo *et al.* 2000; Peter *et al.* 2001; Lilleskov *et al.* 2002). This was also true in a community dominated by the angiosperm *Arctostaphylos glandulosa* (Horton *et al.* 1999). The fact that species in the Russulaceae are dominant is not too surprising since *Russula* and *Lactarius* sporocarps are relatively common in most EM communities. The dominance of *Tylospora* was reported prior to molecular studies (Taylor & Alexander 1989), but this pattern is now reinforced by a large number of additional studies from previously unsampled ecosystems (Erland 1995; Dahlberg *et al.* 1997; Flynn *et al.* 1998; Jonsson *et al.* 1999c; Lilleskov *et al.* 2002). The identified taxa in the Thelephoraceae have been members of the genus *Tomentella*, which, like *Tylospora*, form resupinate sporocarps. These are thin crust-like structures, that are often found on woody debris or litter, and because of this habit, *Tomentella* spp. were at one time thought to be saprotrophic (Larsen 1968). The fruiting structures are cryptic and tend to be ignored in surveys of sporocarps; thus, contributing to the mismatch between above and below ground species lists. Why species in Russulaceae and resupinate taxa are so abundant in many communities is unknown, but it suggests that these fungi are particularly good competitors and play critical functional roles in EM communities. These two important EM groups both require focused taxonomic treatments.

Response to disturbance

Airborne anthropogenic sources of nitrogen are now quantitatively important in many ecosystems (Vitousek *et al.* 1997) and several studies have highlighted the response of fungi to N deposition. High N deposition appears to have a dramatic effect on fruiting (Arnolds 1988; Brandrud 1995; Jonsson *et al.* 2000; Peter *et al.* 2001). Kårén & Nylund (1997) found that in contrast to previous studies based on sporocarp records, data based on EM roots showed that nitrogen deposition did not appear to affect the number of species observed in a stand. However, nitrogen deposition was found to alter the species composition and reduce fine-root biomass (Kårén & Nylund 1997; Peter *et al.* 2001). In contrast, Lilleskov *et al.* (2002) report that increasing nitrogen deposition reduced the species richness as measured by fungal occurrence on EM roots, with certain species disappearing and others becoming more abundant.

Other studies have investigated the response of EM fungi to fire. Visser (1995) reported that 75% of jack pine root tips were colonized by *Suillus brevipes* 6 years after a stand replacing fire, and that there was an increase in species richness as stands aged to 41 years, with a concomitant decrease in dominance by *Suillus*. Bishop pine seedlings that established in the first year after a stand replacing fire

were largely colonized by fungi present as resistant propagules in the soil (Baar *et al.* 1999). This resistant propagule bank had a similar composition before (Taylor & Bruns 1999) and after the fire (Baar *et al.* 1999), and was composed primarily of *Rhizopogon*, *Wilcoxina*, and *Tuber* species that were rare EM types in the prefire community (Taylor & Bruns 1999). This behaviour has striking parallels to seed banks in plant communities. Interestingly, in both fire adapted pine communities, suilloid fungi (*Suillus* and *Rhizopogon*) became dominant EM types during the early stages of community development.

Studies have also been conducted to investigate the effects of ground fires that allow trees and some EM roots to survive. Following fires that did not completely burn off the litter layer, EM fungal communities showed little change in species richness but a reduction in species evenness in burned stands (Jonsson *et al.* 1999b). In a study where the litter layer was completely burned off, the most abundant species were reduced to undetectable levels, thereby increasing species evenness in the stand (Stendell *et al.* 1999). In this latter study, it was suggested that EM types that were in high abundance before the fire preferentially colonized the organic layer.

Fungal networks and plant succession

Mycorrhizal fungi directly and indirectly influence plant community dynamics and succession (Perry *et al.* 1989; Allen 1991; Smith & Read 1997; van der Heijden *et al.* 1998; Horton *et al.* 1999). One way they influence plant communities is through fungal carbon transfer between plant hosts. The use of radioactive labels and more recently, stable isotopes, have demonstrated that interhost connections occur in nature and that net transfer of carbon can occur between plant species (Björkman 1960; Simard *et al.* 1997a,b; McKendrick *et al.* 2000).

