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Mycorrhizas in ecological interactions

Introduction

As shown in Chapter 15, mycorrhizal symbioses are prevalent in all major terrestrial biomes. The reasons for this prevalence are not always clear from our current understanding of function of the symbioses determined in pot experiments using single plant species, particularly in relation to whole plant nutrition. In consequence, researchers are now actively addressing the questions posed in the second edition (Smith and Read, 1997), that relate to the biology of mycorrhizal plants in natural environments (see Chapter 15). In particular, we asked whether there are impacts of mycorrhizal colonization on the fitness of individual plants? Do mycorrhizal fungi influence the outcome of competitive interactions? If so, under what circumstances and in what ways are the effects mediated? Investigations have explored the contributions of mycorrhizas to fitness of individuals and species, to outcomes of competitive interactions between species and to the possibility that mycorrhizal status may change both the ability of plants to coexist and the diversity of plant assemblages. With the wider recognition of structural and functional diversity among different mycorrhizal partnerships has come the appreciation that the interdependence of plant and fungal partners may lead to changes in fungal, as well as plant communities.

Experimental approaches to determining which interactions are important in field situations and to understanding the mechanisms that underlie them at physiological, biochemical and molecular levels are by no means easy. In particular, the widespread, indeed universal, occurrence of mycorrhizas means that the appropriate non-mycorrhizal control treatments that are necessary to satisfy experimental designs, require elimination or marked reduction of mycorrhizal colonization. As yet no wholly satisfactory field treatments have been found (Hartnett and Wilson, 2002). The commonly-utilized fungicide applications are not specific to mycorrhizal fungi, with consequent problems of determining whether the outcomes are related to reduction in activity of mycorrhizas or other susceptible groups, such as plant pathogens or saprotrophs. The consequence has been that many physiological ecologists, interested in unravelling details of the interactions and their underlying mechanisms, have necessarily relied on pot or 'microcosm' experiments to increase their

understanding of natural communities or the outcomes of field manipulation. The failure of some pot experiments to demonstrate benefits in terms of plant growth and nutrition, coupled with field experiments which showed no increases in the efficiency with which roots took up nutrients, led to the suggestion that there may be other benefits of the symbioses that partially explain their persistence in natural vegetation. Even though it is now appreciated that mycorrhizal fungi may make marked contributions to nutrient uptake in the absence of growth or nutrient responses (see Chapters 4, 5 and 15), one of the consequences of this research has been increased understanding of multitrophic interactions involving soil microflora and fauna, herbivores, plant pathogens and mycorrhizal plants. Moreover, ubiquity of mycorrhizal colonization does not necessarily imply universal benefit or increased fitness. It might simply mean that there is no strong selection against the symbiosis.

This chapter will consider some of the observations and experiments that have been carried out in the field and in pots with the aim of increasing our understanding of mechanisms by which mycorrhizas influence plant and fungal communities. It will also briefly cover multitrophic interactions that are even more difficult to study experimentally, but are likely to have significant impacts in natural ecosystems.

Those interested in following the extensive literature covering development of ideas and investigations relating to the parts played by mycorrhizas in ecological interactions may find three books (Allen, 1991, 1992; van der Heijden and Sanders, 2002) and a number of key reviews particularly valuable. The work of Mike and Edith Allen in the USA played an important part in broadening ecological thought to include the roles of mycorrhizal associations in plant succession and competitive interactions (Allen and Allen, 1984; Allen, 1991, 1992). Major contributions, particularly emphasizing the importance of mycorrhizas in facilitating access to recalcitrant sources of nutrients and hence diversifying pathways of nutrient cycling, have been made by David Read and Jonathan Leake (Leake *et al.*, 2004a; Leake and Read, 1997; Read *et al.*, 2004), while important roles in recognizing non-nutritional benefits of mycorrhizas and in questioning received wisdom generally have been played by Alistair Fitter and collaborators (Fitter, 1991, 2001, 2005; Robinson and Fitter, 1999). The most recent compilation of a wide range of topics is to be found in 'Mycorrhizal Ecology' (van der Heijden and Sanders, 2002), a multi-author volume that reflects much current thought in this area.

