

15

The roles of mycorrhizas in successional processes and in selected biomes

Introduction

Studies of the species composition and community structure of assemblages of land plants and their dependent heterotrophs over the last century have enabled the delineation of distinct biomes at the global scale (Odum, 1971). They have also revealed some of the successional dynamics involved in progression from immature and disturbed to mature and stable states in some of these biomes. Over broadly the same time period, commencing with pioneers like Janse (1897) and Gallaud (1905), extensive below-ground surveys have confirmed the presence of the mycorrhizal symbioses in most, but not all, successional stages and in most of the plants that make up the mature biomes. They have also revealed some segregation between plant families, both in the extent to which they are colonized by mycorrhizal fungi and the types of mycorrhiza that they support (Trappe, 1987; Newman and Redell, 1987; Fitter and Moyersoen, 1996; Wang and Qui, 2006).

While it has been relatively easy to achieve the sampling necessary to describe both the species composition of biomes above ground and the nature of mycorrhizal communities below ground, it has proved more demanding to determine relationships between records of the occurrence of mycorrhizas and the possible contributions of the symbioses to the dynamic properties of the biomes in which they occur. Despite these difficulties, some progress has been made. What is emerging is a picture suggesting that the functions of the symbioses go far beyond simple scenarios involving facilitation of mineral nutrient capture by individual plants or of organic C by the associated fungi. It can be hypothesized that while soil and climate have combined to configure the distinctive composition of the autotroph community in each biome, selection will also have favoured mycorrhizal symbioses and mycobionts that are appropriate to that particular set of environmental circumstances. There follows an analysis of the extent to which the emerging evidence supports this hypothesis.

After commencing with a consideration of the roles of mycorrhizas in successional dynamics, attention is turned to arctic-alpine, heathland, boreo-temperate forest and tropical forest biomes, each in turn viewed as a stable climax community occurring

along a latitudinal gradient from the poles to equatorial regions.¹ The extent and nature of mycorrhizal colonization in each case is considered with a view to elucidating the functions that might be important under the conditions prevailing in each system. At this stage, there are more questions than there are answers. It is to be hoped that identification of the questions can at least be of assistance to the next generation of researchers who will continue attempts to test hypotheses of the kind presented above.

The roles of mycorrhizal colonization in primary succession

The sequential development of plant communities following major environmental perturbations such as glaciation (Crocker and Major, 1955) and volcanic activity (Simkins and Fiske, 1983) are well documented. It is acknowledged also that scarcity of nutrients in the poorly weathered materials exposed by such events may determine the early stages of the primary succession initiated on them (Gorham *et al.*, 1979). Under these circumstances it seems likely that mycorrhizal fungi would play a role in facilitating the succession but, until recently, there was little direct evidence for such a role in nature. Work of Kazuhide Nara and his group towards the summit of Mount Fuji in Japan has now gone some way to describing their involvement in a volcanic environment. Here, at an altitude of 1500–1600 m, the slopes, which are covered to a depth of up to 10 m with volcanic tephra deposited in the course of an eruption in 1707, support sparse patchy vegetation within which the shrub *Salix reini*i occurs as an ectomycorrhizal pioneer species.

Nara *et al.* (2003a) began by describing the succession of fungal sporocarp production associated with naturally colonizing plants of *S. reini*i. Two *Laccaria* and one *Inocybe* species were the first colonizers and these were succeeded by other species as hosts grew with age (Figure 15.1). This pattern is not unlike that recorded in secondary successional sites (Last *et al.*, 1983; see Chapter 6), but there was no evidence of replacement of any of the fungi with tree development. The biomass production of the sporocarps was exceptionally large, yields equivalent to as much as 633 kg/ha/year being recorded in association with the largest *S. reini*i trees. Comparative analysis of above- and below-ground composition of these fungal communities, based upon sequencing of the ITS region of nuclear r-DNA (Nara *et al.*, 2003b), revealed that species dominating the sporocarp population were also the most widely encountered ectomycorrhizal (ECM) associates of *S. reini*i roots (Table 15.1).

While confirming the early presence of ECM fungi and providing evidence of correlations between formation of the symbiosis and performance, these studies do not directly address questions relating to the facilitation of establishment. These were considered in experiments involving seedling transplants. It was first shown by planting seedlings close to healthy or to unhealthy *S. reini*i, in bare ground or in vegetation patches lacking the shrub, that ECM formation occurred prolifically only in association with healthy plants (Nara and Hogetsu, 2004). Furthermore, at the end of the three-month growing season, seedlings associated with healthy willows were of significantly greater biomass as well as N and P contents than those

¹We recognize the importance of grasslands as major terrestrial biomes. They are not discussed in detail here, but aspects of plant interactions in them are considered in Chapter 16.

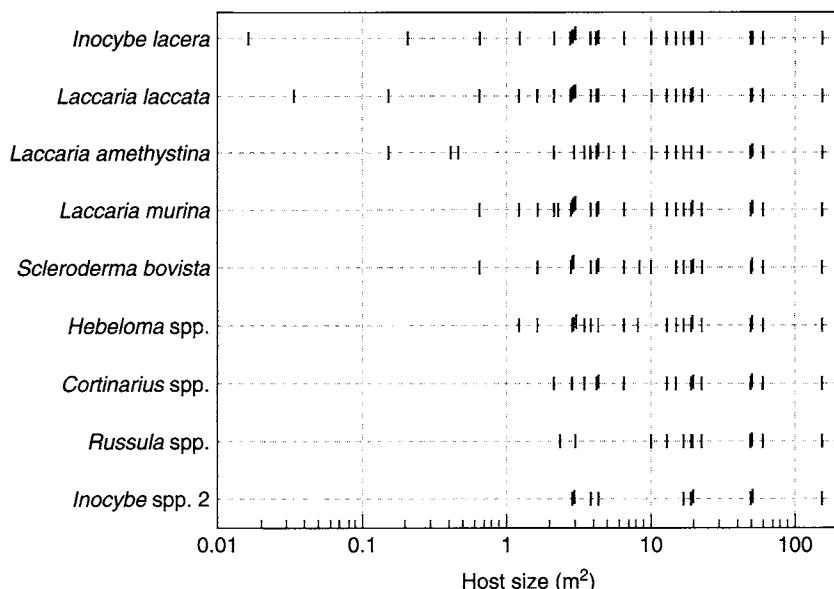


Figure 15.1 The recruitment of ectomycorrhizal fungal species, as revealed by measurements of sporocarp succession, over a period during which the shrubs of the autotroph *Salix reinii* gain size (m^2 ground area covered) with age. From Nara et al. (2003a).

Table 15.1 Species composition in the sporocarp and underground ectomycorrhizal (ECM) communities associated with individuals of *Salix reinii*, in an early successional volcanic desert.

Patch No.	Host size (m^2)	Sporocarp abundance rank*					ECM abundance rank†				
		1	2	3	4	5	1	2	3	4	5
Small hosts											
8	0.03	Ll					Ll				
125	0.15	Ll	La				Ll	La			
41	0.21	II					II	Tl			
127	0.47	La					La	Lm	Ll		
Middle-sized hosts											
131	2.83	Sb	Ll	Hp	Il	Lm	Il	Lm	Sb	L	Tl
61	3.87	Sb	Ll	Hm	Lm	La	La	Sb	Ll	Lm	Tl
120	4.31	Sb	Ll	La	Il	Lm	Lm	La	Il	Sb	Li
142	6.58	Il	Sb	Lm	La	I2	Il	Lm	Sb	Ll	La
Large hosts											
89	48.45	Ll	La	Hm	Lm	Sb	La	Ll	Sb	Lm	Ud
83	50.23	Ll	La	Hm	Sb	Il	Il	Ll	Sb	La	Lm
90	59.84	Ll	Sb	Hm	La	Il	La	Lm	Ud	Sb	Li
139	153.73	Ll	La	Hm	Cd	Sb	Lm	T2	Sb	Il	Li

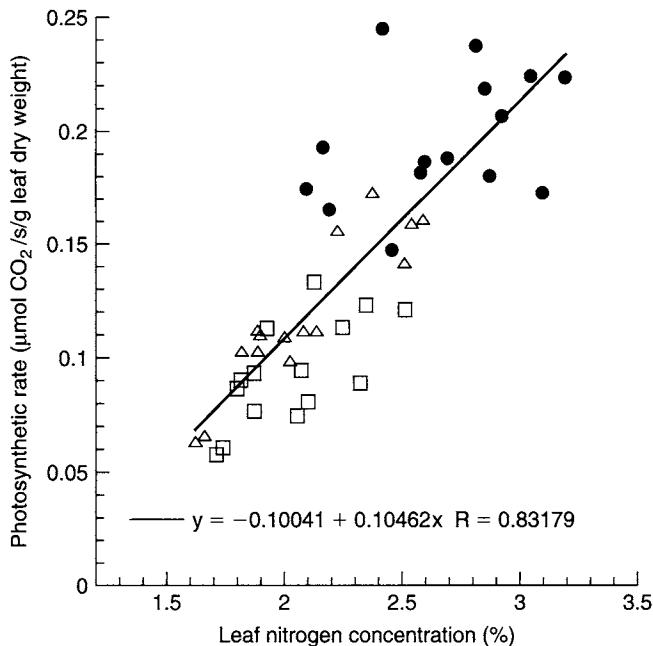
*Abbreviations of the ECM fungal species are Cd, *Cortinarius decipiens*; Hm, *Hebeloma mesophaeum*; Hp, *Hebeloma pusillum*; Il, *Inocybe lacera*; I2, *Inocybe* spp.; L, *Laccaria* spp.; La, *Laccaria amethystina*; Ll, *Laccaria laccata*; Lm, *Laccaria murina*; Sb, *Scleroderma bovista*; Tl, *Tomentella* spp.; Ud, Unidentified D morphotype spp. *The data for sporocarps were from Nara et al. (2003a). †The ECM abundance rank was based on the relative abundance of ECM root tips in three soil samples collected from each of the small and middle-sized host and in twelve soil samples collected from each large host. From Nara et al. (2003b).

planted in bare ground or in patches lacking established willow plants (Table 15.2). Since linear relationships had been shown earlier (Nara *et al.*, 2003a) between photosynthetic rates of established *S. reinii* plants and their tissue N (Figure 15.2a) and P (Figure 15.2b) concentrations, the importance of early colonization by appropriate ECM symbionts was confirmed. The growth of transplant seedlings was most strongly correlated with their N content. Because photosynthetic activity at this site is closely linked to tissue N content (Figure 15.2a), Nara and Hogetsu (2004) suggest that access to this element is the key determinant of survival and growth at this site.

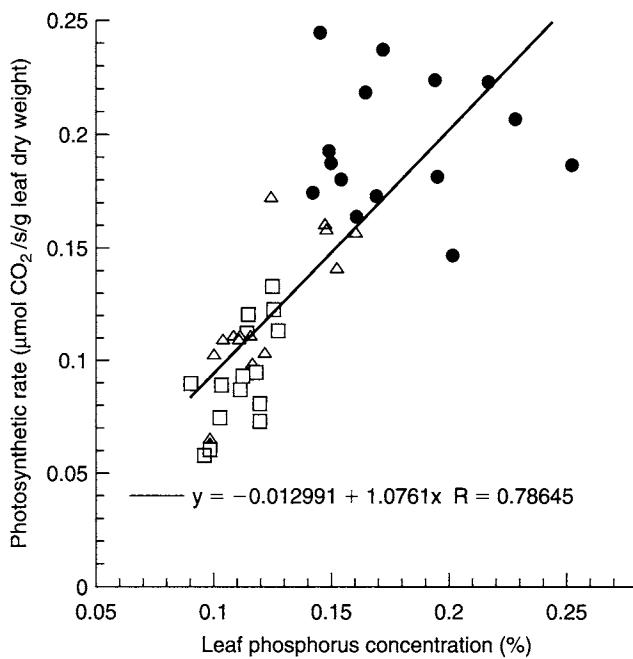
The role of mycelial networks produced by 11 of the commonest mycorrhiza-forming fungi at the site in facilitating growth and nutrient acquisition of pioneer plants was investigated by transplanting seedlings into the field with pre-formed connections to 'mother' plants or lacking any fungal associations (Nara, 2006a). Shared ECM mycelial networks involving fungi isolated from the field site were first established on plants grown in microcosms for some weeks in the laboratory. Alongside these 'mother' plants were then planted seedlings of the same species, so that they became colonized by the shared ECM inoculum. When these microcosms were transplanted intact at the field site so that the roots and mycelia were free to extend into the natural environment, the impacts of the individual inoculant fungi could be assessed and the performance of their plant partners compared with that of non-mycorrhizal transplants. Only eight of fifteen non-mycorrhizal seedlings had survived at the end of the four-month growing period, whereas the majority survived in all other treatments. None of the non-mycorrhizal seedlings survived the ensuing winter (Nara, personal communication). There were significant differences between the biomass, N and P contents of seedlings, depending first upon whether they were mycorrhizal and secondly, upon the fungal species originally inoculated (Figure 15.3). In almost all cases growth, N and P contents were greater in mycorrhizal plants. Plants inoculated with *Laccaria amethystina*, in which no parameter was significantly different from the mycorrhiza-free plants, proved to be exceptions. Seedlings responded particularly strongly to colonization by *Hebeloma leucosarx*, *Russula sororia* and *Inocybe lacera*.

These results provide strong support for the suggestion that early colonization by ECM fungi is essential for survival of the associated willows in this harsh environment. It is likely that the establishment of the first tree seedlings will be dependent upon chance association with spores of pioneer fungi. These plants having established support vigorous mycelial networks that facilitate later recruitment around them. Most of the pioneer fungi found at the Mount Fuji site are generalists in terms of their host preference (see Chapter 6) and so are able also to colonize and facilitate the recruitment of other ectomycorrhizal tree species. Nara and Hogetsu (2004) observed that the later ECM plant colonists, *Betula ermanii* and *Larix kaempferi*, established successfully only in patches containing pre-established *S. reinii* with which they shared ECM symbionts. These fungi are thus effectively driving the succession process in this environment.

A prerequisite for any fungal impacts upon primary succession is that propagules are transported to the newly exposed substrates. Cazares *et al.* (2005) investigated the possible role of propagule availability in determining primary succession along a chronosequence of soils exposed by a retreating glacier. They carried out *in situ* assays of plant mycorrhizal status and also bioassays of 'bait' seedlings grown in



(a)



(b)

Figure 15.2 The relationships between photosynthetic rate and the concentration of leaf nitrogen (a) and phosphorus (b) of *Salix reinii* with reference to the production of associated ectomycorrhizal sporocarps. ● indicates leaves collected from patches in which ectomycorrhizal sporocarp production was relatively high; △ indicates those from patches with middle productivity; □ indicates those from unproductive patches. The R-values in (a) and (b) indicate statistically significant correlation ($P < 0.01$). From Nara et al. (2003a).

Table 15.2 Effects of established willow (*Salix reinii*) shrubs on ectomycorrhiza (ECM) formation and the performance of transplanted *S. reinii* seedlings in a volcanic desert on Mt Fuji, Japan.

Transplant sites [†]	No. seedlings [‡] (ECM/total)	No. ECM root tips/seedling [§]	Shoot dry mass [§] (mg/seedling)	Shoot N amount [§] (µg/seedling)	Shoot P amount [§] (µg/seedling)
Bare ground	0/14	0.0 ^a ± 0.0	0.8 ^a ± 0.1	10.8 ^a ± 1.0	4.6 ^a ± 0.4
No-willow patch	1/17	0.2 ^a ± 0.2	0.8 ^a ± 0.1	11.0 ^a ± 1.2	4.5 ^a ± 0.3
Unhealthy willow	10/16	9.3 ^b ± 2.5	1.1 ^a ± 0.2	20.4 ^b ± 3.7	4.7 ^a ± 0.4
Healthy willow	17/17	25.5 ^c ± 2.9	2.2 ^b ± 0.3	27.7 ^b ± 1.6	6.4 ^b ± 0.5

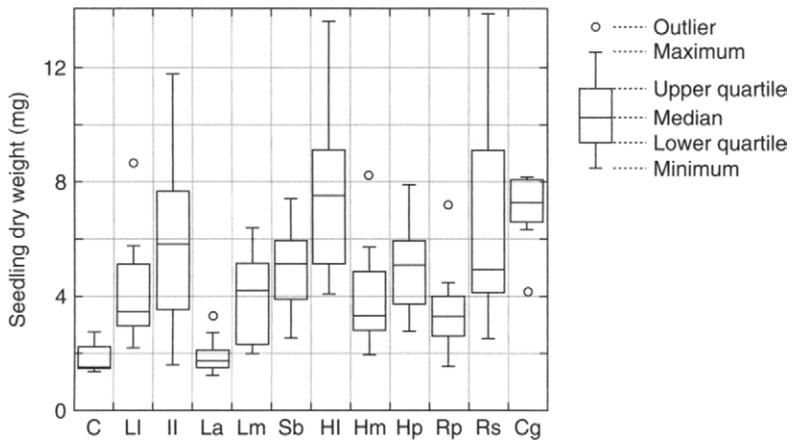
From Nara and Hogetsu (2004). [†]Seedlings were transplanted into four habitat types: bare ground, the periphery of vegetation patches lacking willow shrubs (no-willow patch), the periphery of vegetation patches containing normally growing middle-sized (10 m² canopy coverage) willow shrubs (healthy willow) and the periphery of vegetation patches containing apparently unhealthy middle-sized willow shrubs (unhealthy willow). [‡]The number of ECM seedlings followed by the total number of sampled (surviving) seedlings. [§]Mean ± 1 SE values followed by different letters within a column differ statistically (Tukey's hsd test, $P < 0.05$).

soils collected along the gradient. The soils exposed for the shortest period, between 0–15 years, supported no plants in nature but were shown in the bioassays to contain propagules of dark septate (DS) fungi (see Chapter 7). In soils exposed for longer periods, four distinct groups of plants were recognized. The first, on soils exposed for approximately 25 years, consisted of plants of families generally considered to be non-mycorrhizal, such as Cyperaceae, Juncaceae, Caryophyllaceae and Onagraceae. These were confirmed to be lacking mycorrhizas, but to support low levels of DS colonization. The third and fourth groups consisted of plants, some of which were mycorrhizal. They contained those with AM, ECM and ERM mycorrhizas but, even in the oldest soils sampled, of ~65 years exposure, only 20% of the samples examined supported each of these types of colonization. Such observations provide tantalizing views of the possible roles of propagule availability in determining primary succession, but only combinations of inoculation and transplantation experiments of the kinds described by Nara *et al.* (see above) can establish cause and effect relationships.

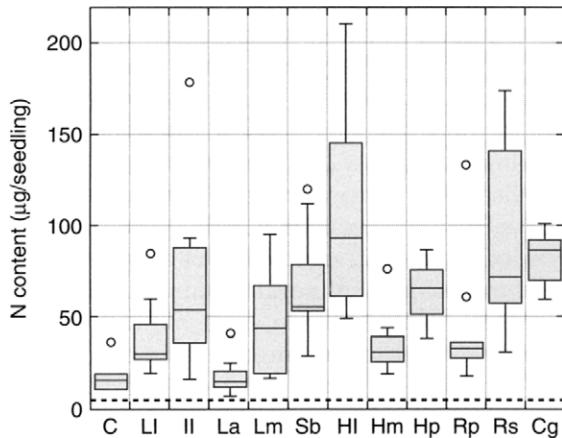
We still know too little about the factors determining the transport of fungal propagules to primary successional environments. While wind is almost certainly the primary vector of spores produced by epigeous sporocarps, animals act as vectors of hypogeous species (see also Chapter 6).