The quintessential example of such transfers involves mycoheterotrophic plants; these are nonphotosynthetic plants that obtain their carbon from fungal associates. Those that are associated with ectomycorrhizal fungi, ultimately obtain their carbon from surrounding photosynthetic hosts that are connected to the same fungi. This has been known since Björkman's fieldwork in 1960, and has been recently demonstrated under laboratory conditions (McKendrick *et al.* 2000). What was unappreciated prior to molecular ecology, is that these plants have highly specific fungal associations, such that each plant species only associates with a narrow range of closely related fungi. This pattern has now been demonstrated for two distantly related genera of orchids (Taylor & Bruns 1997), and several genera in the Monotropoideae (Cullings *et al.* 1996). In fact, among the mycoheterotrophs examined to date, there are no exceptions to the rule that they have specific associations (Kretzer *et al.* 2000a).

Photosynthetic plants typically associate simultaneously with a large number of unrelated ectomycorrhizal fungi, providing a great potential for connections with dissimilar plants through shared mycorrhizal networks. Based on data from sporocarp occurrence, pure culture synthesis experiments, and soil bioassays, it had been hypothesized that EM fungi may affect competitive interactions among ectomycorrhizal hosts (Kropp & Trappe 1982; Molina & Trappe 1982; Perry *et al.* 1989; Smith *et al.* 1995; Simard *et al.* 1997a; Massicotte *et al.* 1999). Until recently, field data were lacking which allowed definitive statements about individual fungi colonizing multiple hosts. We now know from three settings that roots of dissimilar plants that overlap in space tend to be colonized by the same fungi (Horton & Bruns 1998; Horton *et al.* 1999; Cullings *et al.* 2000). In one case, fungi that formed mycorrhizae on both *Pseudotsuga menziesii* and *Pinus muricata* were in the majority below ground, but whether this impacted competitive interactions between these species was unclear (Horton & Bruns 1998). This pattern was observed again with *Pinus contorta* and *Picea engelmannii* (Cullings *et al.* 2000). A similar pattern was observed in a third case where preferential establishment of *Pseudotsuga* in *Arctostaphylos* patches had been observed, implying the EM interaction influences plant succession (Horton *et al.* 1999). Whether carbon transfer occurred between *Arctostaphylos* and the shaded *Pseudotsuga* seedlings was not tested, but even without carbon transfer, it is a clear benefit to seedlings to simply tap into a mycelial network already developed and supported by other plants (Newman 1988). However, net carbon transfer between *Betula papyrifera* and shaded *Pseudotsuga* via shared EM fungi has been demonstrated (Simard *et al.* 1997b). Taken together these examples show that shared networks are common and the potential for them to effect plant competitive interactions should not be ignored.

Population level advances — delineation of individuals

Our understanding of the autecology of individual EM species have been advanced in several ways by recent population level studies that have been directed toward identifying the physical extent of individual genotypes. Prior to the development of molecular tools for population studies, somatic incompatibility was used for this purpose on some of the few taxa that could be cultured. However, it was shown by Jacobson *et al.* (1993) that somatic incompatibility tests do not discriminate individuals as well as molecular approaches. Based on sporocarp collections and a variety of genetic markers useful in identifying individuals, researchers have delineated the spatial extent and temporal persistence of *Amanita francheti*, *Laccaria bicolor*, *Suillus pungens*, *Pisolithus tinctorius*, *Hebeloma cylindrosporium*, *Lactarius xanthogalactus*, *Russula cremoricolor*,

Suillus grevillei, and *Cortinarius rotundisporus* individuals in a variety of settings (De La Bastide *et al.* 1994; Gyrtta *et al.* 1997; Anderson *et al.* 1998; Bonello *et al.* 1998; Selosse *et al.* 1998a,b, 1999; Sawyer *et al.* 1999; Zhou *et al.* 1999; Redecker *et al.* 2001). Taken together these studies show that different species vary with respect to their temporal persistence and vegetative spread; some species are continuously recruited through sexual reproduction and spore dispersal even in undisturbed forest settings, while others appear to establish rarely or at an early stage in community development, and then spread vegetatively. Nevertheless, even the smallest individuals occupy soil volumes that include intermingling roots of multiple plant hosts.