Roles of mycorrhizas in mediating effects at the level of single plant species

Overview

Searches of the literature have revealed that experimental approaches to infer or determine mycorrhizal function in the field have most often been applied to communities in which many of the plant species form arbuscular mycorrhizas, rather than one of the other major types. Several factors may have contributed to this apparent bias. First, AM-dominated communities, such as grasslands, prairies, meadows, tropical forests and some temperate woodlands, are often more floristically diverse than those forests and heathlands where most of the species form ectomycorrhizal or ericoid mycorrhizas (see Chapter 15). In consequence, the 'AM

communities' hold more fascination for plant ecologists interested in mechanisms underlying interspecific plant interactions and consequences for plant diversity. It is also true that these plant communities are often dominated by grasses and forbes that are easier to manipulate in the field and in pots than the large woody perennials typical of 'ECM communities'. Nevertheless, large numbers of experiments have been done with economically important ECM trees, directed towards improving forestry production (see Chapter 17). A third reason for prevalence of research on roles of arbuscular mycorrhizas is that it is in these symbioses that major variations in occurrence of colonization and plant response have been highlighted and hence questions relating to evolutionary advantage of 'facultative' mycorrhizal status have been raised and explanations sought. In contrast, it is generally accepted that the ECM or ERM states are an essential requirement if the plants are to access the predominant sources of nutrients, particularly organic N, in those ecosystems that they inhabit (see Chapters 9, 11 and 15).

The occurrence (and presumed activity) of mycorrhizas along successional gradients has provided some insights into their possible roles, without resort to manipulation to eliminate the fungal symbionts. Allen and Allen (1984) reviewed available literature and concluded that arbuscular mycorrhizas were likely to be relatively unimportant in disturbed and early successional stages characterized by high availability of nutrients. In contrast, in low nutrient habitats and late successional stages, all plants were found to be colonized, with the implication that the symbioses played significant roles in the success and persistence of species found there. The authors also highlighted the possibility that AM symbioses may be very important determinants of the outcome of plant competition in habitats with high nutrient and water availability because the resultant high biomass production would lead to more intense competition, as suggested by Grime (1979 and see Chapter 15).

Selection appears to have favoured the prevalence of AM rather than ECM colonization in many ecosystems which are primarily P limited. However, it has proved quite difficult to demonstrate experimentally that plants growing in the field under natural conditions benefit from enhanced access to P in terms of whole plant uptake (Fitter, 1985, 1990), leading to the suggestions that impacts of AM colonization in the field are lower than would be expected from pot experiments. One problem stems from the earlier presumption that it is necessary to show either increased tissue P concentrations or efficiency of root uptake (e.g. inflow) in order to demonstrate that arbuscular mycorrhizas play a role in P nutrition. As discussed in Chapter 5, we now know that operation of the AM uptake pathway does not necessarily lead to increased P uptake. Accordingly, failure to demonstrate increased P inflow due to mycorrhizas (based on whole plant measurements) in the field should no longer be taken as evidence that the fungal pathway makes no contribution to plant nutrient acquisition. Indeed, studies employing radioactive tracers to track hyphal P uptake from root-free hyphal compartments buried in field soil have shown considerable AM contributions to P uptake, even in non-responsive crops (Schweiger and Jakobsen, 2000) (see Chapters 5 and 17). The approach could usefully be applied in natural environments. It remains true that the contribution of AM fungi may have no effect on overall growth or whole plant P uptake and that for positively responsive plants effects observed in pots may be greater than those seen in the field (Fitter and Merryweather, 1992). It is a major challenge of current and future research to elucidate what benefits operation of the AM nutrient uptake pathway may have under these conditions.

Several other explanations have been put forward to explain the discrepancy between field and pot experiments. Rates of growth of many plants in nature may be limited by environmental factors other than P deficiency, for example water shortage. In stress-tolerant species, growth rates may be inherently low, so that their P requirements can be satisfied by diffusion processes without involvement of mycorrhizal hyphae. However, even this idea may be challenged on the basis that a slow growing and long-lived root may develop a considerable zone of depletion as a consequence of prolonged nutrient uptake from the same soil zone (Smith FA *et al.*, 2003b). Grazing of extraradical hyphae by arthropods, particularly collembolans, has been demonstrated to eliminate responses to AM colonization in some circumstances (Warnock *et al.*, 1982; Fitter and Sanders, 1992). However, damage might be offset by enhanced nutrient turnover as senescent mycelium is grazed, with consequently increased nutrient availability (see below). It is important not to be defensive about apparent lack of field-based effects. Interactions are extremely complex and difficult to unravel. Increasingly, where appropriate experiments have been carried out, the importance of mycorrhizal fungi in mediating plant interactions, productivity and diversity are being revealed. In any event, the evolutionary persistence of mycorrhizal fungi as symbionts that make considerable demands on their plant partners for C, argues for benefits in the field that confer selective advantages.