Warner *et al.* (1987) demonstrated that spores of AM fungi could be wind blown for up to 2 km, whereas animals were able to transport AM propagules over several miles

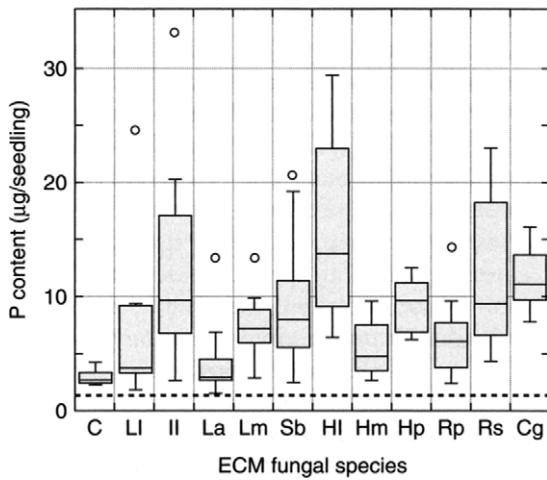
Figure 15.3 Effects of mycorrhizal networks of individual ectomycorrhizal fungal species on (a) seedling dry weight, (b) nitrogen content and (c) phosphorus content of current-year *Salix reinii* seedlings in an early successional volcanic desert on Mount Fuji, Japan. C, control; Li, *Laccaria laccata*; II, *Inocybe lacera*; La, *Laccaria amethystina*; Lm, *Laccaria murina*; Sb, *Scleroderma bovista*; Hl, *Hebeloma leucosarcx*; Hm, *Hebeloma mesophaeum*; Hp, *Hebeloma pusillum*; Rp, *Russula pectinatoides*; Rs, *Russula soraria*; Cg, *Cenococcum geophilum*. Broken lines indicate the mean for *S. reinii* seeds. From Nara (2006a).



(a)



(b)



(c)

Figure 15.3 (Caption opposite)

of sterile pumice on Mount St Helens after its eruption (Allen, 1988). In all likelihood, the smaller spores of ECM fungi and the microspores of DS fungi, such as *P. fortinii*, will be transported over even greater distances (see Chapters 2 and 7).

The resupinate sporocarps of *Tomentella* spp., that are widespread ECM formers, have been found in recently deglaciated soils of circumpolar regions (Koljalg, 1995). Since these sporocarps are eaten by a wide range of soil invertebrates, there is the possibility that arthropods act as both long- and short-distance vectors of these fungi. Lilleskov and Bruns (2005) observed that the spores of *T. sublilacina* retain viability and can initiate mycorrhiza formation after passage through the intestines of a number of invertebrate species.

The proximity of established vegetation probably frequently complicates the successional process by providing local sources of mycorrhizal propagules. Allen (1988) found such undisturbed patches of the original plant communities in the centre of the main region of pyroclastic flow on Mount St Helens following its eruption. Evidence from species lists compiled for isolated islands formed by volcanic eruptions does little to clarify the picture, though non-mycorrhizal or facultatively mycorrhizal species, the latter often grasses, figured more prominently as early colonists on Krakatoa. These were succeeded by species likely to be more responsive to mycorrhizal fungi including orchids and a *Casuarina* spp. (Simkin and Fiske, 1983). Critical questions concerning the role of mycorrhizal colonization in facilitating establishment of plants in virgin sites can ultimately only be answered by manipulative experimentation, involving addition of plants to such sites and monitoring over a chronosequence the relationship between colonization nutrient capture, growth and survival.

Primary succession on sand-dune ecosystems is a more predictable process in which relationships between soil quality disturbance and mycorrhizal status are apparent (Read, 1989). In the disturbed and nutrient enriched conditions of the drift line, non-mycorrhizal species predominate, particularly those in the families Chenopodiaceae and Brassicaceae. Under more stable conditions, a succession of communities made up of species that are responsive to AM colonization are found, ranging from open grassland dominated by *Ammophila arenaria* on the fore-dunes, to herb-rich closed communities on the more stable dunes. The succession from drift line to stable back-dunes typically covers a gradient of decreasing pH and increasing soil organic matter content (Figure 15.4) over which communities dominated by plants with ectomycorrhiza or ericoid mycorrhiza become increasingly important, forest or heathland replacing grassland as the climax vegetation type.

Some doubts have been expressed about the validity of this model. On the basis of their observation that *Salix repens* retains a predominantly ECM association on calcareous and acidic dunes in the Netherlands, van der Heijden and Vosatka (1999) and van der Heijden (2001) concluded that this gradient-based model may not be widely applicable. There is no doubt that *S. repens* has the ability to colonize calcareous soil, although it is not normally a colonist of the mobile fore-dunes that are occupied by AM grasses. It remains likely that once it has colonized stabilized calcareous sand, the accumulation of its litter in the superficial layers will begin the process of acidification that drives the system in the direction favouring first ECM and then ERM plants. The compatibility of *S. repens* with both AM and ECM fungi (van der Heijden, 2001; see Chapter 6) may contribute to its ability to establish on calcareous sand and so to accelerate successional processes.

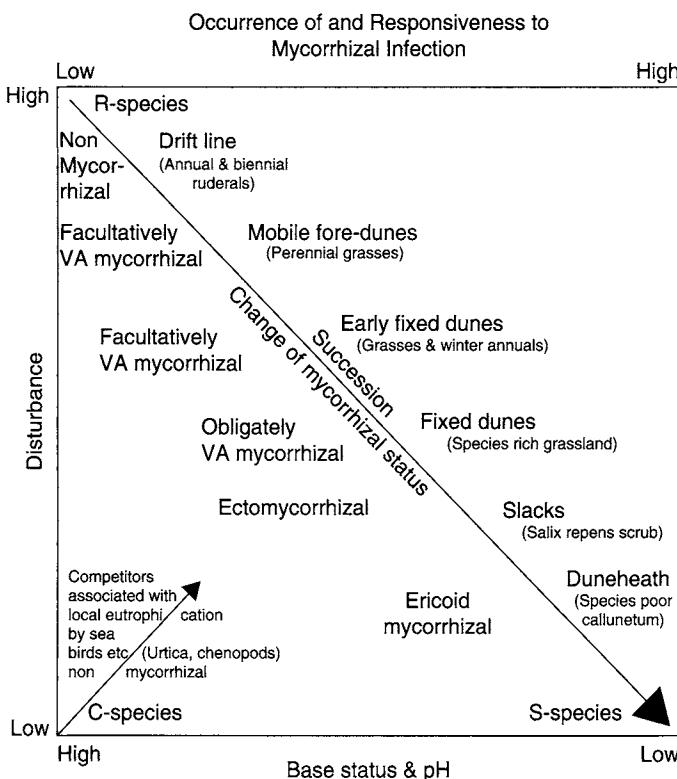


Figure 15.4 Diagrammatic representation of succession of mycorrhizal communities in a coastal sand dune, along an axis representing decreasing disturbance, pH and availability of mineral nutrients and increasing soil organic matter. C = competitor; R = ruderal; S = stress tolerant; VA = arbuscular (AM). From Read (1989), with permission.

The role of mycorrhiza in secondary successions

While primary successions often commence under conditions of nutrient impoverishment, those processes referred to as secondary succession, which follow disturbance of existing vegetation, are normally initiated in an environment of relatively greater enrichment, a pulse of N and P being produced by mineralization of residues left by the previous community (Walker and Syers, 1976). The disturbed soil is characteristically first occupied by weedy annuals, especially of families such as Chenopodiaceae, Brassicaceae and Polygonaceae, which are effective colonizers and which, since the early work of Stahl (1900), have been considered to be largely non-mycorrhizal. The conventional interpretation of the basis of the success of these ruderal plants is that as 'r' strategists they have a high fecundity, short generation time and an ability rapidly to exploit pulses of nutrient availability (Grime, 1979). However, the sensitivity of many such plants to the presence of AM mycelium or to competition from associated AM plants (see Chapter 16) raises the possibility that reduction of inoculum potential of these fungi, which is known to arise from disturbance (see Chapters 2 and 17), is an essential prerequisite for their success.

Decline of nutrient availability as the initial flush of minerals is utilized or lost by leaching leads progressively to competition between plants for resources and to a

situation in which mycorrhizal colonization could be expected to provide a nutritional advantage to plants. A possible role for mycorrhizas in determining the trajectory of successional processes was acknowledged by Gorham *et al.* (1979) who proposed that plants characteristic of a particular stage of succession may have a higher 'affinity', through their fungal associates, for nutrients at a particular stage. However, few ecologists have considered mycorrhizas as possibly playing a pivotal role in the successional dynamics. The hypothesis that mycorrhizal colonization might provide hosts with a greater competitive ability and that this leads to acceleration of successional processes was tested by Allen and Allen (1988), who introduced inoculum of AM fungi to a high-altitude soil which had been disturbed by open-cast coal mining and was colonized largely by annual 'non-host' species. The presence of inoculum had the effect of reducing the growth of the ruderals and so, in some plots, led to increases in rates of succession. However, in others, loss of cover provided by the ruderals led to exposure-damage to those species, mostly grasses, that had the potential to respond to colonization. Consequently, the rate of succession declined (Allen, 1989). Experiments of this kind demonstrate the complexity of interacting factors that can influence the successional process and emphasize that above ground, as well as below ground, factors can affect plant response (see Chapter 16).

Much emphasis has been placed by ecologists upon secondary succession in old fields where progressive decrease of availability of N is believed to be the factor driving the process (Odum, 1960; Golley, 1965; Tilman, 1987). Following the ruderal phase, a succession of grass species with increasing ability to compete for N is recognized (Tilman, 1990). Although these grasses are likely to be colonized by AM fungi, the role of mycorrhizas in determining the outcome of competitive interactions between them appear not to have been considered until recently. There is much scope for work which includes the natural symbionts of these organisms. On theoretical grounds, because the decline in N availability arises partly through progressive inhibition of nitrification and replacement of mobile nitrate by relatively immobile ammonium as sources of mineral N (Robertson and Vitousek, 1981), advantages should increasingly accrue to mycorrhizal plants. In this context it is worth noting that the prairie grasses discussed above, were typical of habitats limited by N rather than P availability.

A response seen in some ecosystems to changing N status is the appearance, as transient occupants, of N₂-fixing shrubs and trees (van Cleve and Viereck, 1981). In the succession from grassland to boreal or temperate forest, other trees to appear early are members of the genera *Salix* and *Populus* which, in addition to having light-weight propagules that enhance their capacity for dispersal into successional environments, are characterized by a plasticity which enables them to form both arbuscular and ectomycorrhizas. Compatibility with AM fungi may be a factor facilitating their incorporation into a turf dominated by AM grasses or herbs, while associations with ECM fungi should be advantageous in the situation where a progressively greater proportion of the soil N is present as ammonium or in organic form.

It has been suggested (Read, 1993) that the change in N status of an ecosystem from one in which inorganic N predominates, to the later condition in which accumulating plant residues sequester N largely in organic form, may be the key factor selecting in favour of ECM trees in late stages of succession, be they members of the Fagaceae as in many temperate forests, or of the Pinaceae in boreal forests

in the northern hemisphere. By the same logic, where for reasons of climatic stress, for example, at high elevation or latitude, growth of ECM trees is restricted, shrubs with ericoid mycorrhiza that also have the ability to mobilize nutrients from organic sources are favoured (see Chapter 11 and below).

As succession towards ECM forest or ERM heathland proceeds, there are inevitably stages during which cohorts of species with different types of mycorrhiza coexist. Indeed, even in stable forest communities, soil and light conditions may permit the persistence of a herbaceous understorey of plants with arbuscular mycorrhizas beneath a canopy of predominantly ECM trees. However, different patterns of root distribution can provide niche separation. Merryweather and Fitter (1995b) show that seedlings of the herb *Hyacinthoides non-scripta* germinating in the organic matter in ECM *Quercus* woodland are largely non-mycorrhizal. With time, the developing bulb, and the roots produced from it, descend into mineral soil where they develop arbuscular mycorrhizas in isolation from the largely surface-rooting trees. In effect, there are two separate communities, the constituent species of each of which, through their mycorrhiza, are exploiting different resources. Such differentiation can even be seen at the intraspecific level. Reddell and Malajczuk (1984) observed that *Eucalyptus marginata* plants formed AM associations when rooted in mineral soil, but ectomycorrhiza if grown in litter. Plasticity of this kind may be of particular value in fire-susceptible ecosystems of the kind in which *Eucalyptus* spp. occur, these being characterized by a cyclical pattern of accumulation and loss of organic resources due to fire.

If, as proposed by some ecologists (e.g. Clements, 1916; Odum, 1971; MacMahon, 1981), succession is a series of predictable processes, the trajectories of which are primarily influenced by nutritional constraints, a potential clearly exists for mycorrhizal colonization to play a significant role in determining both the rate and direction of the processes. The need, therefore, is for more field-based experiments which investigate the effects of manipulation of mycorrhizal status on the outcome of interaction between species at different stages of the succession. Only by these approaches can the real impact of the symbiosis upon the dynamics of the process be evaluated.

From what has been written earlier in this chapter, it is evident that some pattern can be recognized in the relationship eventually established in stable, climax communities, between biome and predominant mycorrhizal type. On this basis, it was proposed (Read, 1984, 1991) that the combination of climatic and soil factors found at any position along a gradient of latitude or altitude selects in favour of that mycorrhizal type having the functional attributes necessary to enable success of both partners in that environment (Figure 15.5). There is no doubt that, on a global scale, in the absence of disturbance, biome-related segregation of predominant mycorrhizal types can be seen even though a given type rarely if ever occurs to the exclusion of all others. The extent and nature, if any, of the involvement of the mycorrhizal symbiosis in determining these observed patterns remains to be investigated by experiment.

Mycorrhizas in Arctic, Antarctic and alpine biomes

The small number of species occurring, usually as individual plants, in the very high Arctic, in continental and sub-maritime Antarctic and in the nival zone of the Alps are only intermittently snow-covered. They grow in mineral soils that are

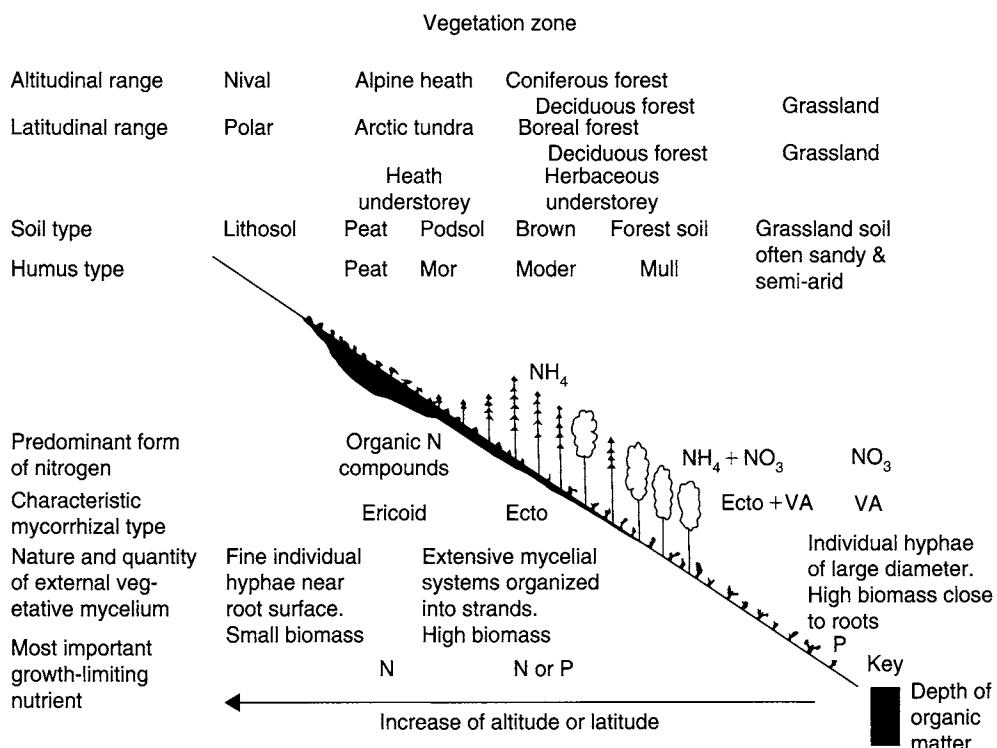


Figure 15.5 Diagrammatic representation of the postulated relationship between latitude or altitude, climate, soil and mycorrhizal type, together with the development of vegetative mycelium associated with mycorrhizas. VA = arbuscular (AM). From Read (1984), with permission.

constantly kept moist by melt water and appear to be largely uncolonized by mycorrhizal fungi. Haselwandter and Read (1982; Haselwandter *et al.*, 1983) found that one of the very few plants to occur in this zone of the Austrian alps, *Ranunculus glacialis*, appeared to be free of all fungal colonization. They concluded that this plant was adequately supplied with N and P contained in melt water during its short growing season and that physical rather than nutritional constraints determined its success in these habitats. Väre *et al.* (1992) reported that none of the six *Ranunculus* spp. examined in the high Arctic at Spitsbergen were colonized by AM fungi. This contrasts with the situation in this genus below the nival zone (Mullen and Schmidt, 1993) and in temperate latitudes (e.g. Harley and Harley, 1987a) where colonization by AM fungi appears to be the norm.

Only two vascular plants are native to mainland Antarctica. These are the graminoid *Deschampsia antarctica* and a member of the Caryophyllaceae, *Colobanthus quitensis*. They occur on thin soils ranging from skeletal gravels to those consisting entirely of acidic organic residues. Extensive analyses of these plants across a latitudinal gradient from 60° to 68°S (Upson, 2006) revealed that they were normally colonized by DS fungi and that, with the very few exceptions of root segments colonized by fungi of the 'fine endophyte' type, AM fungi were absent. This is consistent with the observation of Christie and Nicolson (1983) who could find no AM colonization on *D. antarctica* in the maritime Antarctic. They did, however, find AM colonization

at less extreme sub-Antarctic sites in the Falkland Islands and South Georgia, as did Laursen *et al.* (1997) in another sub-Antarctic location, Macquarie Island, where AM colonization was recorded on 18 of the 40 plant species examined.

Below the nival zone of alpine areas and in those regions of the Arctic that have a consistent snow-free growing season, there can be patches of continuous vegetation cover and accumulation of organic matter, at least at the surface, is normal. In their study of *R. adoneus*, carried out at 3500 m in the alpine zone of the Colorado Front Range, Mullen and Schmidt (1993) showed that, whereas the plant was lightly colonized by coarse and fine AM endophytes throughout the year, arbuscules were present only during the short growing season. Their formation was followed by increases of P concentration in the shoots and roots. It was proposed that P acquired in this period was stored for use during growth and flowering the following spring, both of which occur before soils thaw to release nutrients. More studies of this kind, in which the dynamics of colonization and nutrient acquisition are followed through the year in the natural environment of the plant, are much needed. They provide not only ecologically relevant information, but heighten the need for physiological investigations to sort out the relative significances of arbuscules and hyphae in P transport.