All of the population studies identified above utilized sporocarps as the source of DNA (in some cases cultures were made from sporocarp tissue). It is desirable to generate similar data utilizing mixed DNA extracts such as those from EM root tips or soil hyphae. As the molecular tools are refined, this comparison will be possible, as demonstrated by Kretzer *et al.* (2000b) who developed microsatellite primers for *Rhizopogon vinicolor*, allowing them to amplify fungal DNA from EM extracts. In addition, studies are needed that not only delineate clone boundaries, but also assess the effective population size and the extent of gene flow between populations of EM fungi, as has been done for some plant pathogens. Results from such studies will have direct applications in evolutionary and conservation biology of fungi.

Unresolved sampling issues

Frequency vs. biomass

Dominance in the EM community has often been gauged by either frequency or abundance. The former simply being the proportion of samples that contain a given species (absolute constancy), and the latter often being either the proportion of total EM or proportion of total dry weight for a given species. In the discussion above we have treated the two as equivalent measures, but in fact they provide different views of the community structure. Frequency measures are biased towards those types that are common, even if they contribute little to the overall number or biomass of EM, while abundance measures are biased towards types that form massive clusters of root tips even though the fungus may be infrequently encountered. Just how different the frequency and abundance views are can be seen in Fig. 4. Several species, including *Cenococcum geophilum* (8), *Rhizopogon parksii* (5), and *R. subcaerulescens* (21), look much more important by frequency than by abundance; these species were common and dispersed, but colonized relatively few tips. Species such as *Amanita gemmata* (6), Cortinarioid 1 (7), and Boletoid 1 (17) were more clustered and contributed high biomass from a