Elimination or suppression of mycorrhiza development and functioning in the field to produce non-mycorrhizal controls has most frequently been attempted using the fungicide benomyl as a soil drench. Its addition to alpine grassland reduced colonization by AM fungi in a number of species, but had no effect on tissue P concentrations (Fitter, 1986). One interpretation of results such as this is, indeed, that AM colonization has little impact upon P nutrition of plants in the field. It is necessary, however, to recognize first that tissue concentration may not reflect whole-plant nutrient uptake and even the latter does not reveal the full contribution of the fungal symbionts (see also Chapter 15). Secondly, these studies suffer from the same essential weakness as do those with potted plants, in that most of them probe only a small proportion of the full life cycle of the plant. The extent of this weakness was highlighted by a study of bluebell, *Hyacinthoides non-scripta*, carried out in a deciduous woodland (Merryweather and Fitter, 1995a, 1995b). This work clearly demonstrated, apparently for the first time in a natural population of field-grown plants, that the rate of P uptake necessary to maintain a positive annual P budget can only be achieved in the AM condition. *H. non-scripta* is a perennial, vernal geophyte which characteristically dominates the herb layer of deciduous woodland throughout north-west Europe. It has a coarse root system made up of thick (0.5–10 mm) unbranched elements which are produced annually from the base of the bulb. A new bulb and root system are produced every year. The relationship between AM colonization and P uptake was explored by regularly sampling undisturbed plants of *H. non-scripta* throughout their annual life cycle (Merryweather and Fitter, 1995a). There was a rapid increase in the proportion of root length colonized by AM fungi over the period from root emergence in September (Autumn), to a maximum of over 70% in January and February (Figure 16.1a), even before the shoots appeared above ground. Thereafter, as evidenced by declining numbers of entry points (Figure 16.1b), new colonization slowed. From the time of root emergence, P inflow increased rapidly at a similar rate to that of colonization, although until December values were negative, indicating that net loss of P was occurring (Figure 16.1c).

Maximum inflows were reached during the photosynthetic phase, but these subsequently declined at the same rate as that of colonization. When curves were fitted to data for P inflow and per cent root length colonized, they demonstrated a very similar pattern, with significant correlation between the two variables (Figure 16.1d).

The individual plants of bluebell lose significant amounts of P, particularly at the end of the growing season, in seeds, old leaves and roots as they are shed. Glasshouse grown plants, lacking AM colonization, are unable to capture sufficient P from the soil to balance their P budget. They therefore end the season with a large P deficit, which could not have been sustained in the field. In a subsequent experiment, otherwise undisturbed colonies of *H. non-scripta* growing in the field were drenched with benomyl at two-monthly intervals over two years, a treatment which greatly reduced AM colonization without having any effect on P availability in soil (Merryweather and Fitter, 1996). This led to a large reduction of the P concentration of all vegetative parts, relative to that in colonies drenched with water. However, the flowers and seeds of the benomyl-treated plants had the same P concentration as the controls after the first season, reduction in their P status being observed only after two years (Figure 16.2). This suggests that when P uptake is restricted, *H. non-scripta* protects reproductive structures by selectivity allocating P to them.

Selective allocation to reproductive structures of P acquired by AM plants from P-deficient soils has also been observed in pot-grown plants of wild oats (*Avena fatua*) and tomato (*Solanum lycopersicum*) (Bryla and Koide, 1990; Koide *et al.*, 1988a),