Often, especially in those regions of the Arctic where the water table lies permanently near the surface, organic soils support tussock tundra dominated by a preponderance of species in families like the Cyperaceae and Juncaceae, which are typically non-mycorrhizal. These systems contrast with the heath tundra communities on somewhat drier soils that contain a higher proportion of shrubby ERM and ECM species (see below). While the prevalence of cyperaceous plants in tussock tundra may help to explain the small quantities of AM colonization present (Bledsoe *et al.*, 1990), it does not provide a full explanation, since again many species which are 'hosts' to AM fungi at lower altitudes, are uncolonized or only lightly so in Arctic-alpine situations. Even where arbuscular mycorrhizas are observed, they are often, as in the Antarctic, formed by 'fine' rather than 'coarse' endophytes, there being a progressive increase in colonization by *Glomus tenuis* with altitude (Crush, 1973; Haselwandter and Read, 1980; Olsson *et al.*, 2005). Nevertheless, the presence of 'fine endophytes' must not be dismissed because they can play highly significant roles in increasing plant P uptake (Crush, 1973; Smith and Smith, unpublished).

One striking feature to emerge from studies of plants growing at high latitudes and altitudes is the extensive occurrence on their roots of DS fungi (Haselwandter and Read, 1980; Read and Haselwandter, 1981; Christie and Nicolson, 1983; Currah and van Dyke, 1986; Kohn and Stasovski, 1990; Väre *et al.*, 1992; Treu *et al.*, 1996; Jumponnen *et al.*, 1998; Ruotsalainen *et al.*, 2002; Upson, 2006). In a study of 179 vascular plant species of Alberta, Currah and Van Dyke (1986) found that roots of 87% of alpine species were colonized by DS fungi, in contrast with only 9% in non-alpine situations.

The preponderance of fungi of this general type on plants growing in alpine situations is reflected in analyses of soil microflora, which suggest that in Antarctic (Heal *et al.*, 1967), Arctic (Väre *et al.*, 1992) and alpine (Haselwandter and Read, 1980) soils, fungi with DS hyphae dominate the soil microbial community. Recognition of their quantitative importance in these habitats and in other climatically or nutritionally stressed habitats has driven attention towards their possible taxonomic and functional status. Some progress has been made towards determination of their taxonomic positions, most of them being now recognized as ascomycetes of the

Dermateaceae (order Helotiales) (see Chapter 7). Among these, members of the genus *Phialocephala*, particularly *P. fortinii*, were prominent in alpine situations (Read and Haselwandter, 1981; Currah and van Dyke, 1986). Molecular analysis of the most widely occurring DS endophytes of the Antarctic species *Deschampsia antarctica* and *C. quitensis* have confirmed the importance of taxa within the Dermateaceae in this biome, but have also provided evidence of host preference in the fungi involved (Upson, 2006). Whereas the dominant fungi on the roots of the grass *D. antarctica* had affinities to mollisoid taxa having close relationships with *P. fortinii sensu lato* (see Figure 7.4a), the roots of *C. quitensis* were preferentially colonized by *Cadophora* and *Leptodontidium* species (see Chapter 7).

Much less is known about the possible functions of DS fungi in polar and alpine regions. Haselwandter and Read (1982) isolated DS fungi from healthy, field-collected roots of the alpine sedges *Carex firma* and *C. sempervirens* and obtained a positive growth response in *C. firma* when the plant was inoculated and grown in sand with one of the isolates. However, since neither the substrate nor the climatic condition employed in the experiment reflected those of the alpine habitat, they urged caution in the interpretation of these responses, referring to them as being evidence of an 'association' rather than of a typical mycorrhizal relationship. Upson (2006) carried out an experiment of similar design but under more relevant climatic conditions (day temperature 6°, night temperature 4°C) in which *D. antarctica* was inoculated with DS isolates and grown on perlite supplemented with nutrient solutions containing ammonium or casein hydrolysate as the N source. The results varied according to both N source and fungal inoculant. Yields of the inoculated grass, relative to those of uninoculated controls, were either reduced or unaffected in the ammonium treatment. However, four of the DS isolates produced a significant increase of biomass when the plants were supplied with organic N source (Figure 15.6). These results confirm that there are circumstances in which *D. antarctica* can benefit from the saprotrophic activities of some of its DS associates, but the mechanisms whereby these effects are achieved and the extent to which they would be observed in nature, where the roots would be colonized by a larger number of DS taxa, remain to be elucidated.

Of equally uncertain status are the ECM-like structures which are frequently, but not consistently, found on roots of herbaceous species such as *Kobresia* (Fontana, 1963; Haselwandter and Read, 1980; Kohn and Stasouski, 1990) and *Polygonum viviparum* (Hesselman, 1900; Read and Haselwandter, 1981; Lesica and Antibus, 1986). Where they occur, these associations too are normally formed by fungi with dark mycelia. Among these, *Cenococcum geophilum* appears to be prominent, though the frequent presence of hyphae with clamp connections indicates that basidiomycetous fungi may also be involved. There is a suggestion (Väre *et al.*, 1992) that these plants are more frequently colonized in this way when growing with typically ECM plants. Again, there is a need for experimental analysis of the status of these types of colonization.

There is concern that warming global climate will pose particular threats to Arctic and sub-Arctic ecosystems. Predicted temperature increases of up to 5°C in the Arctic (International Panel on Climate Change – IPCC, 2001) can be expected to lead to northward migration of ECM woody shrubs (Bret-Hart *et al.*, 2001; van Wijk *et al.*, 2004; Clemmensen and Michelsen, 2006; Clemmensen *et al.*, 2006). Associated changes in below-ground activities, including more rapid rates of mineralization of organic matter, might in turn lead to increases of fertility and the release of the

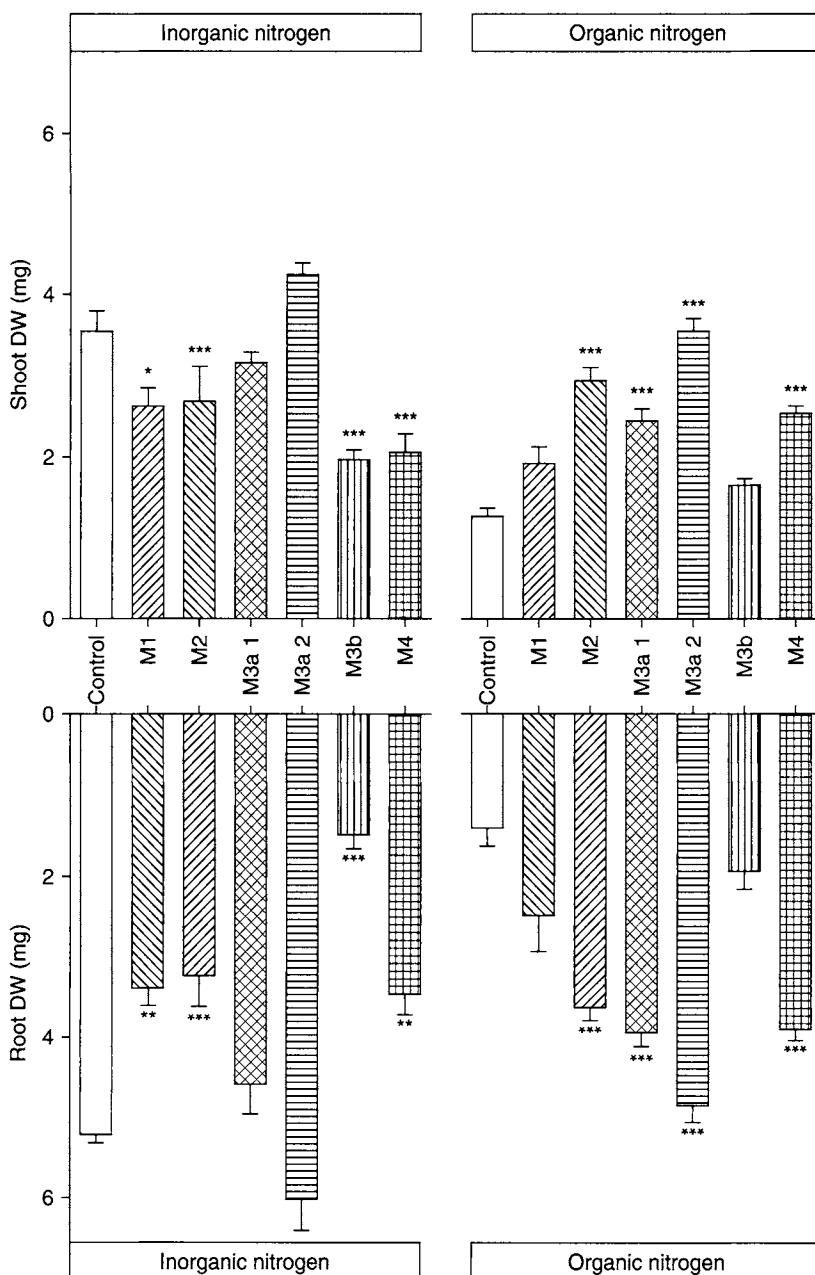


Figure 15.6 Shoot and root dry weight (DW) of *Deschampsia antarctica*, grown in perlite with an inorganic or organic nitrogen form and either (white) uninoculated or inoculated with DS isolates M1, M2, M3a (isolate 1 or 2), M3b or M4. Significant differences between inoculated and uninoculated plants tested by two-way ANOVA (GLM) and Tukey's pairwise comparisons are shown as * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Bars represent the mean (+S.E.) of 5 or 6 replicates. From Upson (2006).

approximately 14% of global soil organic C currently stored in Arctic ecosystems (Mack *et al.*, 2004). Since the ECM mantle and its associated extraradical hyphae occupy the interface between roots and this C reserve, more information on the possible interactions between these compartments is essential. Clemmensen *et al.* (2006) investigated the effects of simulated soil warming and fertilization by N, N and P, or NPK over a 14-year period on the development of ECM mycelium of *Betula nana* growing in tussock and heath tundra. Using ergosterol as a biomarker, they found that fertilizer application increased production of ECM extraradical mycelium at both sites and that temperature increase stimulated its production in the tussock tundra (Figure 15.7). It appears from these results that one consequence of warming and associated eutrophication will be an enhancement of cycling of both C and N in this previously strongly nutrient-limited ecosystem. There is also the possibility that warming will lead to changes in the population structure of ECM fungal communities in the Arctic. In detailed analyses of fungal isolates collected across a gradient extending from the Arctic, through boreal, into temperate forests, it was concluded that *Hebeloma* spp. showed significant physiological adaptation to their local environments (Tibbett *et al.*, 1998a, 1998b). When compared with temperate and boreal forest isolates, the species and races from more northerly latitudes had greater nutrient mobilizing capabilities at low temperatures. These low temperature-adapted fungi may either be displaced northwards as soils warm or, if suitable habitats are not available, driven to extinction.

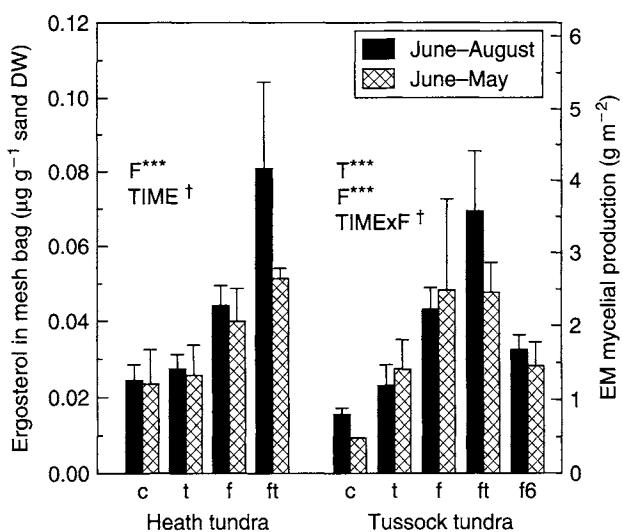


Figure 15.7 Production of external ectomycorrhizal (EM) mycelia associated with *Betula nana* measured as ergosterol concentration in sand-filled ingrowth bags after incubation in the field for one growing season (June–August) or 1 year (June–May) (mean \pm 1 SE) in (c), control; (t), temperature enhanced; (f), fertilized; or combined f and t plots after 14-year treatment at a heath tundra in sub-Arctic Sweden and at a tussock tundra in Arctic Alaska. Significant main factor effects (T, temperature enhancement; F, fertilization; TIME, incubation period) and interactions are indicated: ***, $P < 0.0001$; †, $P < 0.10$. At tussock tundra, additional plots fertilized for 6 years (f6) are not included in statistical analyses. From Clemmensen *et al.* (2006).

Mycorrhizas in heathland

Heathlands occur as major biomes under two environmental circumstances. In the first, the upland or maritime heath is found in both continental and island locations at a distinct altitudinal position between the alpine zone and the tree line (Figure 15.8). The second, lowland heath, occupies areas of particularly impoverished acid soil at low elevation. These biomes are characterized by the presence of shrubby, sclerophyllous, evergreen plants of the family Ericaceae, all of which normally have hair roots colonized by ERM fungi (Read, 1983; see Chapter 11).

Analysis of environmental gradients across which plants with ericoid mycorrhizas become increasingly prevalent has shown that such communities arise primarily in response to nutrient impoverishment (Specht, 1981; Rundel, 1988; Read, 1989). Their occurrence in warm Mediterranean climate zones as 'dry-heath' or 'sand-plain' formations, as well as in sub-alpine environments, serves to emphasize the fact that nutritional rather than climatic factors play the primary role in determining the distribution of these communities. The response to low availability of N and P is to allocate increasing proportions of fixed C to the structural components lignin and cellulose, rather than to molecules rich in protein or P, a process which leads directly to sclerophylly (Specht and Rundel, 1990) and to the release of residues of high C:N ratio and considerable recalcitrance. These accumulate at the soil surface to provide the matrix in which ERM roots proliferate.

In northern heaths, the hair roots of dominant plants such as *Calluna vulgaris*, *Erica* spp. and *Vaccinium* spp. are characteristically confined to the top 10 cm or less of the soil profile, where they are closely associated with the litter (Reiners, 1965; Gimingham, 1972; Persson, 1980). Interestingly, when herbaceous species such as *Molinia caerulea*, *Eriophorum vaginatum* and *Carex* spp. coexist with ericaceous shrubs,

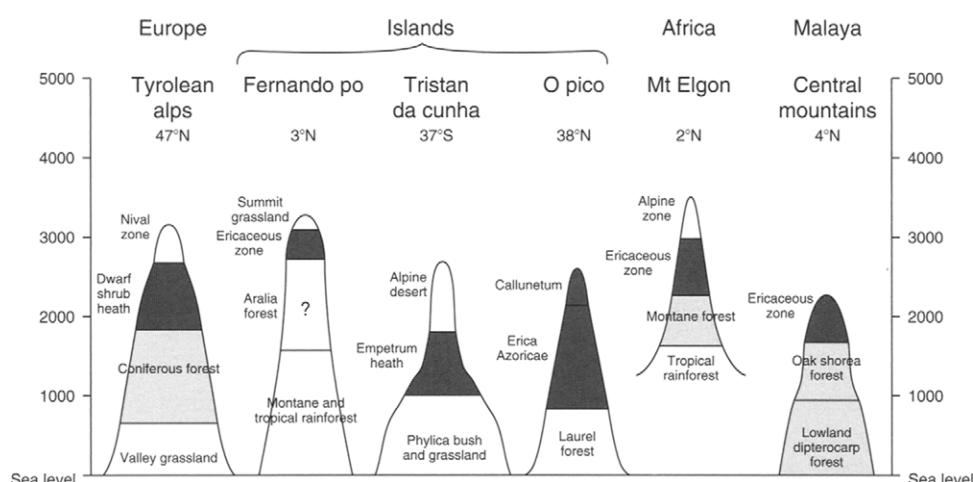


Figure 15.8 Simplified global pattern of distribution of major biomes, highlighting the segregation of predominant mycorrhizal types in association with distinctive types of plant community. Mycorrhizal types: black shading, ericoid; grey shading, ECM; no shading, AM.ecto. Note that in the dipterocarp forests there will be important canopy and understorey plants that are AM (see text). From Read (1993), with permission.

their roots are concentrated at greater depths in the soil profile (Gimmingham, 1972), so the two groups of plants are not competing for the same resources. Indeed, the grasses may be colonized by AM fungi, again emphasizing separate strategies of resource acquisition. Read (1993) presented a schematic view of the manner whereby distinctive mutualisms, together with modifications of root distribution and anatomy, might promote species diversity in northern heaths by enabling exploitation of different sources of the critical growth limiting element N. In this (Figure 15.9), the coexistence of ericaceous, leguminous and carnivorous species, typically seen in heaths of moderate acidity, was facilitated by their abilities to use sources of N derived, respectively, from soil organic matter, the atmosphere and captured animals. Structural modifications, in particular production of aerenchyma, enables roots of cyperaceous species like *E. vaginatum* to penetrate water-logged horizons where they exploit N sources untapped by the other groups that are essentially surface rooting.

Questions have been raised concerning the extent to which non-mycorrhizal plants may compete for organic N with co-occurring ERM and ECM fungi in heathland and heath tundra environments. Whereas there is no doubt that some of these non-mycorrhizal plants have the potential to assimilate simple forms of organic N (Chapin *et al.*, 1993; Nasholm *et al.*, 1998), direct comparisons between these and species with ERM and ECM roots suggest that their abilities to do so are relatively

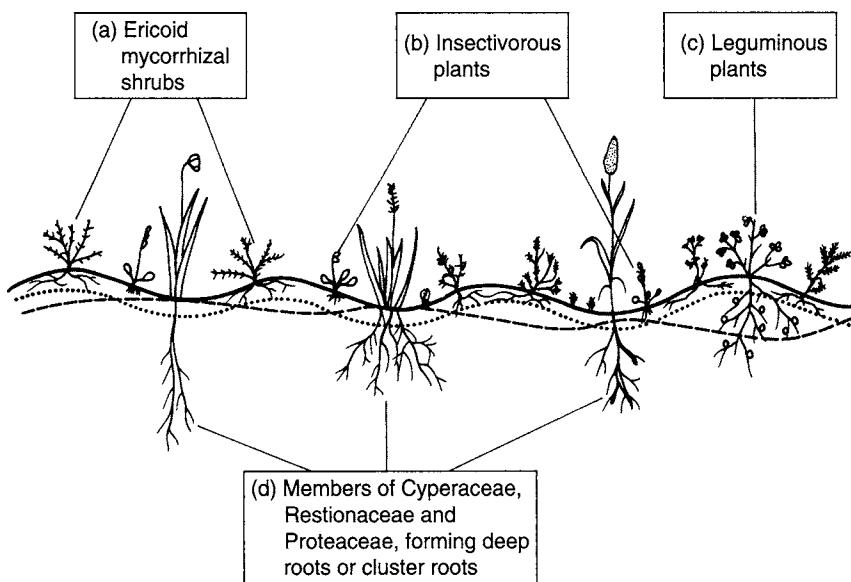


Figure 15.9 Schematic representation of compartmentation of resource acquisition in heathland ecosystems, based on the occurrence of distinctive mutualistic associations or root specializations: (a) Ericoid mycorrhizas occur in dwarf shrubs and play an important role in the mobilization of N in plant litter and microbial protein. (b) Insectivorous plants capture insects, from which they release N. (c) Leguminous plants form nodules which fix N₂. (d) Members of the Cyperaceae, Restionaceae and Proteaceae either produce deep roots that tap N in low soil horizons and/or proteoid or cluster roots that are important in capture of nutrients (particularly P, but also possibly N in the surface horizons). From Read (1993), with permission.

limited (Emmerton *et al.*, 2001b). When supplied with ^{15}N -labelled glutamic acid and glycine under aseptic conditions, the ERM plant *Vaccinium vitis-idaea* and the ECM *Betula nana* assimilated these N sources readily, whereas their non-mycorrhizal counterparts *E. vaginatum* and *Luzula wahlenbergii* captured very limited amounts of N from them. It has been confirmed that the ability of *V. macrocarpon* to assimilate amino acids so effectively is expressed only when the plant is growing in the ERM condition (Read *et al.*, 2004). In any event, it must be recognized that because small organic molecules like amino acids can be readily assimilated or mineralized by the assemblage of soil saprotrophs, they will have short half lives in the soil. Under these potentially competitive circumstances, the advantages conferred upon ERM (and some ECM, see Chapter 9) plants by their extensive fine root and extraradical mycelial systems are likely to be important.