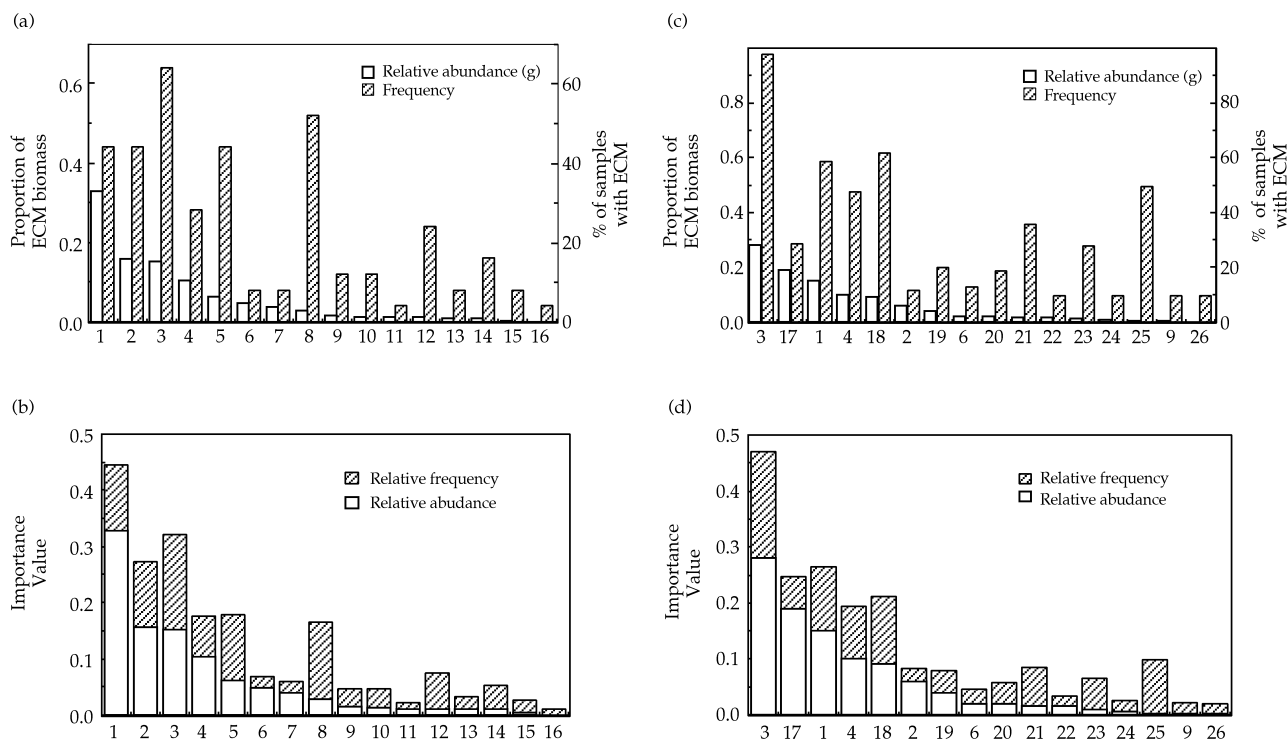


Fig. 4 Different views of community structure provided by species abundance vs. frequency. Relative abundance (black) and relative frequency (white) of mycorrhizae are shown for data from *Pinus muricata*/*Pseudotsuga menziesii* and *Pinus muricata* sites of Horton & Bruns (1998, a and b) and Gardes & Bruns (1996a, c and d), respectively. Species data are ranked by abundance as in the original papers. The top graphs show relative abundance and frequency separately, bottom shows relative abundance and relative frequency summed into an importance value. Relative abundance = tips or dry weight of a species/total tips or dry weight for all species. Absolute frequency = number of samples in which a species occurs/total number of samples. Relative frequency = absolute frequency of individual species/sum of absolute frequencies for all species. 1, *Tomentella sublilacina*; 2, Thelephoroid 2; 3, *Russula amoenolens*; 4, *R. xerampelina*-like 1; 5, *Rhizopogon parksii*; 6, *Amanita gemmata/pantherina*; 7, Cortinarioid-1; 8, *Cantharellus geophilus*; 9, *Amanita muscaria*; 10, unknown ascomycete; 11, *Laccaria amethysteo-occidentalis*; 12, Amanitoid; 13, Thelephoroid-3; 14, *Xerocomus chrysenteron*; 15, Thelephoroid-4; 16, Russuloid-2; 17, Boletoid-1; 18, *Amanita francheti*; 19, *Clavulina* sp.; 20, Cortinarioid-2; 21, *Rhizopogon subcaerulescens*; 22, Boletoid-3; 23, *Suillus pungens*; 24, *Russula xerampelina*-like 2; 25, boletoid-2; 26, suilloid.

relatively small number of samples. Either a frequency or abundance view alone would not have revealed these patterns. Other species, such as *Tomentella sublilacina* (1), *Russula amoenolens* (3), and *R. xerampelina*-like 1 (4), were both frequent and abundant, and these species behaved this way at both sites. In these cases either an abundance or a frequency comparison would have identified these as dominant species, but presenting an importance value using relative dominance and relative frequency (Fig. 5b, 5d) provides an effective presentation for the contribution of each species in the community (Mueller-Dombois & Ellenberg 1974).

How much to sample

In stands with a high level of diversity, we may never be able to sample enough to detect treatment effects on the rarest species. Thus, with the current technology, it may be most appropriate to accept this and settle for analyses

concerning only the dominant species; much of the data presented above highlights this approach. It will be beneficial to reduce the number of root tips collected per sample to a manageable number, and then increase the number of samples taken in studies conducted in complex communities (Gehring *et al.* 1998; Jonsson *et al.* 1999a,b). It is also helpful to gain some concept of the level of diversity in a community prior to developing a sampling approach for a particular study site (Gehring *et al.* 1998). In ecosystems where EM fungal diversity is expected to be low or where species are expected to be more uniformly distributed, the problem is not as acute. This can be expected in relative harsh environments such as sites that have been recently disturbed (Baar *et al.* 1999), those that are undergoing primary succession (Gehring *et al.* 1998), or those that involve introductions of EM communities into non-EM habitats such as *Pinus radiata* plantations in paramo grasslands of Ecuadorian Andes (Chapela *et al.* 2001).