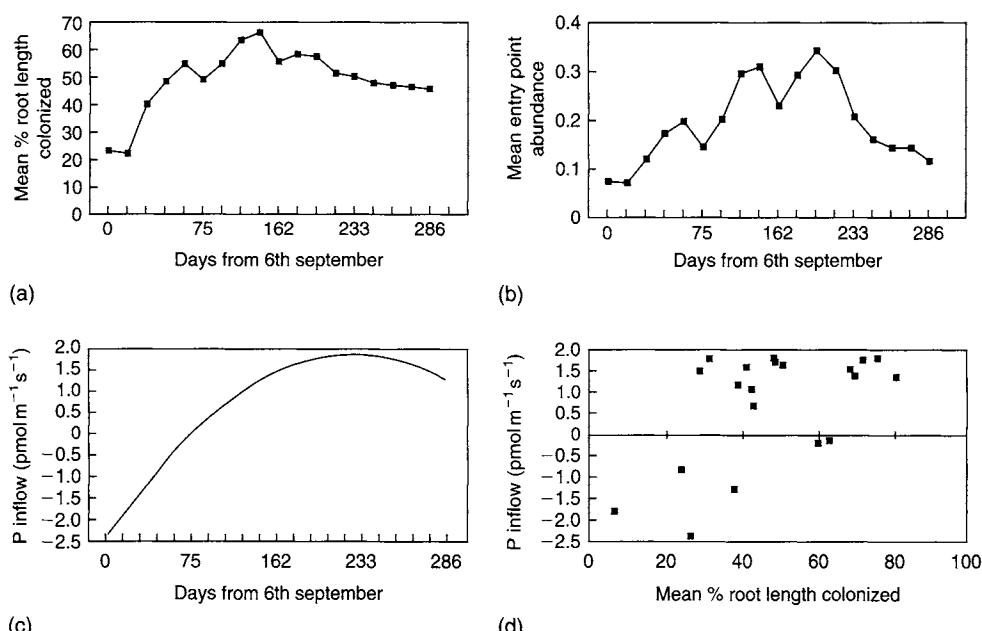


Figure 16.1 Seasonal pattern of AM colonization and P inflow in *Hyacinthoides non-scripta* growing in the field. (a) Mean per cent colonization; (b) mean abundance of entry points; the points on the curves are three-harvest running means; (c) fitted values for P inflow in the field; (d) correlation between P inflow and per cent colonization. Linear regression: $r^2 = 28.8$, $P = 0.15$. From Merryweather and Fitter (1995a), with permission.

wheat (Li *et al.*, 2005) and *Campanula rotundifolia* (Nuortila *et al.*, 2004). In the last case, although seed output was reduced in the AM plants, the seeds that were produced had higher relative growth rates in the next season than non-mycorrhizal counterparts. Detailed investigations of both male and female function in tomato have demonstrated considerable positive effects on total flower production, fruit mass, seed number and pollen production per plant and per flower, as well as *in vitro* pollen tube growth rates, which were again related to improved P nutrition (Poulton *et al.*, 2001, 2002). By these means, AM colonization may significantly influence fecundity of plants and so play a direct role in determination of fitness in the field, but more work on wild plants in natural environments is required. An extreme example of this effect is seen in the mast fruiting of ECM trees which may possibly be dependent on the apparently luxury accumulation of paid N and as a result of mycorrhizal activity (see Chapter 15).

Plants such as bluebell, with very coarse root systems, can be predicted on theoretical grounds to be responsive to AM colonization, but questions remain as to the role played by the symbiosis in plants such as grasses which, despite the fibrosis of their root systems, retain high levels of colonization in nature. As in the forbs, there are many glasshouse experiments with grasses demonstrating that enhancement of P capture can lead to increases of productivity, but such effects have been difficult to observe in natural communities. Hetrick *et al.* (1988, 1990), examining the responsiveness of two grass species that dominate the tall grass prairies of the USA, found evidence in the C3 species *Bromus inermis*, that, despite greater P acquisition in the AM condition, there was little or no growth response. In nature, *B. inermis* makes most of its growth in the cool seasons of autumn and spring and it is then that arbuscule production is at a maximum. Hetrick *et al.* suggested that early season growth enabled the plant to avoid competition with the other dominant species, *Andropogon gerardii*, a C4 plant which grows in the warm season and is extremely responsive to AM colonization. The authors proposed that benefits of P acquisition may only be expressed at a later growth stage in terms of increased fecundity and improved offspring performance. As the experiments were carried out with *B. inermis* alone in pots, it is likely that competitive advantages were missed and, in this case, it could be that luxury consumption of P early in the growing season enhanced the

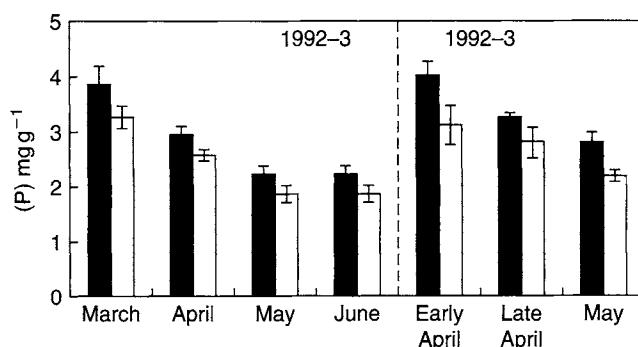


Figure 16.2 Effect of benomyl drench on the concentration of P in the leaves of *Hyacinthoides non-scripta*, measured at intervals over two growing seasons in the field. Black columns, control; white columns, benomyl. From Merryweather and Fitter (1996), with permission.

competitive ability of this grass by pre-empting availability of the element to other species. It cannot be emphasized too strongly that responsiveness to AM colonization determined in single plants in pots, whether positive, neutral or negative, is likely to be strongly modified by both inter- and intra-specific interactions in the field.