Irrespective of differences between non-mycorrhizal plants and those colonized by ERM and ECM fungi in terms of their abilities to compete for and assimilate simple organic N compounds, it is likely that the direct attack on macromolecular organic N sources facilitated by proteolytic activities (see Chapters 9 and 11) will be the distinguishing advantage for ERM and ECM plants when growing in competition with species lacking this attribute (Read *et al.*, 2004).

The extent to which $\delta^{15}\text{N}$ signatures of plant shoots can provide indications of the extent of organic N use by different mycorrhizal types has been investigated. Comparative analysis of $\delta^{15}\text{N}$ -enrichment of leaf tissues of ERM (*Vaccinium vitis idaea*), ECM (*Picea mariana*) and AM (*Calamagrostis canadensis*) species all growing in the tundra heath-boreal forest transition zone of Alaska revealed significant differences of enrichment between these species. *P. mariana* had a significantly lower $\delta^{15}\text{N}$ value (-6.496) than *V. vitis idaea* (-3.837) and *C. canadensis* ($+0.585$) (Schultze *et al.*, 1994). Distinctive rooting depths may have contributed to these differences, but the possibility of discrimination facilitated by ECM, ERM and AM colonization, respectively, was suggested as a possible basis for the observed effects. Some support for the latter possibility was obtained in a field-based study of sub-Arctic plants (Michelsen *et al.*, 1996). The extent of mycorrhizal colonization was examined in fell-field and heathland communities before $\delta^{15}\text{N}$ enrichment of leaf tissue was determined in plants of known mycorrhizal status, representative of ERM, ECM, AM and non-mycorrhizal categories. In the fellfield, the mean $\delta^{15}\text{N}$ of the ERM species was -5.5 , that of ECM species was -4.1 and of AM or non-mycorrhizal species zero. In the heath, the mean $\delta^{15}\text{N}$ values of the same groups were -7.6 , -6.4 and -1.8 , respectively. In all cases the values obtained from the ERM and ECM plants were significantly different from those of the AM or non-mycorrhizal species. Though the differences between ERM and ECM species were not significant ($P = 0.051$ in fell-field and 0.270 in heath), the ECM plants appeared to occupy an intermediate position in the hierarchy. It was concluded that ERM and ECM colonization facilitates access to organic sources of N which were not only the predominant forms of N in the soil but also had significantly lower levels of $\delta^{15}\text{N}$ enrichment.

More recent work has cast doubt on the extent to which $\delta^{15}\text{N}$ signatures of shoots can provide information concerning the sources of N assimilated by ERM roots. Using defined N sources of known $\delta^{15}\text{N}$ enrichment, it has been shown that considerable fractionation of N isotopes occurs in the course of assimilation both by the fungi in pure culture (Emmerton *et al.*, 2001a) and by the mycorrhizal plants (Emmerton *et al.*, 2001b). Of greatest interest in this connection was that root, shoot

and whole plant ^{15}N abundance values frequently showed significant differences from those of the source N compounds supplied to the mycorrhizal plants. Furthermore, there were differences between mycorrhizal categories in the extent to which N fractionation occurred in the fungus–root–plant pathway. The interpretation of $\delta^{15}\text{N}$ data is further discussed in the context of boreal forest biomes later in this chapter.

The ability of ERM fungi to release a wide range of polymer-degrading enzymes and so to facilitate mobilization of the N and P contained in organic resources of various kinds has been emphasized earlier (see Chapter 11). From the biome perspective, the most important attribute of the ericoid mycorrhizas could be the provision of direct access to the detrital remains of the dominant components of the system. Berendse (1994) emphasized the role of litter quality as a determinant of fitness in heathland plants, but envisaged saprotrophs as being the agents facilitating this recycling. Experiments demonstrating the abilities of ERM fungi to release N from residues of their own mycelia and those of ECM fungi (Kerley and Read, 1997), as well as from necromass of their autotrophic associates (Kerley and Read, 1998), have confirmed their potential to effect significant decomposer activities. Whereas ERM plants of *V. macrocarpon* were able to recover 40% of the N contained in *Vaccinium* necromass over 60 days, non-mycorrhizal plants recovered only 5% over the same period (see Chapter 11). Obviously, detrital material produced aseptically by desiccation is not a precise surrogate for that found in nature, but experiments of this kind provide some evidence that ERM fungi can be expected to contribute to the tightly coupled recovery of nutrients from the otherwise recalcitrant residues of their host plants.

In view of the prediction that Arctic and sub-Arctic biomes, including heaths, may be subject to particularly marked temperature increases over the next century (see above), Olsrud *et al.* (2004) have examined the impacts of elevated temperature and CO₂ concentration on aspects of ERM root colonization. They found that the extent of ERM colonization increased in association with greater rates of photosynthesis under conditions of both higher temperature and elevated CO₂. However, in CO₂-enriched plots, leaf N contents of the ericaceous species were reduced. Olsrud *et al.* (2004) propose that declining availability of inorganic N under conditions of elevated CO₂ will limit impacts upon productivity of ericaceous ecosystems.

Whereas plants with ERM are well adapted to the organic N-enriched soils of high latitudes, they have responded negatively to the anthropogenic enhancement of nitrate and ammonium availability seen in industrialized regions further south (Aerts, 2002). The widespread replacement of *Calluna*-dominated heaths by grasslands composed of more productive AM-associated species can be ascribed to a combination of factors, among which the greater competitive ability of the herbs under N-enriched conditions and the failure of ERM systems to assimilate nitrate effectively are likely to be prominent (Aerts and Bobbink, 1999; Aerts, 2002; Read *et al.*, 2004).

Pate and Hopper (1993) point out that the great species diversity of the epacrid-containing heathlands of the southern hemisphere is in striking contrast to the situation seen in northern heaths which are often dominated by monospecific stands of ericaceous species. It appears that with the diversity comes a commensurate increase in the range of root specializations (Lamont, 1982, 1984; Pate, 1994; Lambers *et al.*, 2006), suggesting that niche separation will be even more marked in these systems than it is in less stressed environments. Among the distinctive plants of southern

heaths, and some other vegetation types growing on severely P-limited soils, members of the non-mycorrhizal Proteaceae, Restionaceae, as well as mycorrhizal *Casuarina*, are characterized by the production of 'proteoid' or cluster roots. These structures are formed by a highly specialized branching of lateral roots, so that a dense mat of rootlets of narrow diameter is formed (Lamont, 1984; Lambers *et al.*, 2006). The clusters are transitory, undergoing growth, maturation and senescence over a period of around 2–3 weeks, at least under the artificial experimental conditions. The developmental changes are accompanied by distinct changes in excretion of organic acids which have been shown to play a significant role in increasing the availability of P, and probably also of Fe, tightly held in inorganic forms (Dinkelaker *et al.*, 1995; Comerford, 1998; Lambers *et al.*, 2006). It has also been suggested that the location of the cluster roots, near to the soil surface and sometimes in the litter layers, may increase capture of nutrients leached through the soil. Present evidence suggests that formation of cluster roots is an adaptation to extreme soil P limitation. Indeed Lambers *et al.* (2006) show that members of the Proteaceae occur in extremely P-impoverished soils in Western Australia, which apparently do not contain enough P in forms available to plants with AM associations. The latter are found, in the same geographic region, on soils which are still P-limited, but not extremely so. Ericaceous species in the same environments should have the potential to exploit organic residues, via the activities of their associated ERM fungi, whereas insectivorous and N₂-fixing species make use of other adaptations (see Figure 15.9). It is worthy of note that *Casuarina* seems to have evolved ways of maximizing acquisition of scarce resources in many forms, forming AM and sometimes ECM symbioses, as well as having N₂-fixing nodules and cluster roots.

Stewart *et al.* (1993) provide evidence of some functional segregation with respect to N nutrition in ecosystems able to support plants with a range of strategies. In the fire-prone habitats that they studied, nitrate was relatively abundant immediately after burning, although some ammonium was also present. Sufficient nitrate reductase activity (NR) was observed in shoots and roots of three proteaceous genera (*Banksia*, *Petrophile* and *Stirlingia*) to suggest that they would assimilate nitrate in nature when available. In contrast, two epacrid species, *Astroloba macrocalyx* and *Conostephium pendulum*, both of which have ericoid mycorrhizas, showed barely detectable NR, even after feeding with nitrate via the transpiration stream. These species would be dependent on assimilation of ammonium, which was always present in soil and predominated at sites not burnt for several years, or on utilization of organic N via ERM associates.

Other plant families with widespread representation in epacridaceous heaths, but which are absent or of little importance in the northern hemisphere, are the Rutaceae, Dilleniaceae and Compositae, most members of which would be expected to be colonized by AM fungi. This type of mycorrhiza has been reported, for example, in *Boronia* (Rutaceae), *Hibbertia* (Dilleniaceae) and *Helichrysum* (Compositae) (Lamont, 1984). These plants have less fibrous root systems than epacrids and, in addition, penetrate the sandy soils more deeply (Dodd *et al.*, 1984; Pate, 1994), a feature providing spatial separation of root activity. Further, while AM colonization will enhance their ability to scavenge for P, members of two of the genera, *Helichrysum* and *Hibbertia*, have been shown to develop significant NR activity (Stewart *et al.*, 1993), again suggesting the likelihood of nutritional as well as spatial niche differentiation between these plants and those with ericoid mycorrhizas. However, the demonstrations that AM colonization may increase uptake of ammonium and especially in dry soils of nitrate may be

relevant here. It is in just such habitats as these that N acquisition is important (see Chapter 5). The presence of these physiological patterns suggests that selection favouring a range of specializations has been an important factor enabling the coexistence of taxonomically distinct species in Australian heathland systems, where the greatest diversity is found on the least fertile soils (Pate and Hopper, 1993). Tilman's equilibrium model of plant competition (1982, 1988) predicts that, in resource-poor environments, diversity will be low because few species can tolerate extremes of nutrient deprivation. In the sand-plain heaths it appears that, on the contrary, selection of distinctive mutualisms and nutrient acquisition strategies over very long periods of evolution can facilitate coexistence of species in very diverse assemblages. Indeed, recent work, admittedly in artificial systems, suggests that mycorrhizal colonization may be of greatest advantage in competition where resources are poor and plant density low (see Chapter 16).

Mycorrhizas in boreal and temperate forest biomes

Communities of ECM trees, in particular members of the Pinaceae, Fagaceae, Betulaceae and Salicaceae, are the natural dominants of the boreo-temperate biomes of the world. These are the world's largest vegetation system. They stretch as a continuous 1000–2000 km wide circumpolar belt around the Northern Hemisphere (Odum, 1971). As in northern heathlands, the diversity of host species in these ECM forests is characteristically low but, in contrast to the heathland situation, there is a very great diversity of fungal symbionts associated with the plants (see Table 6.2, Chapter 6). The cool climates prevailing across much of the biome lead to low rates of both evapotranspiration and decomposition, with the result that acidic organic residues of plants accumulate either as raw humus in superficial layers of the soil or as peat deposits which can be of considerable depth. Under these circumstances, dwarf shrubs with ericoid mycorrhiza are commonly found as understorey components. However, within this predominantly ECM habitat there are areas in which base enrichment of the soil can provide conditions suitable for the development of plants with AM colonization. These may occur as dominants in the case of forests of *Acer* and *Fraxinus*, as understorey shrubs, or as a herb layer (Brundrett and Kendrick, 1988, 1990a, 1990b; Giesler *et al.*, 1998; Höglberg *et al.*, 2003). The changing nutritional circumstances driving change of mycorrhizal type along such gradients are discussed below.

An understanding of the pattern of distribution of roots within the soil profile is important because this determines the nature of the substrates that are accessible to them and, in particular, to their fungal symbionts. In the podzolic and peaty soils, which characterize the boreal zone, as well as in the moder and mull soils of the temperate zone, ECM roots are predominantly found in the superficial organic layers (Meyer, 1973; Harley, 1978; Persson, 1978; George and Marschner, 1996). In the case of the peats which cover so much of the boreal zone, anaerobiosis associated with a high water table and permanent or winter freezing of the deeper layers, restricts the development of mycorrhizal roots, which are essentially aerobic in their requirements, to superficial layers. In black spruce (*Picea mariana*) forests formed on soils of this kind in interior Alaska, almost 100% of first-order fine roots were ECM and 84% of their production occurred within 20 cm of the surface (Ruess *et al.*, 2003). In a sandy podzolic system supporting pine (*Pinus sylvestris*) with an ericaceous understorey,

Persson (1983) observed that the presence of the shrubs influenced the distribution of ECM roots. Within the surface organic (O) horizon, fine roots were largely concentrated in the superficial L (litter) and F (fermentation) layers where the trees grew in the absence of understorey shrubs. However, in areas where they were present, ECM proliferation was depressed by a few centimetres towards the F-H (humus)-mineral soil transition.

An analysis of the distribution of *Picea abies* roots across a latitudinal gradient through Europe from the boreal to the temperate zones (Stober *et al.*, 2000) revealed that the bulk of the fine roots were located in the top 10 cm of the soil profile in every stand. However, there was some evidence of a greater proliferation in the organic horizons of the boreal (93%) than in two temperate sites (75% and 78%). Further, whereas most of the fine roots in the upper 10 cm of the profile were living, those in the deeper layers were mostly dead.

The advance of molecular technologies has now made it possible to combine analysis of fine root distribution with characterization of their fungal associations. Rosling *et al.* (2003), working in a boreal *P. sylvestris*-*P. abies* forest, confirmed the earlier observations that the superficial organic layers of the podzol are the most intensively exploited by fine roots (Figure 15.10). However, they also showed that considerable numbers of ECM roots occur in the mineral horizons and that half of the identified fungal taxa were associated with these substrates (Table 15.3). The mineral horizons most extensively occupied by ECM roots were the eluviated

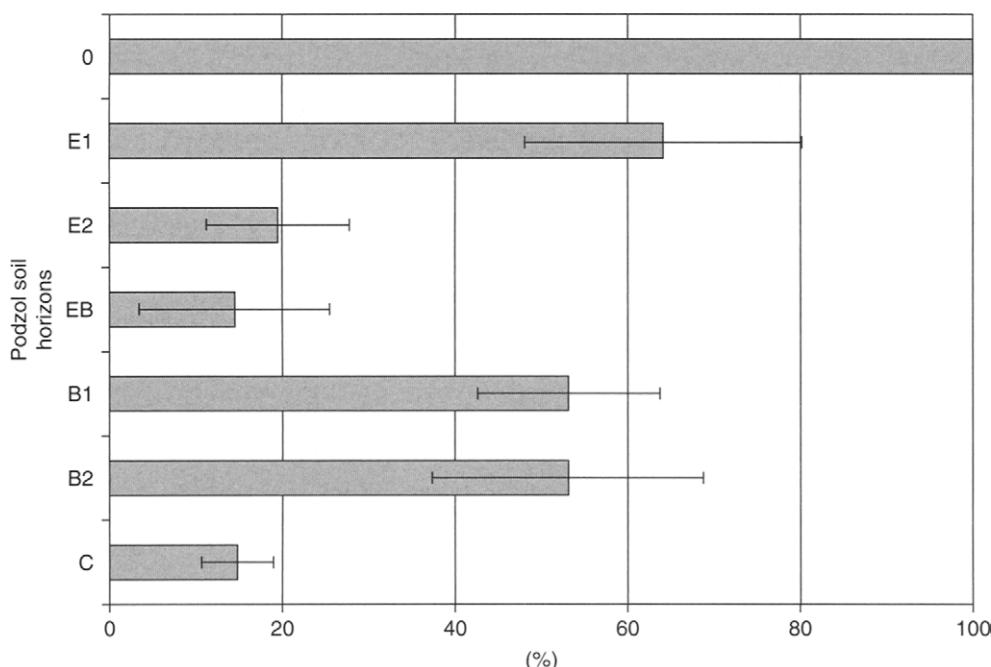


Figure 15.10 Average total number of root tips in each soil horizon of a boreal forest podzol (O = organic; E1, E2, EB = eluviated; B1, B2 = illuviated; C = subsoil) expressed as the percentage of total number of root tips in the organic horizon. From Rosling *et al.* (2003).

Table 15.3 Vertical distribution of ectomycorrhizal fungal taxa in a podzol soil profile.

	O	E1	E2	EB	B1	B2	C
<i>Tylospora</i> spp.	X	X	X	X		X	X
<i>Cortinarius</i> spp.	X		X	X	X	X	X
<i>Piloderma reticulatum</i>	X	X	X	X		X	X
<i>Piloderma</i> spp. JS15686	X	X			X	X	
<i>Piloderma byssium</i>	X						
<i>Inocybe</i>	X						
<i>Tomentellopsis submollis</i>	X	X					
<i>Piloderma fallax</i>	X	X	X				
<i>Hygrophorus olivaceoalbus</i>	X	X	X				
<i>Russula decolorans</i>	X	X	X	X			
<i>Dermocybe</i> spp.	X	X	X	X			
Tomentelloid			X				
<i>Lactarius utilis</i>			X	X	X	X	
<i>Piloderma</i> spp.2			X	X	X	X	
<i>Piloderma</i> spp.3				X	X	X	
<i>Piloderma</i> spp.1				X	X	X	
<i>Suillus luteus</i>			X	X	X	X	X
unID ≠ 15						X	
unID ≠ 12						X	
<i>Wilcoxina</i>							X
<i>Russula adusta</i>							X
<i>Tricholoma portentosum</i>							X

Data from Rosling *et al.* (2003). O, organic horizon; E1 and E2, elluviated horizon; B1 and B2, illuviated horizons; C, subsoil; unID, unidentified.

(E1), located immediately below the O horizon and both of the illuviated (B) layers. These are regions from which organic materials are leached (E1), or into which they may be precipitated (B1 and B2). It is of particular interest that the fungal communities occupying the organic and mineral horizons are distinct (Table 15.3). While *Dermocybe* spp., *Tomentellopsis submollis*, and three *Piloderma* spp. were found predominantly in the organic horizon, *Suillus luteus*, *Lactarius utilis* and three different species of *Piloderma* were associated with the mineral layers.

Further recent studies, one using DNA analysis of hyphal fragments recovered from the L, F, H and B horizons of a pine forest soil (Dickie *et al.*, 2002), another a combination of sequencing with morphotyping in boreal forest substrates of different quality (Tedersoo *et al.*, 2003) and one using morphotyping alone (Koide and Wu, 2003), indicate clustering of distinctive types of ECM fungi in specific niches of coniferous forest soils. In the study of Tedersoo *et al.* (2003), a strong preference of resupinate thelephoroid and atheliod fungi for woody debris was observed.