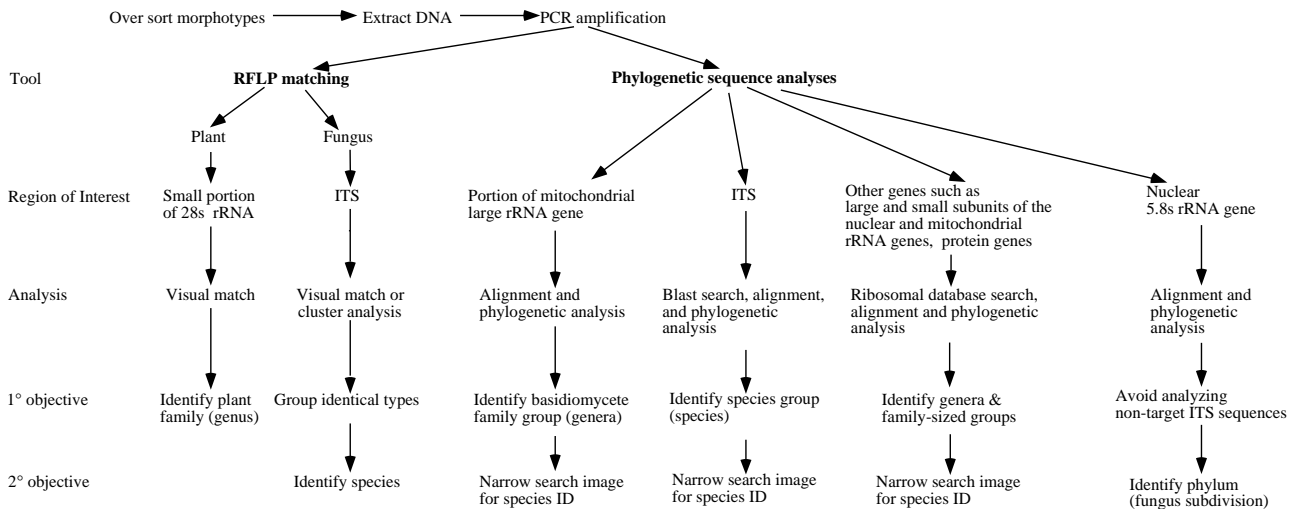


Fig. 5 Flowchart of a combined morphological-molecular typing approach to identify EM fungi from root tips. Rapid sorting of EM is conducted utilizing morphological characters, visible under a dissecting microscope: colour, presence/absence of extramatrical hyphae, branching pattern, presence/absence of setae or cystidia. EM from each soil sample are sorted into as many groups as possible at the expense of splitting species into multiple morphotypes. Each soil sample is analysed separately to further avoid lumping of species at the morphotyping step. The sorting of EM should be completed within two weeks after removal from the field to maintain high quality DNA for PCR amplification. To identify EM types, two general techniques are employed, RFLP-matching and phylogenetic sequence analysis. For those fungi that remain unknown after comparison to a database of ITS-RFLPs generated from sporocarp DNA, phylogenetic analysis of various gene regions provides increased taxonomic information from the phylum to the species group level. Identical types within and across soil samples are grouped after RFLP analysis and the data are then adjusted accordingly.

How much to morphotype

At one extreme, detailed descriptions of each morphotype can be generated whereby identical types can be recognized by morphology across samples (Shaw *et al.* 1993; Smith *et al.* 1995; Visser 1995; Helm *et al.* 1996; Luoma *et al.* 1997; Simard *et al.* 1997a,b; Goodman & Trofymow 1998a,b; Kranabetter & Wylie 1998; Qian *et al.* 1998; Massicotte *et al.* 1999). This can amount to a tremendous effort; Luoma *et al.* (1997) characterized 198 morphological types from 200 soil samples. Unfortunately, despite the effort, the species identification for all but a few types is left unclear when morphology is used exclusively.

At the other extreme, one or a few root tips for ITS-RFLP identification from each soil sample can be selected without the morphotyping step (Gehring *et al.* 1998; Jonsson *et al.* 1999c). This can be very fast and is especially useful in settings where multiple species are known to form mycorrhizae with a similar morphology (e.g. smooth brown, Jonsson *et al.* 1999c). It is probably the method of choice for questions involving large spatial scales, but resolution will be essentially limited to frequency changes in the dominant species.