Density-dependent effects

For a plant population, density and pot size have effects on the productivity of individuals. A number of ecological studies indicate that the biomass (or other measure of success) of an individual plant is greatest when it is grown alone and declines as the planting density increases. Although the mycorrhizal status of the plants was not recorded in many of these investigations, the assumption must be that potential mycorrhizal hosts were colonized in untreated field soil and results should be interpreted accordingly. Experiments investigating the effects of AM colonization on intraspecific interactions of positively responsive species consistently confirm that AM plants perform best (as individuals) at the lowest planting densities and compete severely for soil resources at higher densities (Koide, 1991b; Allsopp and Stock, 1992b; Hetrick *et al.*, 1994a; West, 1996; Facelli *et al.*, 1999; Schroeder and Janos, 2004). In contrast, there is apparently little or no competition between individuals of the same species when grown without AM inoculum. These plants grow relatively poorly (as individuals), even at the lowest planting densities when non-mycorrhizal and their success (again as individuals) is not changed as density increases (Figure 16.3). In contrast, the negative responses of some plants to AM colonization, apparent with single plants or at low density, is sometimes lessened at high density (Hartnett *et al.*, 1993; Schroeder and Janos, 2004; Li *et al.*, unpublished), not because AM plants grow better at high densities but because the negative effects of density are greater for the larger, non-mycorrhizal plants. This can be explained by greater overlap of roots and hence zones of resource acquisition among the larger non-mycorrhizal plants, leading to greater competition.

Effects of density interact not only with AM colonization but also with P availability and the identity of the AM fungal symbionts, with outcomes varying again with responsiveness of the plants to AM colonization and P supply when grown

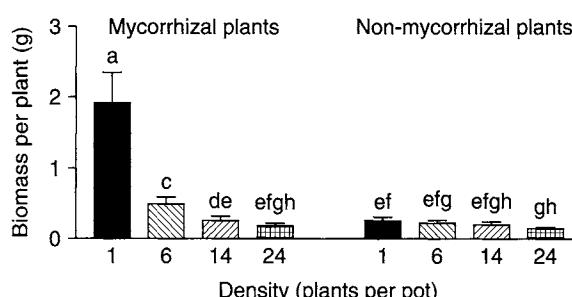


Figure 16.3 Effects of plant density on biomass of plants of *Trifolium subterraneum*, inoculated with *Gigaspora margarita*, or not inoculated. Bars with different letters are significantly different. Results from E. Facelli. See Facelli *et al.* (1999).

singly (Schroeder and Janos, 2004; Li *et al.*, unpublished). Rather surprisingly Facelli *et al.* (1999) found no influence of reduced irradiance on the effects of density on AM responsiveness. In their investigations, intraspecific competition intensity was increased by AM colonization at both low and high irradiance, due to an increase in capture of soil P. The increase in competition was reflected in the greater size-inequality of low-density AM treatments. They came to the important conclusion that the influence of AM colonization at the level of individual plants cannot be expected to be apparent at the population level, because of the large modifying effects of density-dependent processes.

Results such as these carry several implications for effects of density on responsive plants:

- 1 potentially AM species may be unable to use soil resources effectively in the absence of colonization, so that increased population size increases root length density and the acquisition of nutrients by the population, but not the individuals, until a plateau is reached
- 2 colonized plants have a combined root and hyphal length density which permits effective use of a much greater volume of soil than non-colonized plants, with the consequence that interplant competition is potentially strong
- 3 responses to AM colonization will be greatest at the lowest planting densities, both on an individual and a population basis.

Applying similar arguments to negatively responsive species, explains why increasing density can minimize the extent of the negative response.

Most investigations have involved plants of the same age, but the situation when seedlings establish in competition with adult plants may be quite different. In non-competitive treatments, seedlings of *Hypericum perforatum* were more responsive to AM inoculation than adults, although the effect was positive in both cases. When adults and seedlings were in competition, the responses were reduced, more so in seedlings (Moora and Zobel, 1997). The authors concluded that the outcome was influenced by the fact that the negative effect of competition on the (small) seedlings was stronger than the positive effect of AM colonization. These results contrast with reports of benefits of AM on seedlings germinating in established plant communities (Grime *et al.*, 1987; Read, 1991; Gange *et al.*, 1993; Francis and Read, 1994; van der Heijden, 2004). The discrepancies are likely to relate to the nutrient uptake strategies of the competing species and their mycorrhizal responses in mixtures, both of which also influence the impact of AM colonization on plant biodiversity (see below).