While proliferation of the mycelia of individual fungi in association with substrates of a particular resource quality have been described previously (Carleton and Read, 1991; Agerer, 1991b; Perez-Moreno and Read, 2000, see below), interspecific differences in occupancy of soil horizons and substrates had not been fully appreciated until recently. It may be of considerable importance if it turns out that these patterns reflect different potentials for exploitation of the different substrates (see below). Lindahl *et al.* (2007) have used DNA-based methods in combination with ^{14}C dating of the organic matter, measurements of C:N ratios and of $\delta^{15}\text{N}$ natural

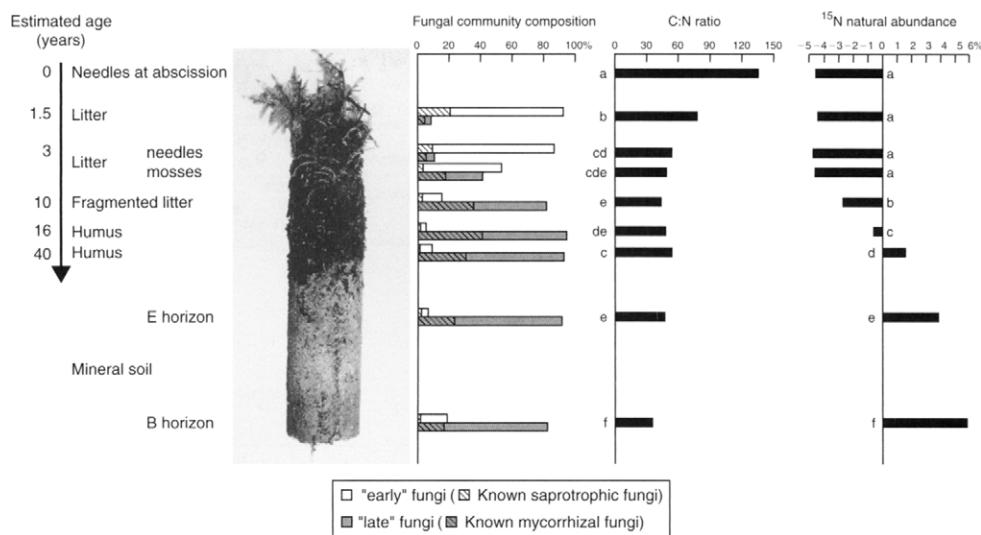


Figure 15.11 Fungal community composition, carbon:nitrogen (C:N) ratio and $\delta^{15}\text{N}$ natural abundance ($\delta^{15}\text{N}$) throughout the upper soil profile of a Scandinavian *Pinus sylvestris* forest. Different letters in the diagram indicate statistically significant differences between the horizons in C:N ratios and $\delta^{15}\text{N}$ abundance, and the standard error of the mean was $<0.3\%$ for $\delta^{15}\text{N}$ natural abundance and <3 for C:N ratio ($n = 19\text{--}27$, for recently abscised needles, $n = 3$). The age of the organic matter is estimated from the above average $\Delta^{14}\text{C}$ of three samples from each horizon (five samples of the litter 2 (needles) fraction) and needle abscission age (3 years) is subtracted. Community composition data are expressed as the frequency of total observations. 'Early' fungi are defined as those occurring with a higher frequency in litter samples compared with older organic matter and mineral soil. 'Late' fungi are those occurring with a higher frequency in older organic matter. From Lindahl *et al.* (2007).

abundances to discriminate between different functional groups of fungi involved in C and N cycling down a boreal forest podsol profile. A clear shift in fungal community composition was identified between the surface litter (L horizon) and the underlying F horizon in which the litter had lost its structural integrity (Figure 15.11). The recently-shed litter (less than 4 years old) near the surface of the profile was dominated by saprotrophic fungi, among which non-mycorrhizal ascomycetes of the Helotiales were prominent. This was a zone in which organic C was mineralized and N was retained. ECM fungi, members of a group referred to as 'late fungi' (Figure 15.11), first appeared as a major component of the fungal community in older fragmented litter and humus in the fermentation horizon. The humus zone was characterized by small increases of C:N ratio with age of organic matter, indicating removal of N by organisms using root-derived C. Selective exploitation of N is suggested by the large increases of $\delta^{15}\text{N}$ at this level. This enrichment of $\delta^{15}\text{N}$ is thought to be driven by fractionation against the heavier isotope during transfer of N from soil through ECM fungi to their host plants (Högberg *et al.*, 1996, 1999; Lindahl *et al.*, 2002; Berg and McClaugherty, 2003; Hobbie and Colpaert, 2003; Hobbie *et al.*, 2005). Among the first of the ECM fungi to appear in the profile were *Cortinarius* spp., which selectively colonized dead moss shoots. Selective exploitation

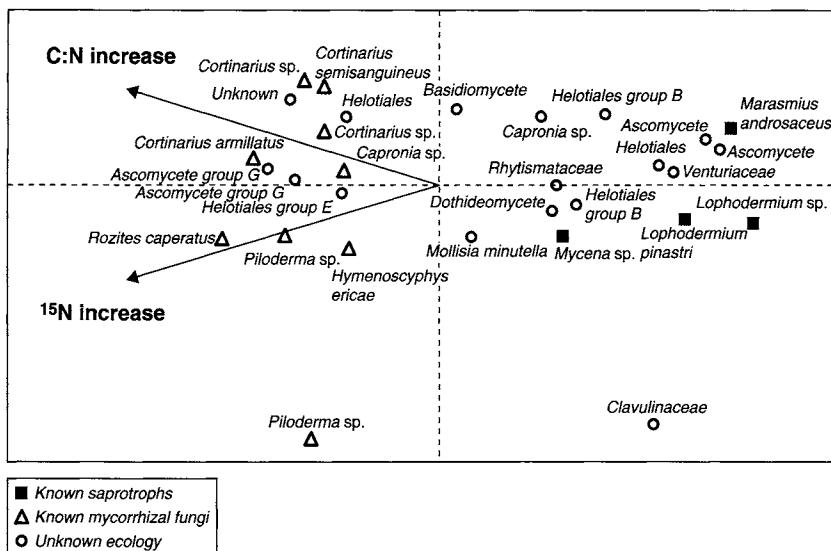


Figure 15.12 Canonical correspondence analysis depicting the relationship between fungal community composition and changes in carbon:nitrogen (C:N) ratio and ^{15}N natural abundance in a boreal forest soil. The arrows represent increasing C:N ratio or ^{15}N abundance with depth and age of the organic matter. ■, known saprotrophic taxa; △, known ectomycorrhizal taxa; ○, taxa with unknown ecology. From Lindahl *et al.* (2007).

of senescent parts of bryophyte shoots by ECM fungi has been reported previously (Carleton and Read, 1991, see Figure 15.16; Genney *et al.*, 2006) and may be particularly important in those boreal systems characterized by a dominance of feather mosses in the forest floor community. As in the case of the Rosling *et al.* study, the species composition of the ECM assemblage changed in the transition from organic to deeper mineral horizons. In a canonical correspondence analysis in which shifts of fungal community composition were compared to changes in the C:N ratio and ^{15}N content of the soil horizons (Figure 15.12), it was shown fungal taxa known to be ectomycorrhizal were most strongly associated with positive changes of C:N and large increases in ^{15}N natural abundance. Saprotrophic fungi, in contrast, were associated with negative changes in C:N and only small changes of ^{15}N natural abundance.

Despite the complexities involved in interpretation of tissue $\delta^{15}\text{N}$ signatures, data that have emerged from recent studies enable some cautious generalizations to be made about the likely patterns of use of soil N sources both within the ECM functional group and between this group and saprotrophic fungi. These are based upon awareness that $\delta^{15}\text{N}$ fractionation in the course of mineralization of soil N leads to a depletion of $\delta^{15}\text{N}$ in the mineral sources of the element and an enrichment in the recalcitrant organic N compounds of the humus (Nadelhoffer and Fry, 1994; Kohzu *et al.*, 1999). Some comparative analyses of the ECM and the saprotrophic groups have suggested that the former are normally enriched in $\delta^{15}\text{N}$ relative to the latter (Hobbie *et al.*, 1999, 2001; Kohzu *et al.*, 1999), the implication being that ECM fungi are using relatively more of the recalcitrant N source. Deeper insights have been

gained from studies in which the $\delta^{15}\text{N}$ signatures of the substrates have been directly compared with those of the fungi growing upon them (Gebauer and Dietrich, 1993; Gebauer and Taylor, 1999). These suggest that gross distinctions between N-use patterns of ECM and saprotrophic groups on the basis of their different $\delta^{15}\text{N}$ signatures may be oversimplistic. Comparing $\delta^{15}\text{N}$ signatures of soil components considered to be supporting mycelia of different fungi with those of their sporocarps, Gebauer and Taylor (1999) identified four functional groups of fungi in a Central European mixed forest environment. ECM fungi known to be capable of using organic N, and saprotrophs capable of using humus N made up two of these groups, both of which had sporocarps enriched in $\delta^{15}\text{N}$. The remaining two consisted of ECM fungi lacking the ability to use polymeric N sources and saprotrophs growing on decomposing wood. In both of these, sporocarps showed depletion of $\delta^{15}\text{N}$. The suggestion is clearly that while the $\delta^{15}\text{N}$ signature reflects that of the N source used, it may not provide an adequate discrimination between ECM and saprotrophs as functional groups.

Further evidence in support of the view that the $\delta^{15}\text{N}$ signatures of ECM sporocarps reflected those of the substrate has been provided by comparing relationships between these signatures measured in field-grown specimens and the ability of the same fungi to use organic N when grown in pure culture (Lilleskov *et al.*, 2002a). Taxa such as *Tricholoma inamoenum*, which grew readily on protein and which have been shown to be sensitive to the presence of high levels of inorganic N in soils (Lilleskov *et al.*, 2001), had the highest sporocarp $\delta^{15}\text{N}$ contents, while those, including *Laccaria* spp., which are relatively insensitive to high mineral N inputs and failed to use organic N in culture, showed the lowest levels of $\delta^{15}\text{N}$ enrichment.

It appears from data presented in Chapters 9 and 10 that ECM fungi isolated from boreal and temperate habitats are little different from saprotrophs in their preferences for and abilities to assimilate the mineral forms of N and P. When mineral forms are present in the soil solution the major function of the mycobiont is to facilitate their capture by providing a dynamic and extensive absorbing system in the form of the extraradical mycelial network. However, it has become increasingly evident that these elements and, in particular, mineral N are often present in such low amounts in the soils of these systems as to limit their productivity (Tamm, 1991). As a consequence of this recognition, increasing attention has been paid over the last two decades to the possibility that ECM fungi of boreal and temperate biomes may have the abilities to degrade some of the macromolecular organic residues present in their immediate natural environments, so gaining access to additional sources of N and P (Read and Perez-Moreno, 2003).

Attempts to deduce the wider functional capabilities of ECM fungi have relied heavily upon analysis of their abilities to mobilize more complex substrates as expressed in pure culture (see Chapters 8, 9 and 10). Because the readily culturable fungi which have been used for these studies represent only a tiny minority among the many species now known to be able to form mycorrhizas, circumspection is required when attempting to interpret the results obtained from them. Nonetheless, it is evident that some abilities to degrade structural polymers representative of those present in plant and fungal residues have been detected. Activities of these kinds can be expected to expose N and P sources that are locked into or protected by the polymeric carbon frameworks. However, the abilities of ECM fungi to perform decomposer functions should not be exaggerated. When comparisons are made between

this functional group of fungi and those representative of the ericoid symbiosis (Bending and Read, 1996a, 1996b; see Chapter 11) or saprotrophs (Maijala *et al.*, 1991; Colpaert and van Laere, 1996; Colpaert and van Tichelen, 1996), the abilities of ECM fungi to depolymerize complex C sources are invariably lower than those of the other groups. The failure of most ECM fungi to penetrate the cell walls of their autotrophic partners may be a reflection of their low cellulolytic capabilities. Furthermore, claims that genes indicative of ligninolytic potential have been detected in ECM fungi (Chen *et al.*, 2001) have now been retracted (Cairney *et al.*, 2003). There remains the possibility that some of the so-far unculturable ECM fungi will be shown to express ligninolytic activity, but the likelihood would seem to be that, after making the switch from the saprotrophic to the mycorrhizal habit, these capabilities were lost (see Chapter 6).

On the basis that ECM roots and their associated mycelia proliferate selectively in organic layers present immediately below the surface litter horizons of boreal and temperate forests, several experiments have now investigated the extent of nutrient mobilization from these types of substrate. Entry *et al.* (1991a) compared N and P contents of organic matter colonized by the mat-forming fungus *Hysterangium setchellii* with those of adjacent uncolonized material. They observed a greater than 30% reduction of both elements in the organic residues colonized by the fungus (Table 15.4). Laboratory mesocosm experiments (Bending and Read, 1995a) (see Colour Plate 6.2) investigating the nutrient mobilizing properties of *Suillus bovinus* and *Thelephora terrestris* growing from pine (*P. sylvestris*) to forage in pine litter collected from the FH horizon-derived, revealed that both fungi were able to export some N and P from the organic matrices but that there were interspecific differences between them (Table 15.4). *S. bovinus* exported significantly more of both elements than *T. terrestris*. When these properties were again examined using *Betula pendula*-*Paxillus involutus* systems and a range of litter types, a marked exploitation of litter P resources but little reduction of their N contents was observed (Perez-Moreno and Read, 2000) (Table 15.4). Such differences between studies may reflect interspecific variation in the abilities of ECM fungi to mobilize N from litter or differences in the incubation times used in the different experiments. They emphasize that generalizations from one mycorrhizal system and substrate type to another must be made with great caution.

Koide and Wu (2003) buried residues of the L, F and H layers in a *Pinus resinosa* plantation and observed that a decrease of C:N ratio was still evident after 16 months of incubation. As they emphasized the duration of the C:N shift and the stage at which the potential for N release is greatest will be influenced by local environmental factors. Among these, availability of moisture, which in some sites can be reduced by the activities of the roots themselves, will be a significant factor affecting the extents and rates of microbial activity. While this activity is likely to be higher under the permanently moist and sometimes elevated temperatures of laboratory incubations, the mesocosm studies confirm the presence of pathways leading both to removal of N and P from litter by ECM fungi and to the increases in C:N and C:P ratios that arise as a consequence. Effective N and P removal during decomposition is indicated by the extremely small residual levels of these elements found in the deeper humic layers of these forest soils (Stevenson, 1982).

These observations leave open to question the identities of the N- and P-enriched substrates that are exploited by the mycorrhizal fungi in and around the FH layer. It is widely accepted that organic sources of N released into the humic soils will be rapidly co-precipitated with polyphenolic materials (Handley, 1954; Northup *et al.*, 1995)

Table 15.4 Nutrient mobilization expressed as percent loss of nitrogen (N) and phosphorus (P) from different organic natural substrates by ectomycorrhizal fungi grown in association with different host plants (bold characters) and in parallel controls with mycorrhizal mycelium absent or very weakly developed.

Type of substrate	Nutrient mobilization (%)			Host plant – ectomycorrhizal fungus combination	Reference
	N	P	Time (days)		
Plant detrital materials					
Douglas fir litter	32	33	365	<i>Pseudotsuga menziesii</i> –mats of <i>Hysterangium setchellii</i>	Entry et al., 1991b
Douglas fir litter	16	19	365	Control (no host plant)–mycorrhizal hyphal mats absent	
Pine FHM	23	22	120	<i>Pinus sylvestris</i> – <i>Suillus bovinus</i>	Bending and Read, 1995a
Pine FHM	13	3	120	<i>Pinus sylvestris</i> – <i>Thelephora terrestris</i>	
Pine FHM	5	0	120	Control (no host plant)–mycorrhizal fungus absent	
Birch FHM	0	40	90	<i>Betula pendula</i> – <i>Paxillus involutus</i>	Perez-Moreno and Read, 2000
Pine FHM	1	35	90	<i>Betula pendula</i> – <i>Paxillus involutus</i>	
Beech FHM	14	37	90	<i>Betula pendula</i> – <i>Paxillus involutus</i>	
Pine FHM	25	63	90	<i>Betula pendula</i> – <i>Pinus sylvestris</i> linked by <i>Paxillus involutus</i>	Perez-Moreno and Read (unpublished data)
Pine FHM	25	54	90	<i>Betula pendula</i> – <i>Pinus sylvestris</i> linked by <i>Paxillus involutus</i>	
Pollen					
Pine pollen	76	97	115	<i>Betula pendula</i> – <i>Paxillus involutus</i>	Perez-Moreno and Read, 2001a
Pine pollen	42	35	115	Control (non-mycorrhizal plant)–mycorrhizal fungus absent	
Soil animals					
Nematodes	68	65	150	<i>Betula pendula</i> – <i>Paxillus involutus</i>	Perez-Moreno and Read, 2001b
Nematodes	37	25	150	Control (non-mycorrhizal plant)–mycorrhizal fungus absent	

Note: FHM, fermentation-horizon material.

and laboratory based results (Bending and Read, 1996a; Wu *et al.*, 2003) indicate that the ECM fungi tested so far have a very low ability to release N and P from such complexes. As a result, the inherently large requirement of ECM fungi, particularly for N, would be expected to drive their scavenging activities in the direction of N sources that are not co-polymerized or precipitated with polyphenolic materials. In boreal and many temperate forest soils that are heavily loaded with phenolic residues, this must require that the fungi attack N-enriched substrates before they become involved in the immobilization processes.

In recognition of these constraints, a new generation of experiments has investigated the abilities of selected ECM fungi to mobilize N contained in substrates that are both of low phenolic content and likely to be present in quantitatively significant amounts in boreal forest soils. Among the materials used in these studies are dead mycelia (necromass) of the ECM fungi themselves (Andersson *et al.*, 1997), pollen (Perez-Moreno and Read, 2001a), seeds (Tibbett and Sanders, 2002) and quantitatively important representatives of the boreal soil mesofauna, namely nematodes (Perez-Moreno and Read, 2001b) and collembolans (Klironomos and Hart, 2001). The rapidity and intensity with which the mycobionts colonize these nutritionally enriched substrates is striking (see Colour Plate 6.3b). In the case of both pollen and nematodes, the colonization leads to significant removal of both N and P from the source materials relative to that lost from the same materials colonized only by saprotrophic fungi (see Table 15.4).