A combined approach using rapid sorting by EM morphology followed by molecular identification can be employed (Fig. 5; Erland 1995; Mehmman *et al.* 1995; Gardes & Bruns 1996b; Dahlberg *et al.* 1997; Kårén & Nylund 1997; Kernaghan *et al.* 1997; Pritsch *et al.* 1997; Jonsson *et al.*

1999a,c). This can be coupled with a complete sampling of roots within a soil sample, which has the advantage of increasing the likelihood of finding rare types (Gardes & Bruns 1996a; Horton & Bruns 1998; Horton *et al.* 1999; Taylor & Bruns 1999; Bidartondo *et al.* 2000). However, if one uses a crude morphotyping approach, it should be limited to sorting differences within soil samples rather than using it to relate types between samples. Identical types encountered in separate cores, or that were inadvertently separated within a core, are numerically combined only after RFLP analysis. The two reasons for structuring the morphotype analysis in this way are that direct visual comparisons are convenient for all morphotypes within a soil sample, and the spatial scale will limit diversity when crude morphological differences are used as the primary distinction. This lessens the lumping of similar looking types, such as the 'brown type' in Erland (1995) or the white types in Jonsson *et al.* (2000), and it results in multiple RFLP replicates of common types. Furthermore, if morphotypes are later found to contain two or more RFLP types, it is relatively easy to analyse additional archived samples of the morphotype and correct the error by estimating the proportion of each RFLP type found with a single morphotype (Horton & Bruns 1998; Jonsson *et al.* 1999a,b).

Using a combination of rapid morphological sorting, RFLP matching, and sequence analysis has proven a successful approach for identifying EM fungi from root tips.

On average, our laboratory groups have identified EM types to the species level for about 40% of our EM by biomass, with another 40% that were sorted to species group level by RFLP and then identified to family group through sequence analysis (Table 1). Similar levels of identification were also achieved by Peter *et al.* (2001) using the same approach.

Mycelial view — back to quantification by molecules?

The active soil mycelium has clear functional significance, as demonstrated by the acquisition and transfer of nutrients throughout the thallus and to associated plants (Melin & Nilsson 1950, 1953; Finlay & Read 1986; Finlay *et al.* 1989; Bending & Read 1995a,b; Perez-Moreno & Read 2000). Yet, the distribution and abundance of fungal mycelium in the soil remains largely undocumented in the field. It seems likely that the mycelial view of the EM community will be different from both root and fruitbody views. While we know that some fungi form hyphal cords, rhizomorphs, fans, or mats, and others do not, data are lacking for most species, and the relationship between mycelial investment and root dominance is unknown.

If quantifying species by mycelia is the goal, then the techniques used will likely become more similar to those used in bacterial systems. Obvious approaches would include: (i) denaturing gradient gel electrophoresis, temperature gradient gel electrophoresis, or terminally labelled characterization of amplified portions of the rDNA (Muyzer & Smalla 1998; Moeseneder *et al.* 1999); and (ii) quantification of rRNA via hybridization probes including microarrays and *in situ* hybridization (Bruns & Gardes 1993; Amann 1995; Zheng *et al.* 1996) coupled with microscopic quantification of mycelium. Because the first set of techniques involve PCR amplification, there will be inherent biases that make quantification approximate at best; nevertheless these kinds of approaches have been very useful in bacterial systems for identifying the dominant species in a system. The second two approaches, typically target the unamplified ribosomes, and so would not have PCR biases, but they would require a set of well characterized oligonucleotide probes that have not been developed yet for most EM fungi.

Concluding remarks

We have come a long way from the 'black-box' approach in terms of identifying the basic structure of EM communities. However, methodological improvements, particularly in the use of sequence-level characterization, need to be employed to move beyond the large number of unknown 'RFLP-types'. We also need ways to increase sample sizes so that broad-scale questions can be addressed more efficiently. Linking the detailed efforts in identi-

fication outlined here with research directed towards understanding the functional roles of various species is required. To date, most of the physiological and autecological studies have been focused on a handful of fungi that grow well in culture or that are readily collected as sporocarps. Now that we have identified a different set of dominant taxa, increased effort is needed to understand their unique roles and behaviour.

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The authors are generally interested in ectomycorrhizal fungi and their roles in plant community dynamics. Primary topics of interest include elucidating: (i) the population and community structure of EM fungi; (ii) the role of EM fungi in plant establishment following disturbances and during primary and secondary succession; and (iii) delineating the phylogenetic relationships of EM fungi.