Broadly based types of characterization which see N mobilization by ECM fungi as a key nutritional feature of boreal forests run the risk of obscuring more subtle patterns associated with distinctive and localized changes of soil condition. Nowhere have such subtleties been better demonstrated than in analyses of local-scale gradients across which, for geological, topographic or hydrological reasons, soil pH, N form and understorey vegetation change dramatically. Along one such gradient of 90 m length at Betsele in northern Sweden a transect revealed a shift from extreme acidity (soil pH 3.5) at one end which was rainwater fed, to near neutrality (soil pH 6.4) at the other, where base-enriched groundwater was discharged (Giesler *et al.*, 1998; Höglberg *et al.*, 2003) (Figure 15.13a, b, c). The acidic to neutral pH gradient was also one of markedly decreasing C to N ratio (Höglberg *et al.*, 2003, 2006). Along the transect, large changes in the structure and composition of the overstorey and ground flora as well as the mycorrhizal and general microflora occur. These appear to be driven by an N-source gradient, in which organic N dominated at the acidic end, with mineral N, predominantly ammonium, in mid-gradient and nitrate at the discharge end. Pine (*P. sylvestris*) with ericaceous dwarf shrub understorey at the acidic end was progressively replaced by spruce (*P. abies*), first with a short herb and then with a tall herb community in the nitrate-enriched zone. While neither soil respiration nor total soil microbial biomass changed along the gradient, analyses of the phospholipid fatty acid (PFLA) signatures (see Chapters 2 and 6) of this biomass suggested that the physicochemical transition was driving significant qualitative changes in the nature of the microbial populations (Höglberg *et al.*, 2003, 2006). These signatures showed drastic decline in fungus to bacteria ratio as pH (Figure 15.14a) and mineral N availability (Figure 15.14b) increased across the gradient. They also indicated a shift in the predominant fungal population from one in which ECM and ERM fungi were likely to be the dominants in the dwarf shrub-pine and short herb environments (Figure 15.15a) to one in which the putative AM fungal

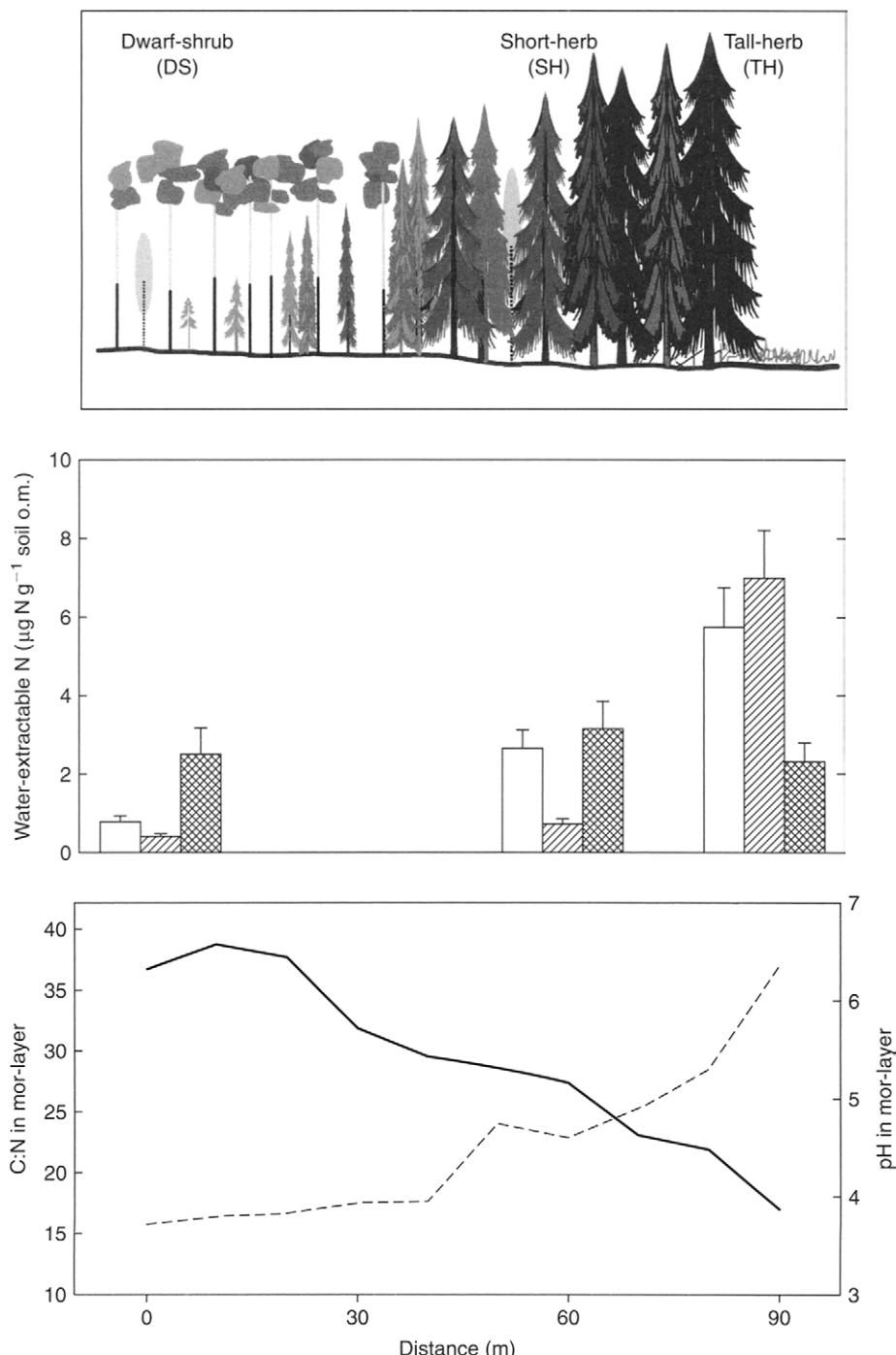


Figure 15.13 The Betsele plant productivity gradient: (a) Forest types: dwarf shrubs understorey (DS) between 0 and 40m, short herb understorey (SH) between 50 and 80m and tall herb understorey (TH) at 90m. (b) water-extractable N forms: open bars, ammonium; hatched bars, nitrate; crossed bars, amino acids. (c) the C:N ratio of the mor-soil (solid line) and pH of the soil solution (dashed line). From Höglberg et al. (2003).

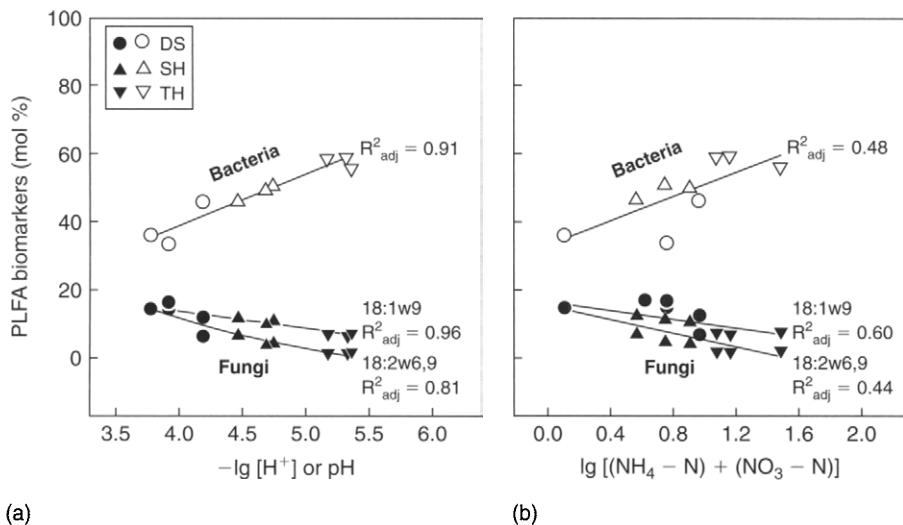


Figure 15.14 Partitioning between major functional microbial groups in forest with three types of understorey. The relationship between the bacterial and the fungal signature lipid biomarkers and (a) $-\log_{10}(H^+)$, i.e. soil pH and (b) \log_{10} (inorganic N). Twelve PLFAs were used as biomarkers for bacteria and two PLFAs for fungi. DS, dwarf shrub; SH, short herb; TH, tall herb. From Högberg *et al.* (2007).

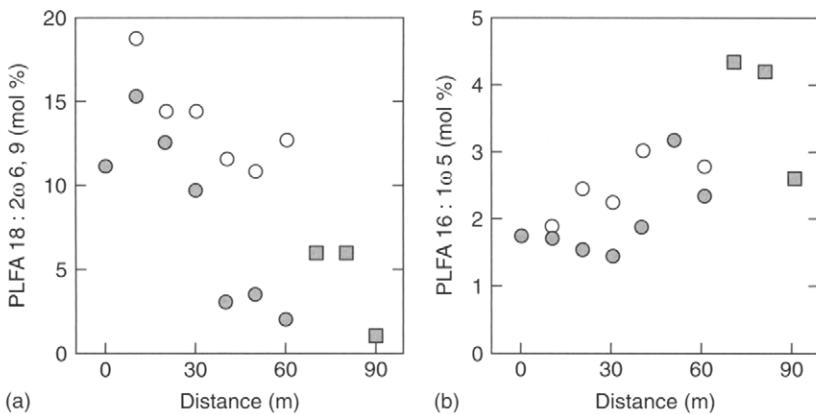


Figure 15.15 Mol percentage of individual phospholipid fatty acids (PLFAs) in the F horizon (O), H horizon (●), and F + H horizon (■) of the mor layer along the Betsele forest gradient. (a) The ERM + ECM fungal indicator 18:2ω6,9; (b) The AM fungal indicator 16:1ω5. From Högberg *et al.* (2003).

signature 16:1ω5c dominated in the tall herb community (Figure 15.15b). These studies appear to confirm the predominant effect of N availability as a determinant both of the type and functions of mycorrhizas in the boreal biome. Högberg *et al.* (2003) hypothesize that under conditions of low N supply at the acidic end of the gradient, autotrophs are induced to increase allocation of photosynthate below ground. This, while stimulating the activity of their fungal symbionts, will in turn contribute to

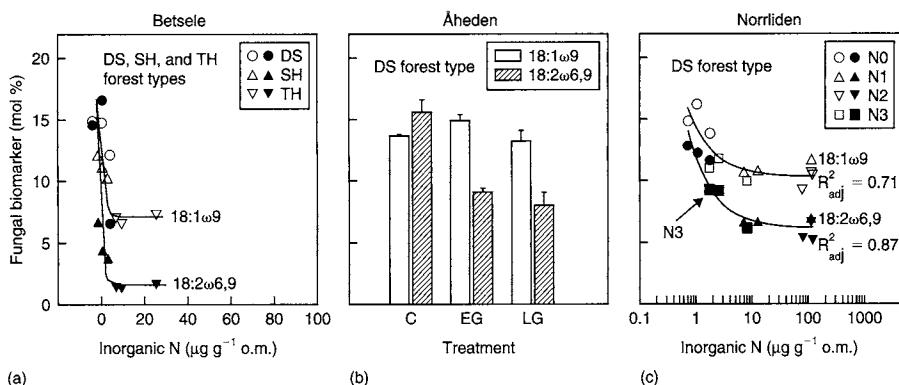


Figure 15.16 The effects of soil N concentration and tree girdling upon the absolute quantities and relative amounts of the two phospholipids fatty acid (PLFA) fungal biomarkers 18:1 ω 6,9 and 18:2 ω 6,9 in boreal forests. (a) The marker 18:2 ω 6,9 declines more strongly than 18:1 ω 6,9 along the natural gradient of increasing mineral N from dwarf shrub (DS), through short herb (SH) to tall herb (TH) communities at Betsele. (b) Whereas the 18:1 ω 6,9 signature does not decline in response to early (EG) or late (LG) stem girdling, that of the 18:2 ω 6,9 is reduced by c.50% in the Åheden experiment with dwarf shrub (DS) understorey. (c) Application of mineral N as a fertilizer at three levels (N1, N2, N3-plus control N0) leads to progressively larger reductions of 18:2 ω 6,9 relative to 18:1 ω 6,9 in another dwarf shrub forest type at Norrliden. From Högberg *et al.* (2007).

the production of litter of high C:N ratio. In contrast, it is generally accepted that in boreal forest systems, increases of N loading, however they may arise, lead to a reduction in C allocation belowground (Waring and Running, 1998). This may be to the relatively strong detriment of ECM fungal symbionts with high C demand, while having lesser effects upon bacteria and AM fungi. Such a scenario is one that again fits with determinations of PLFA signatures in this type of ecosystem. Högberg *et al.* (2006) observed that of the two markers 18:1 ω 6,9 and 18:2 ω 6,9 considered to represent the ECM and ERM mycelial biomass, the 18:2 ω 6,9 was strongly sensitive to availability of mineral N. This was the case not only along the natural gradient of N concentrations at Betsele (Figure 15.16a), but also under three levels of mineral N application in a nearby pine-dwarf ericaceous shrub community (Figure 15.16c). The pattern of differential response of the two markers was repeated following girdling of pine with an ericaceous understorey at Åheden (Figure 15.16b). Since, at this last site, C supply to the ECM community was specifically targeted by the treatment, the indication emerges that the 18:2 ω 6,9 is a better marker for ECM fungi. The extent to which the 18:1 ω 6,9 signature encompasses fungi of the ERM and of saprotrophic habit remains to be elucidated. In combination, these observations suggest that under circumstances where C supply from their autotrophic partners is maintained, the structures of the fungal symbiont populations in boreal forests are largely determined by qualitative and quantitative aspects of soil N economies.

Notwithstanding the observation (Figure 15.15b) that under the localized influence of raised pH and N enrichment there can be enhancement of the AM mycelial component of boreal forest soils, there have been few measurements of the extent or function of AM colonization in the herb communities that develop under these circumstances.

Opik *et al.* (2003) observed that *Pulsatilla* spp. growing in soils of pH 8.6 in the boreal region in Estonia were extensively colonized by AM fungi. While there were site-dependent differences in AM fungal community composition, preliminary analysis of different *Pulsatilla* spp. indicated that the associations lacked specificity. When seed of two species of the genus were sown in soil either with or without AMF inoculum, lower establishment was observed in the AM inoculum circumstance (Moora *et al.*, 2004). In this study, an AMF inoculum from grassland was more effective than one from boreal forest soil in colonizing roots, promoting biomass production and elevating P concentration in one *Pulsatilla* species, whereas a boreal forest inoculum was slightly more effective in another. It was concluded here that the distinctive responses of the congeneric species may be attributable to fungal specificity. Further studies of these kinds are required because the herbaceous communities of the boreal forest contain a high proportion of the plant biodiversity seen in the biome.

While P is rarely the primary growth limiting element in such communities, it is of interest that the processes involved in its immobilization and release in litter appear to be the same as those of N (Berg and McClaugherty, 1989). As in the case of N, the onset of energy limitation in the saprotroph population should enable ECM fungi, with their ability to produce a range of phosphomono- and di-esterase (see Chapter 10) to compete effectively for P as well as N. Indeed, the retention of the ECM habit in mull humus forests, in many of which active nitrification occurs (Aber *et al.*, 1989; Ellenberg, 1988), may be attributable to the ability of such fungi to release P from the organic residues. In addition to the general observation that ECM mycelia proliferate most intensively in material from the FH horizon, there is evidence at a finer scale of hyphal growth patterns which are likely to provide intimate contact with resources of a particular quality. Ponge (1990), using the light microscope, observed selective exploitation of pine needles, *Pteridium* leaflets and animal corpses by hyphae of ECM fungi. In an attempt to simulate conditions prevailing in the ECM conifer-feather moss communities that cover large area of the boreal forest zone, Carleton and Read (1991) grew mycorrhizal *Pinus* seedlings in association with the feather moss *Pleurozium schreberi*. Hyphae of the fungal symbiont *Suillus bovinus* selectively colonized and formed a sheath-like structure around senescing parts of the moss shoot (Figure 15.17). It was shown that such colonization provided the potential to capture resources from the moss shoot. In nature, this colonization is likely to provide a key link in the nutrient cycle since most of the elements arising at the forest floor in these ecosystems are intercepted by the moss carpet.

At an even finer scale, Agerer (1991b) has demonstrated structural modifications of the hyphal tips of ECM fungi at their point of contact with particular materials in soil. An appressorium-like structure attaches the tip of its substrate (Figure 15.18) providing an enlarged surface of contact for biochemical interaction. It seems that the walls at the tips of ECM hyphae are less hydrophobic than they are behind the tip (Unestam and Sun, 1995; Sun *et al.*, 1999). It is likely to be at the tip that most of the active interactions with the environment occur. In view of the long-distance transport role of the hyphae, which serve as pipe-lines through which movement of C and minerals occurs to and from the growing tip, the need for their walls and membranes to be effectively impermeable at maturity may be paramount.

In addition to interactions with organic materials, the possibility that release of protons, organic anions or CO₂ from hyphal tips might lead to the release of minerals has received increasing attention. The recognition (see above) that in podzolic

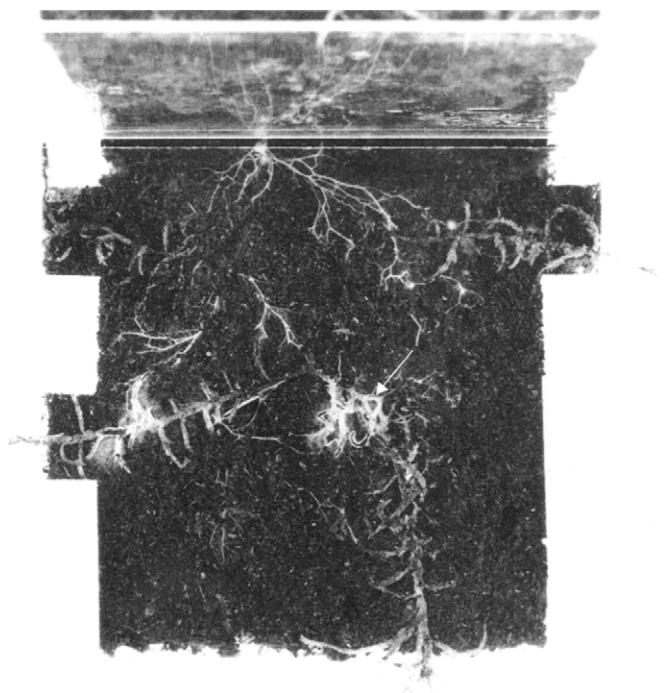


Figure 15.17 Observation chamber showing the colonization of senescent parts of the shoot of the feather moss, *Pleurozium schreberi*, by mycelium of *Suillus bovinus* (arrowed, growing from a colonized plant of *Pinus contorta* (not shown). From Carleton and Read (1991), with permission.

soils a significant proportion of ECM roots can occur in the mineral horizons and that some of the fungi involved are localized in this environment has led to the suggestion that they may be directly involved in mineral dissolution and so contribute to the podsolization process (van Breemen *et al.*, 2000b; Smits, 2006; Wallander, 2006). Interest in this aspect of mycorrhizal physiology was strengthened by the description of small (3–10 µm wide) tunnel-like features in hornblende and feldspar grains of Swedish boreal forest podsol soils (Jongmans *et al.*, 1997). It was postulated that the tunnels were created by mineral dissolution through the activities of low molecular weight organic acids released from ECM hyphae. It was further suggested that the products of dissolution, in particular P from apatite inclusions in feldspars, as well as Ca, Mg and K, if transported to the roots, would enable a bypass of the bulk soil solution (van Breemen *et al.*, 2000a; Landeweert *et al.*, 2001). Further circumstantial evidence in favour of a possible involvement of these hyphae in tunnel formation was provided by the description of a positive linear relationship between tunnel length and density of ECM root tips (Hoffland *et al.*, 2003) and the observation that the tunnels are almost entirely restricted to ECM forests of boreal and temperate biomes (Hoffland *et al.*, 2002). To date, however, there appears to have been no direct observational or experimental verification of the involvement of ECM hyphae in tunnel formation. Hyphae occupy less than 1% of the tunnels observed and there is no confirmation that these are of ECM origin. Further, calculations (Smits, 2006) and models (Sverdrup *et al.*, 2002) indicate that tunnelling

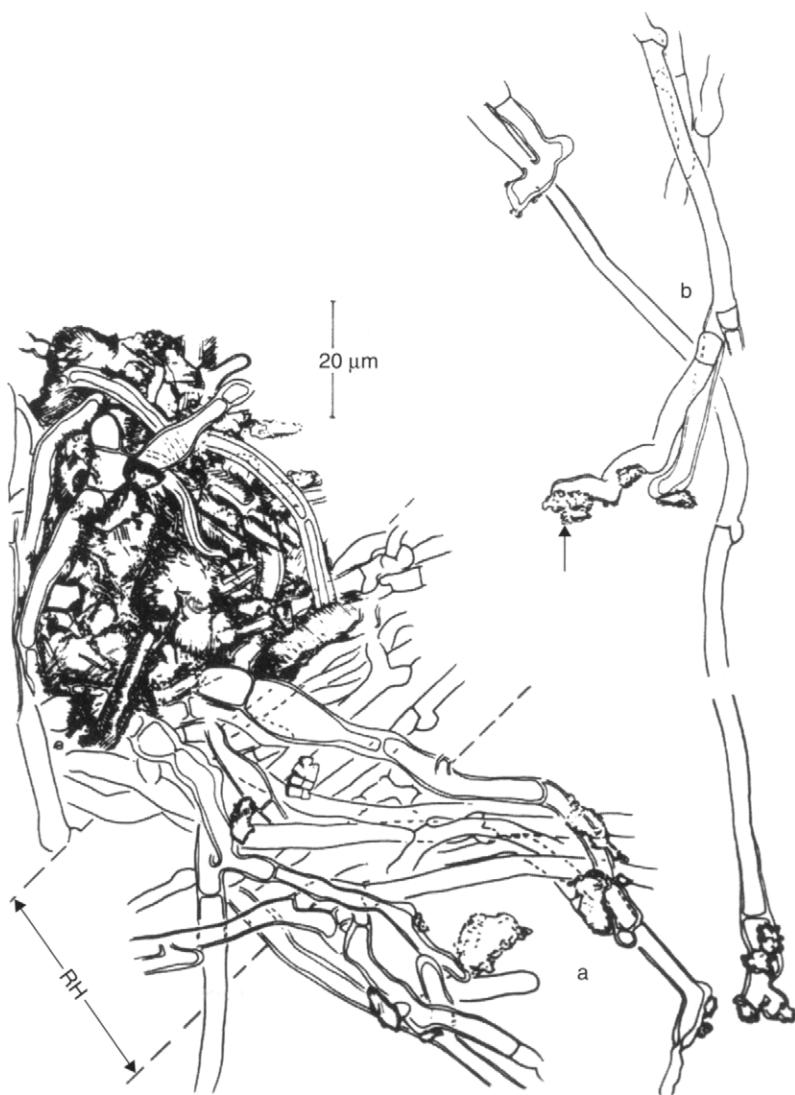


Figure 15.18 Thick-walled hyphae of *Sarcodon imbricatus*: (a) growing into a humus particle. (b) The hyphae in close contact with the soil debris are thick-walled and have somewhat swollen, appressorium-like tips adhering to the soil particles (arrowed). From Agerer (1991), with permission.

of mineral grains contributes very little to total weathering in soils. The possibility remains, however, that in view of the generic property of fungi to release low molecular weight organic anions (Gadd, 1999), as well as to acidify their environments through proton release (Rosling *et al.*, 2004b), the ECM fungal community may be influential in broader soil mineralization processes (see Chapter 10).

The threats posed to boreo-temperate forest biomes by anthropogenically-related increases of atmospheric C and of N deposition are increasingly recognized and the responses of ECM systems to elevation of both elements have been investigated. It

has been shown that, under conditions of CO₂ enrichment, the relative abundance of ECM morphotypes changes significantly, there being a shift towards types with greater quantities of rhizomorphs and extramatrical mycelium (Godbolt *et al.*, 1997; Godbold and Berntson, 1997). Doubling of CO₂ concentrations around shoots of *Betula pendula* was shown to stimulate growth of ECM mycelium of both *Paxillus involutus* and *Suillus bovinus* in microcosms but, since *P. involutus* was more responsive to the treatment than *S. bovinus*, the need to be aware of interspecific effects on the ECM community was again emphasized (Rouhier and Read, 1998). Through its effects upon availability of C, enhancement of ambient CO₂ can be expected to influence the production and longevity of mycorrhizal roots and associated mycelia. Rygiewicz *et al.* (1997) observed that elevation of CO₂ concentration over microcosms supporting ECM *P. ponderosa* led to enhancement of production but to no impact upon the life span of roots. The observation that abrupt rather than gradual exposure to CO₂ can produce large and probably unrealistic responses (Klironomos *et al.*, 2005; see Chapter 2) should be borne in mind when interpreting results of the experiments reported above.

The responses of ECM systems to elevated CO₂ have been the subject of recent reviews (Gorissen and Kuyper, 2000; Treseder and Allen, 2000; Rillig *et al.*, 2002a) as well as of meta-analyses (Treseder, 2004; Albertson *et al.*, 2005). Conclusions from the analyses made to date must be made with caution because both the experimental conditions and the responses observed in studies of CO₂ impacts vary greatly. This situation reflects that seen in studies of AM systems (see Chapter 2). Despite the variability, taken overall, the results indicate that both the ECM plants and their fungal partners respond positively to elevation of ambient CO₂ levels. Perhaps not surprisingly, however, in a given symbiont pairing the extent of the response seen in the autotroph and the fungus can be quite different (Albertson *et al.*, 2005). Response ratios calculated on the basis of a large number of morphological and physiological parameters, indicate that the activities of the fungal partners in the ECM symbiosis are particularly stimulated by CO₂ enrichment. The calculations of Albertson *et al.* (2005) indicate that across the experiments ECM fungi showed a 34% response-ratio increase under elevated relative to ambient CO₂ exposure. Since this value is considerably larger than the 21% increase seen in experiments involving AM fungi, it can be predicted that ecosystems dominated by ECM partnerships will be more strongly affected by the predicted global increases of CO₂ than those consisting largely of AM symbionts. ECM plants are generally less CO₂-responsive than their fungal partners being, at +26%, only 1% more so than AM plants. In view of the greater responsiveness, particularly of ECM fungi, to elevated CO₂, Albertson *et al.* (2005) emphasize the need to incorporate myco-centric perceptions in considerations of ecosystem responses to the emerging CO₂ scenarios.

There has been a plethora of studies on the impacts of pollutant N enrichment on mycorrhizas of boreo-temperate forests. These have been extensively reviewed (Jansen and Dighton, 1990; Colpaert and van Tichelen, 1996; Wallenda and Kottke, 1998; Cairney and Meharg, 1999; Avis *et al.*, 2003) and will be only briefly described here. A decline of ECM sporocarp production was described as the first impact of N deposition (Arnolds, 1991). The involvement of N deposition in this decline was strongly implicated by experiments in which N fertilization of forest plots was shown to inhibit sporocarp yields (Rühling and Tyler, 1991; Termoshuizen, 1993). The effects of N enrichment observed on sporocarp production above ground are

likely to be a product of impacts of N on below-ground patterns of C allocation to ECM mycelial systems. Experimental analyses of these impacts have produced variable results. Some have shown that N additions to microcosms lead to modest increases of mycelial development (e.g. Arnebrandt, 1994), others to a decrease (e.g. Wallander *et al.*, 1994) and others to no effect (e.g. Wallander *et al.*, 1999). This is not surprising since, as described above, the impacts of N are likely to be mediated at least in part through its effects on canopy photosynthesis and C allocation. They will also be influenced by the initial N status of the system. N enrichment has been increasing slowly across the industrialized world over the last century so, as in the case of CO₂, experiments investigating its effects should reflect the long-term nature of the deposition processes. The cumulative effects of N deposition at the biome level have been examined in spruce (*Picea abies*) forests along a gradient of N deposition, and hence soil mineral N content, from pristine boreal forest environments in Northern Sweden where N mineralization is almost undetectable (Persson *et al.*, 2000) to heavily polluted stands in central Europe (Taylor *et al.*, 2000). As extractable soil N increased in a southerly direction, there was both a decrease in diversity of ECM fungi and a reduction in the proportion of mycobionts that are identified as having proteolytic potential (Figure 15.19). Associated with the enhancement of soil mineral N concentration was an increasing prevalence of the resupinate corticoid fungus *Tylospora fibrillosa* as an ECM symbiont, indicating that the decline of sporophore-producing ECM fungi in these polluted forests is a result of species replacement rather than of complete elimination of symbionts (Taylor *et al.*, 2000).

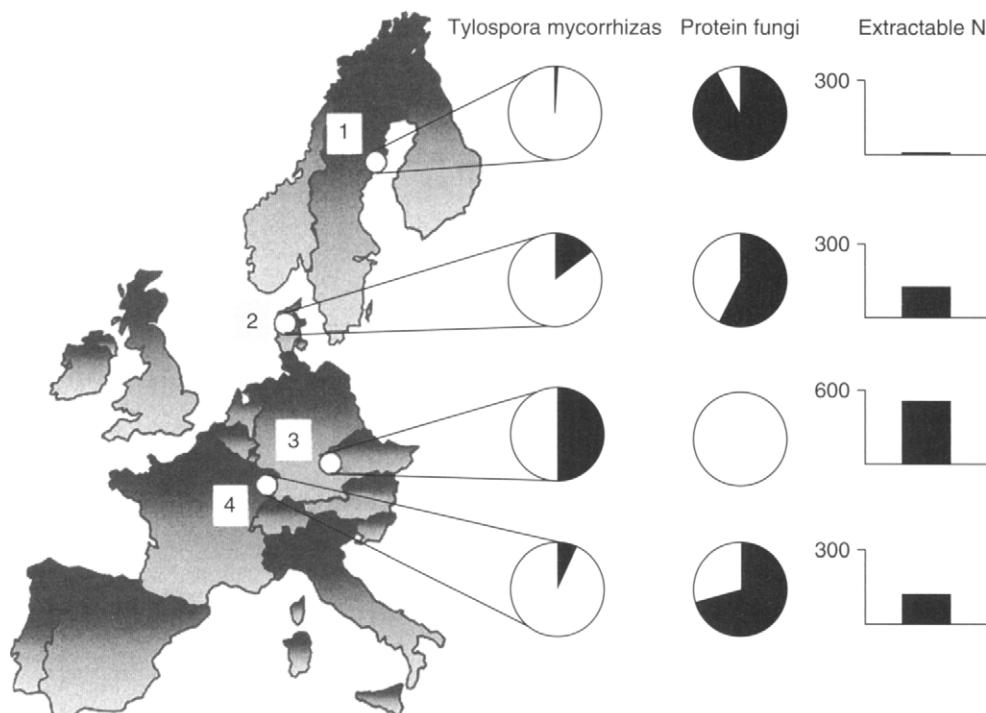


Figure 15.19 (Caption opposite)

An experimental application of N over 16 years in an oak-savanna system dominated by ECM oaks led to loss of diversity and a 50% reduction of overall sporocarp abundance indicating that effects of anthropogenic inputs are similar in boreal and more temperate systems. However, a distinctive feature of the oak savanna was that the treatment ($17\text{ g N/m}^2/\text{year}$) led to a 5-fold increase of sporocarp production by *Russula* spp. (Avis *et al.*, 2003). Such results are indicative of the likelihood that ecosystems may respond in subtly different ways to nutritional perturbations.

Mycorrhizas in tropical and subtropical biomes

Mycorrhizal associations in the wet tropics

Highly productive tropical rainforest ecosystems cover much of the equatorial belt spreading across South-Central America, West Africa and South-East Asia. These forests are widely threatened by anthropogenic activity, both directly through logging activities and indirectly as a result of global climate change. Sadly, despite these threats, we remain largely ignorant of some of the most basic aspects of rainforest biology. The gaps in our knowledge are nowhere better illustrated than in consideration of the mycorrhizal status of these systems. While we recognize that almost all rainforest trees so far examined have mycorrhizal symbionts and have made progress towards understanding of the distribution of mycorrhizal types (Alexander, 1989a), we are still better placed to raise questions about the identity and functions of the fungal symbionts involved than we are to provide answers (Janos, 1996; Alexander and Lee, 2005).

A defining feature of tropical rainforest is the enormous diversity of plant species in the communities. In the tree flora alone, Wright (2002) recorded 1175 species in a 0.52 km^2 plot of Borneo rainforest. This is more than occur in all of the boreo-temperate forests of the northern hemisphere. Within this diversity it is possible to recognize some patterns with respect to the distribution of mycorrhizal types. While most rainforest tree species associate with AM fungi, there are families of tropical plants which are predominantly or entirely ectomycorrhizal (Alexander, 1989a). These include the Dipterocarpaceae (Lee *et al.*, 1996), Fagaceae (Corner, 1972), many legumes in the Caesalpinoideae (Alexander, 1989b) and members of the subfamily Leptospermoideae in the Myrtaceae (Moyersoen *et al.*, 2001). Circumstantial evidence suggests that there is a relationship between mycorrhizal type and soil conditions. Whereas trees with

Figure 15.19 Summary of an analysis of the ectomycorrhizal communities at four Norway spruce (*Picea abies*) forests along a north-south European transect. Pie charts show the proportion of root tips at each site colonized by *Tylopoma fibrillosa* and the proportion of species isolates from each site that could utilize protein as a source of nitrogen (protein fungi, *sensu* Abuzinadah and Read, 1986a). None of the isolates from site 3 could utilize protein as an N source. The bar charts show KCl-extractable inorganic N ($\mu\text{g N/g LOI}$) in the LFH soil horizons (note that the scale at site 3 is twice that at the other three sites). Sites: 1, Åheden, northern Sweden; 2, Klosterhede, western Jutland, Denmark; 3, Waldstein, Fichtelgebirge, Germany; 4, Aubure, Vosges Mountains, NE France. Data from Taylor *et al.* (2000), Persson *et al.* (2000).

AM colonization predominate over large areas of such systems, there are localized occurrences in South American (Singer and Araujo, 1979, 1986; Henkel, 2003), West African (Newbery *et al.*, 1988) and South-East Asian (Whitmore, 1984) forests of communities dominated by ECM species. Although these are often restricted to the most nutrient-poor soils with a surface accumulation of litter and raw humus (Torti *et al.*, 2001; Henkel, 2003), deductions concerning possible ecological relationships between mycorrhizal and soil types must be drawn with caution in view of the background phylogenetic histories of each family. This complication is best illustrated by the generally ectomycorrhizal Dipterocarpaceae which dominate the rainforests of South-East Asia almost irrespective of soil type (Whitmore, 1984), and apparently without particular association with nitrogen-limiting conditions (Alexander and Lee, 2005). This taxonomically-based ECM trait differentiates the fundamentally ECM forests of the Paleotropics from the primarily AM forests of the Neotropics. Despite these reservations, it can be said that, especially in the cases of trees which form uniform groves of the kind seen in many ECM tropical forests, feedbacks between litter quality and quantity, types of mycorrhizal colonization and nutrient supply are likely to be important factors in maintaining the stability of the ecosystem.

Some knowledge of the taxonomic status of the fungal symbionts of tropical rain-forest plants is a prerequisite for understanding mycorrhizal function in these ecosystems. In this context it is clearly important to know whether the diversity of mycobionts below ground reflects in any way that observed in the plants. The picture in this regard is still incomplete, but increasingly intensive sampling of ECM fungi (Henkel *et al.*, 2002; Lee *et al.*, 2003) and the application of molecular methods to analysis of AM fungal associations (Husband *et al.*, 2002a, 2002b; Vandenkoornhuyse, 2002) is beginning to indicate that diversity among these communities is much higher than was previously thought. Husband *et al.* (2002a) analysed the AM fungal communities associated with the roots of emergent seedlings of two tree species co-occurring in the rainforest of Panama. A total of 48 plants were examined using AM fungal-specific primers. Compared with communities of these types sampled from temperate habitats and analysed using the same methods, both the overall diversity and the species richness were higher at the rainforest site. A total of 30 AM fungal types was identified, 17 of which were previously unrecorded (Table 15.5). A shift in the AM community was also detected over time (Husband *et al.*, 2002a,

Table 15.5 The number of AM fungal types and the diversity of the AM fungal community in a number of ecosystems, based on phylogenetic analysis of partial SSU rRNA sequences. The relatively high diversity of AM types in the tropical biome is evident.

Ecosystem	No. of roots sampled	No. of host species	No. of AM fungal types	Diversity ^a (H')	Reference
Tropical forest, Panama	48	2	30	2.33	Husband <i>et al.</i> , 2002a
Temperate grassland, UK	47	2	24	1.71	Vandenkoornhuyse <i>et al.</i> , 2002
Temperate woodland, UK	49	5	11	1.44	Helgason <i>et al.</i> , 1998
Arable fields, UK	79	4	8	1.16	Daniell <i>et al.</i> , 2001

From Husband *et al.* (2002a). ^aShannon-Weiner diversity index.

2002b). Those fungi dominant in newly germinated seedlings were replaced over a year by others and seedlings of different ages sampled at the same time supported significantly different AM communities. Even taking into account some variations in sampling protocols between studies, these results suggest not only that AM diversity is much higher than previously acknowledged, but also that it is far greater than that seen in temperate systems. Bearing in mind that a serious decline of AM diversity has been seen to result from agricultural practices in the temperate zone (Helgason *et al.*, 1998; see Chapter 17) and that logging is already known to reduce AM inoculum potential in the tropics (Alexander *et al.*, 1992), there is every reason to expect that diverse rainforest communities of AM fungi will be seriously threatened by these and other commercially-driven activities in rainforest ecosystems (Janos, 1996). There is an urgent need for more intensive and widespread sampling of AM fungal genotypes of tropical soils, so that these communities can be both characterized and archived for possible use by future generations.

In the absence of similar molecular analyses of ECM associations, the diversity of these fungi can only be inferred from records of occurrence of putatively ECM sporocarps and by morphotyping studies.

Lee *et al.* (2003) measured sporocarp occurrence over 7 years in a 20ha lowland rainforest dominated by Dipterocarpaceae and Fagaceae in Malaysia. A total of 296 species were recorded of which 66% were new to science. This diversity is greater than the cumulative total of 265 species of putatively ECM sporocarps determined in weekly visits over 21 years to a North temperate forest in Switzerland (Straatsma *et al.*, 2001) and is more than double that recorded over the same 7 year sampling period in an Austrian forest (Straatsma and Krisai-Greilhuber, 2003). Whereas there are now extensive databases describing ECM morphotypes of North temperate ECM forests (Agerer *et al.*, 1987–2002; Goodman *et al.*, 1996–2002), few such records exist for the tropics. However, recent analyses using these approaches to characterize communities on dipterocarp seedlings in South-East Asia (Lee and Alexander, 1996; Lee *et al.*, 1996; Ingleby *et al.*, 1998) indicate that the numbers of morphotypes present in the tropical systems are at least as great or greater than those of temperate systems (Alexander and Lee, 2005).

Studies involving transplantation of non-mycorrhizal seedlings of the dipterocarp *Hopea nervosa* into logged and unlogged areas of Malaysian forest (Lee *et al.*, 1996) revealed that, after 6 months growth, the amount of ECM colonization was the same in both types of system, but seedlings recovered from the unlogged forest supported 40% more morphotypes than their counterparts from the logged system. In addition, there were twice as many uncommon morphotypes in the undisturbed forest, indicating that relatively rare fungi are most likely to be lost as a result of logging activities. Comparisons with the morphotypes present on seedlings of another dipterocarp species, *Shorea leprosula*, indicated that the most common fungi were equally well represented on both host plants, suggesting a lack of host specificity among dipterocarp fungi. In summary, as far as ECM fungal community structure is concerned, the emerging picture is one in which, as in the case of AM fungi, diversity is higher in tropical rainforest than in temperate forest but that this diversity is threatened by the extensive logging activities.

What have we learned of the functions of these obviously widespread mycorrhizal symbioses? There have been attempts using pot experiments to determine whether tropical trees show growth responses to mycorrhizal colonization. As Alexander and

Lee (2005) point out, the results of these must be treated with caution because they have inevitably been carried out over a short time span, have generally used mineral soil low in organic matter and the inoculum has often been either undefined or of a model species. Yazid *et al.* (1994) demonstrated growth increases and improved P uptake in two *Hopea* species following inoculation with a *Pisolithus* isolate. Using the same two species, Lee and Alexander (1994) also showed that application of ECM inoculum, on this occasion collected from the field, increased P uptake. In this case, the growth response to mycorrhizal colonization was greater than that to added P, indicating that factors other than P supply were involved. A similar effect was obtained by Moyersoen *et al.* (1998b) with the widespread AM species of rainforest in Cameroon, *Oubangia alata*. In the same study, it was shown that in the ECM species *Tetraberlinia moreliana* there was increased uptake of P at both high and low levels of P supply, but there was no growth response to the P addition (see Chapters 5 and 10). In an attempt to reproduce more closely the qualitative aspects of the rooting environment, Brearley *et al.* (2003) grew seedlings of three ECM dipterocarp species in pots of mineral soil with and without litter additions. The seedlings grew better in the presence of the litter and showed a negative relationship between their $\delta^{15}\text{N}$ signatures and the extent of ECM colonization. Since the litter had a lower $\delta^{15}\text{N}$ signature than the soil, this was interpreted as evidence that ECM colonization facilitated uptake of litter-derived N. It can be concluded from these types of experiment that mycorrhizal colonization may facilitate increased access to P and N, but that many, particularly the slow growing shade tolerant species, may not show a growth response as a result of the additional nutrient supplies. This in turn suggests that there may be considerable temporal uncoupling between the absorption and allocation of nutrients, a feature that is further considered below.

In an attempt to determine whether mature stands of tropical trees were limited by P availability, Newbery *et al.* (2002) applied P to a grove of the ECM species *Microberlinia bisulcata* in Cameroon. They found no effects of P addition upon seedling establishment or growth either of seedlings or trees in the fertilized plots, despite the fact that the P was taken up as evidenced by an increased P concentration in the foliage and litter. A fertilizer experiment in lowland ECM dipterocarp forest of Central Malaysia involving factorial application of both P and N produced similar results. While litterfall biomass and P concentrations were increased, indicating that the P at least was taken up, there was no change in tree girth over a 5-year period (Mirmanto *et al.*, 1999). These results do not support the view that P is a widespread limiting element, at least in ECM forests of the lowland tropics. This view is largely supported by studies carried out in the montane forests of the wet tropics (Tanner *et al.*, 1998). However, these authors concluded that there were severe growth limitations in this forest type imposed by low N availability. N was also seen as the critical limiting nutrient in tropical heath forest (Vitousek and Stanford, 1986). Following the contention (Read, 1991b) that the N limitation characteristic of boreal and temperate forests has selected in favour of ECM colonization, it might be suggested that these types of tropical forest would also be dominated by ECM trees. Alexander and Lee (2005) have subjected this hypothesis to scrutiny by examining reports of the mycorrhizal status of trees growing in particularly N-limited tropical environments. They find little evidence to support it. Some of the most intensively examined of these environments in the montane tropics contain few, if any, ECM trees (Tanner, 1977; Tanner *et al.*, 1992; Vitousek *et al.*, 1995).

Similarly, in extremely N-deficient heath forests of Brunei (Moyersoen *et al.*, 2001), Venezuela (Moyersoen, 1993) and Guyana (Bereau *et al.*, 1997), most of the dominant trees associate with AM fungi.

A further possibility, again based upon analysis of stratified soils of the kind found in boreal forest, is that ECM and AM species exploit different horizons, the former occupying predominantly the surface organic layers and the latter the mineral soil. There have been few comparative analyses of ECM and AM root distribution in the tropics. However, Moyersoen *et al.* (1998a) examined the vertical distribution of these root types in a lowland forest of Cameroon and found that fractional colonization of roots by either type was unaffected by horizon. It was later confirmed (Moyersoen *et al.*, 2001), that there were no differences in the relative abundances of ECM and AM roots in organic and mineral layers either in mixed dipterocarp or heath forests of Brunei. It was even apparent that the AM fractional colonization was significantly higher in the organic and acidic soils of the most nutrient stressed heath forests. Observations of these kinds bring into sharp focus the need to investigate the taxonomic and functional status of the AM fungi that dominate such stressed environments. It is also important to view both snap shots of tissue nutrient concentrations and short-term changes in growth (or lack of them) in the context of the whole life span of the trees, not simply the immediate duration of the experiments.

While, when they co-occur, ECM and AM trees may share the same localized soil niches, it remains the case that trees with each of these types of symbiosis are often spatially segregated in tropical forests. Under these circumstances, the structures of the resulting plant communities can be distinctive. Thus, in rainforests of both West Africa (Alexander, 1989a; Hart *et al.*, 1989; Torti *et al.*, 2001) and South America (Henkel, 2003; Mayor and Henkel, 2006), extensive pure stands, often referred to as 'groves', of monodominant ECM trees can be surrounded by species-rich stands of AM trees. On both continents the monodominant ectomycorrhizal species are often caesalpinoid legumes, most of which appear not to be involved in nitrogen fixation. There is little evidence to support the notion that these monodominant stands are associated with particular soil types since they can be found on substrates ranging from leached 'white sand' soils to red tropical oxisols (Nascimento and Proctor, 1997; Henkel *et al.*, 2002; Henkel, 2003). However, there is evidence that they can produce deeper litter layers and greater humic accumulations and that both of these substrates can be intensively exploited by ECM roots (Torti *et al.*, 2001; Henkel, 2003). Working in Guyana on the monodominant ECM caesalpinoid legume *Dicymbium corymbosum*, Henkel (2003) observed extensive root mounds at the base of large trees extending outwards between conspecifics to form forest-wide litter traps with depths of up to 50 cm. These were intensively exploited by ECM mycelia and roots. In a test of the hypothesis that this exploitation would lead to changes of residual litter quality, Mayor and Henkel (2006) examined mass loss from litter bags inserted into *D. corymbosum*-dominated plots without (trenched) and with (untrenched) intact mycorrhizal systems. Leaf litter mass loss was not influenced by the presence of the ectomycorrhizas and the only identifiable change of litter nutrient status was a more effective removal of Ca in the untrenched plots. These results thus argue against the view, originally expressed by Gadgil and Gadgil (1975), that the presence of ECM fungi leads to the suppression of saprotrophic fungi and hence to a reduction of decomposition rate. In the absence of a measurable effect of ECM mycelia on litter decomposition, Mayor and Henkel (2006) concluded that the deep litter layers

characteristic of *D. corymbosa* stands may contribute to the maintenance of monodominance by inhibiting seedling establishment by AM species. Torti *et al.* (2001) working in monodominant ECM stands of *Gilbertiodendron dewevrei* in Congo came to a similar conclusion. In this context, the abilities of many of the species forming these monodominant stands to produce, often in 'mast' years (see below), very large numbers of seedlings that can be rapidly recruited into the ECM mycelial network, may be important. The evidence collected so far thus points to the likelihood that monodominance of ECM stands in the tropics is maintained by competitor exclusion rather than by nutrient competition, but it is also probable that other as yet unidentified biotic effects are involved.

The difficulties experienced in identifying a direct nutritional benefit to trees from mycorrhizal colonization in the moist tropics suggest that a search for alternative functions might be fruitful. Two hypotheses, both indirectly involving nutrition, are worthy of further investigation. The first, supported by observations of a positive impact of mycorrhizal colonization upon seedling recruitment (Alexander *et al.*, 1992; Onguene and Kuyper, 2002) is that young plants may be sustained by C transfer from illuminated overstorey plants (Read *et al.*, 1985; Read, 1997). The second, that colonization enables the cumulative capture and storage of sufficient reserves to facilitate mast fruiting (Newbery *et al.*, 1997, 2006; Henkel *et al.*, 2005).

The suggestion that organic C might be passed from illuminated 'source' plants to support shaded 'sink' seedlings has been controversial (Robinson and Fitter, 1999; Newbery *et al.*, 2000; see Chapters 4 and 16). However, in addition to experimental evidence of the process gained from ECM ecosystems (see Chapter 16), there is now some indirect support for its occurrence in AM systems in the moist tropics. Bidartondo *et al.* (2002) confirmed that all stages of development of fully mycoheterotrophic plants of a number of families growing on the floor of moist tropical forests were colonized by AM fungi (Chapter 13). On the basis that these fungi are obligate mycorrhizal associates of autotrophs, it is most likely that the mycoheterotrophs are secondary recipients of photosynthate from the autotrophic plants. A pathway for C transfer is therefore demonstrated for these plants as it is for a number of other mycoheterotrophs that are routinely colonized by ECM fungi (see Chapter 13). Clearly, fully mycoheterotrophic plants (whether associated with AM or ECM fungi) represent the end point of an evolutionary process enabling them to survive, often as sole occupants of the forest floor, under conditions of extremely low irradiance. Since in the Orchidaceae partially mycoheterotrophic green plants associated with ECM fungi are common, there is reason to believe that intermediate physiological conditions will be found in seedlings that are recruited into ECM networks in the tropics.

Fruiting is a process involving distinct supra-annual bursts of seed production, evidenced in most cases by large peak years separated by periods during which fruiting may not occur. The process is best recognized in ECM tree families such as Dipterocarpaceae, Fagaceae and in the caesalpinoid legumes of tropical forests, but is also seen in temperate zone Fagaceae as well as in Pinaceae and Betulaceae. While the association between mast fruiting and trees of ECM habit is not exclusive, the event being occasionally observed in AM families (Alexander and Höglberg, 1986; Newman and Reddell, 1987), it is predominantly a feature of taxa with the former type of symbiosis. Two non-exclusive theories have been proposed to explain the habit. The first, the resource limitation hypothesis (Isagi *et al.*, 1997; Newbery *et al.*, 1997) proposes that the demands which fruiting places upon the C and mineral

nutrient resources of the tree are so great that annual cycles of seed production are not possible. The second, the predator satiation hypothesis (Janzen, 1974; Kelly and Sork, 2002), suggests that irregular seed production leads to reduction of herbivore numbers and so increases seedling survivorship in masting relative to non-masting species.

There is no doubt that mast fruiting produces a heavy demand on the nutrient resources of the tree. In the monodominant ECM stands of *Dicymbla corymbosa* in the neotropics, the resource allocation to seed production in a mast year was 3.0 t/ha (Henkel *et al.*, 2005). The African ECM caesalp, *Microberlinia bisulcata*, was likewise shown to invest the equivalent of 55% of the annual dry weight of leaves in seed and pod production during a masting event, the relative amounts of N and P being 13 and 21% respectively (Green and Newbery, 2002). In their phenology and climate ECM response (PACER) hypothesis, Newbery *et al.* (1997) proposed that it was the ability of ECM systems to capture and accumulate C, P and N during unusually moist seasons preceding a mast year that sustained the demands of fruiting on this scale. As an indication of the P stress suffered in association with mast fruiting, it was observed that in years following these events leaves shed from the canopy had lower P concentrations than in non-mast years. In general, removal of P from leaves of ECM species prior to leaf fall was approximately half that from those of non-ECM species (Chuyong *et al.*, 2000). Clearly, while some of the arguments in favour of a functional link between ECM associations and mast fruiting are persuasive, the evidence in their favour remains largely circumstantial and further testing of the suggested relationships are required. Nevertheless, as we have pointed out elsewhere (see Chapter 16), experiments only investigate a snap shot of the whole lives of plants. It may well be that it is our lack of understanding of the roles of enhanced tissue nutrient concentrations (apparent luxury consumption) in the eventual success of plants, that has led to the belief that such accumulations are valueless unless accompanied by growth increases.

Mycorrhizas in the seasonally dry tropics

Outside the wet equatorial forest belt, gradients occur towards biomes with pronounced dry seasons. Vitousek (1984) concluded that the changes of climatic regime across gradients of this kind had pronounced qualitative impacts upon the nutrient status of soils. He concluded that, whereas soils of wet tropical rainforest were most likely to be P-limited, those of the seasonally dry savannas, from which N was frequently lost in fire events, were characteristically N limited. If real, these deficiencies would be expected to select in favour of symbioses that optimized access to the limiting nutrient. These generalizations will of course be moderated by geological history so that some soils, weathered and eroded over millions of years, will be highly deficient in all essential nutrients.

An alternative but still indirect method of assessing the role of mycorrhizas in these challenging ecosystems is to determine the response of native plants to inoculation when grown in forest soil under greenhouse conditions. Extensive studies of normally AM colonized woody plants of the seasonally dry Tibaga River Basin of Southern Brazil have revealed a wide range of responsiveness (Siquera *et al.*, 1998; Zangaro *et al.*, 2000, 2003; Siquera and Saggin-Junior, 2001). While significant numbers of these native species were non-mycorrhizal, many were highly responsive

and some apparently dependent upon AM colonization. Pioneer species had small seeds and were found to be both highly susceptible to AM colonization and very responsive in terms of growth. Late successional species with larger seeds were less dependent upon colonization, at least in the early stages of their development (Siqueira *et al.*, 1998). These observations were broadly confirmed in a further study of 80 woody species of the same forest (Zangaro *et al.*, 2003). The responses to AM fungi and the extent of colonization were, respectively, 5.9 and 4.2 times greater in early successional than late successional species. Again, inverse correlations were established between seed weight and responsiveness to AM colonization. Studies of these kinds involving a wide range of native species provide valuable insights into the ecology of hitherto understudied communities and are an important first step towards evaluation of the likely roles of mycorrhiza under conditions of disturbance.

The widespread occurrence of woody N₂-fixing legumes in biomes, notably *Acacia* spp. in Africa, *Prosopis* spp. in America and *Acacia* and *Casuarina* in Australia, might be seen as a response to N limitation of trees in these biomes (Högberg, 1986, 1989, 1992). The legumes, however, comprise only a proportion of the trees in most of the systems in which they occur. In the sub-humid miombo woodlands of Africa, for example, they comprise only 20–25% of the woody species in the community (Högberg and Pearce, 1986). In any event, alleviation of one deficiency is likely to lead to its replacement by another. The dependence of N₂-fixing legumes upon P supplies is demonstrated by experiments showing that application of P to dry subtropical woodlands increased both the yield and nitrogenase activity of leguminous understorey plants in the genera *Acacia* and *Kennedia* in Australia (Hingston *et al.*, 1982). The interdependence of N and P supplies should not be overlooked and may help to explain the observation that N-fixing legumes are generally colonized by AM fungi (Alexander, 1989a, 1989b). The fact that non-nodulated leguminous tree species often successfully co-occur with those that are nodulated in savannah grasslands, suggests, as pointed out by Sprent (1985), that in these relatively arid tropical biomes the production of an extensive root system to enable acquisition of water may be the key determinant of success. This appears to be the strategy of the dominant savannah grasses, the extensive root systems of which are also colonized by AM fungi (Newman *et al.*, 1986).

The absence of large data sets for nutrient status in soils of the dry tropics has led some to measure element concentrations. Högberg and Alexander (1995) examined the leaf N and P concentrations in 98 species and site combinations of Tanzania and north east Zambia, recognizing N-fixing AM species, non-fixing AM species and non-fixing ECM species as three separate functional groups. The concentrations of N and P in each group were expressed in terms of what are widely considered to be their optimal ratios in plant tissues of between 12.5:1 and 10:1. The N-fixing AM species had supra-optimal N:P ratios indicative of P deficiency, non-fixing AM species show suboptimal N:P ratios suggesting P deficiency, whereas ECM species occupied an intermediate category in which the N:P ratio was near optimal. Such a distribution might be considered to support the view that, whereas AM colonization selectively favours the acquisition of P in these systems, the ECM symbiosis provides access to both elements.

Tissue δ¹⁵N signatures have been measured with a view to determining whether the different types of symbiosis found in the dry tropics are providing access to different sources of N (Högberg, 1992; Högberg and Alexander, 1995). The first analyses of nodulated, AM and ECM plants growing in miombo systems of Tanzania suggested that there were indeed differences between the groups. Tissues of ECM

species showed greater $\delta^{15}\text{N}$ enrichment than those that were nodulated, suggesting that they may have access to organic N sources. AM plants occupied an intermediate position (Högberg, 1992). However, subsequent measurements of miombo plants in Zambia have failed to confirm this pattern (Högberg and Alexander, 1995). The difficulties inherent in attempts to interpret patterns of nutrient acquisition from measurements of leaf stable isotope enrichment were described earlier. Progress is most likely to arise through studies of the kind employed for heathland and boreal forest plants, which involve direct parallel analyses of nutrient mobilization and stable isotope composition.

Conclusions

Evidence produced from field experiments is beginning to replace speculation and hence to throw new light on the important role played by mycorrhizal fungi in successional regimes at high altitudes and latitudes. They confirm that while the earliest herbaceous pioneers may well be ruderals that lack mycorrhizal colonization, the invasion of woody pioneers, particularly of the ECM kinds, is dependent upon mycorrhizal colonization. The important role played by ecto- and ericoid mycorrhiza in tundra and heathland environments in providing access to the predominantly organic N resources of these biomes is established. However, there is increasing consciousness of the threat to these delicate ecosystems that are posed by global climate change. Again, field experiments have provided valuable documentation of the responses of the mycorrhizal components of these systems both to elevated temperatures and to anthropogenically induced enhancement of N deposition.

Molecular methods have been applied to evaluate the diversity and distribution of ECM fungi in boreal and temperate forest systems. The possibility emerges that individual fungal species selectively exploit well-defined niches in the mineral as well as in the organic soil horizons of podsolic soils, but knowledge of the functional roles of the fungal symbionts lags behind awareness of their spatial distribution. The important role of soil N as a determinant of the structure and activity of mycorrhizal fungal communities has been assessed by careful evaluation of microbial populations along natural gradients of increasing N availability. Such work highlights the sensitivity of mycorrhizal communities to anthropogenic N depositions and provides a mechanistic basis for evaluation of the impacts of these effects.

Although N is still regarded by many as the most important limiting nutrient in terrestrial biomes, there are certainly many regions where P availability limits productivity. In such environments plants characteristically depend on arbuscular mycorrhizas where inorganic P is the predominant form in soil, but a range of other root adaptations, most of them primarily involved with mobilization and assimilation of P, are also found (Table 15.6). AM associations may play key roles in species with N_2 -fixing symbioses, enabling the plants to establish on highly weathered and nutritionally impoverished soils. However, in P-limited soils, which, nevertheless, accumulate some P (and N) in organic form, ERM and ECM species will gain greater access to these nutrient sources. As anthropogenic N depositions alter the nutrient balance of ecosystems, it will be important to pay attention to the likely changes in vegetation that may come about as a result of variations in accessibility of forms of N, P and other nutrients brought about by different mycorrhizal associates.

Table 15.6 Diagrammatic representation of the types of P and N resources in soil that can be accessed by plants with different root adaptations, including mycorrhizas of various types. The intensity of shading indicates the extent to which the different adaptations shown are thought to be able to access the nutrients. It is assumed that plants without specialized adaptations access nutrients of all types largely from the soluble inorganic pools in soil, although they may also have some capacity for uptake from soluble organic pools.

Strategy Resource \	Roots and root hairs	AM	Exudates/clusters	ECM/ERM
Soluble inorganic		P (N)	P	P and N
Insoluble inorganic		P	P	P and N
Labile/soluble organic		(P)	(P)	P and N
Recalcitrant organic				P and N

While understanding of the functional roles of mycorrhiza in tropical biomes still lags behind that obtained in the temperate zone, considerable progress has been made towards an evaluation of the extent of below-ground biodiversity in these systems. Hitherto, unsuspected diversity has been found in AM as well as in ECM fungal assemblages. The challenge now is to bring some of the hitherto undescribed species of both functional groups into culture, so that their functional attributes can be assessed. Research of this kind is a matter of urgency, not only because we need to gain a better appreciation of the roles of these fungi in the nutrient dynamics of tropical forests, but also because widespread devastation of these biomes through human activity is threatening to drive potentially valuable species to extinction before they have even been described.