A recent investigation of the importance of ECM links in facilitating establishment of seedlings has highlighted similar benefits (Nara, 2006a). The investigation of willow (*Salix reinii*) seedling establishment on a nutrient poor, volcanic desert on the slopes of Mt Fuji, Japan capitalized on the lack of spore-based inoculum at the site. Details of the procedures have been described in Chapter 15. The salient features of the results were that spore-based inoculum had an insignificant role in colonization at the site, common mycelial networks (CMNs) growing from each mother tree facilitated ECM development and that the CMNs appeared to reduce competition for N between the large and small plants, regardless of the identity of the fungal partner and number of ECM root tips. The significance of CMNs for establishing seedlings and in mediating enhanced nutrient uptake and reduced competition has

thus been demonstrated without resort to trenching, which has confounded previous investigations.

Competitive interactions in mixed-species assemblages

AM colonization certainly affects the relative performance of adults of different species in mixtures, altering the species diversity and growth of individuals. In one of the earliest published examples, outcomes of competition between the pasture grass *Lolium perenne* and the legume *Trifolium repens* were shown to be markedly influenced by AM colonization. *L. perenne* became extensively colonized but did not respond to colonization, presumably because its roots were relatively efficient at extracting P from soil. In contrast, the highly responsive *T. repens* only performed well in mixtures with *L. perenne* when mycorrhizal (Hall, 1978). Similarly, AM inoculation in pots changed the relative productivity and survivorship of a number of grassland species grown in mixtures in low P soil (Grime *et al.*, 1987). Analogous findings are commonly reported for groups and pairs of species, indicating how AM symbionts can markedly influence the co-occurrence of species, their competitive interactions and, in consequence, the biodiversity of ecosystems (Herrick *et al.*, 1994a; West, 1996; van der Heijden *et al.*, 2003).

Unravelling the mechanisms by which mycorrhizal fungi alter relative plant performance in interspecific competitive situations is extremely difficult, in part because of the differences in nutrient acquisition and growth strategies of the competing plants and in part because of differences in effectiveness of different fungi. Responsiveness can be strongly modified by potentially competitive interactions and there are considerable dangers of predicting likely outcomes of competition from responsiveness of single plants. This is borne out by experiments with wild species. Growth responses of single species from tall-grass prairie were useful in predicting outcomes when the species were grown in mixtures. In this case, as mentioned above, the warm-season grass *Andropogon gerardii* was highly responsive and this character aligned with its dominance in competition. However, Marler *et al.* (1999), investigating the mechanisms underlying invasiveness of *Centaurea maculata* in grasslands in Montana, observed no positive growth response in this species or in *Festuca idahoensis* with which it successfully competes. However, when paired with large plants of *F. idahoensis*, to reduce the size imbalance of the two species, *C. maculata* was highly responsive to AM inoculation and outcompeted the grass, as it does in the field. The authors suggested that C transfer between the species might have been the basis for the effect in mixtures, but the same group later examined this idea in more detail and found no unequivocal evidence to support it (Zabinski *et al.*, 2002; Carey *et al.*, 2004). The most likely conclusion is that AM colonization conferred on *C. maculata* an enhanced ability to scavenge P which was, in the experiments of Zabinski *et al.* (2002), apparent as increased P concentrations in plant tissues. This may be another example of higher competitive ability, based on pre-emption of limiting resources.

Some insights into mechanisms were obtained from a pot experiment in which wild-type tomato (normally AM, but non-responsive in the soil used) was paired with a mycorrhiza-defective mutant derived from it (Cavagnaro *et al.*, 2004a). When non-inoculated, the two plants (both non-mycorrhizal) grew at the same rate and

showed the same responses to P fertilization and to density of planting, whether as single genotypes or in competition with each other in a replacement series (Cavagnaro *et al.*, 2004a). Thus, the only strategy likely to confer enhanced nutrient uptake from low P soil was the ability of the wild type to form AM when inoculated. Despite potential differences in C balance between the wild type (AM) and the mutant (non-mycorrhizal), the outcome of competition in inoculated treatments in the replacement series was a clear growth benefit to the AM wild type, based on an increased ability to compete for P in the low nutrient soil. The growth response was only apparent in competition; the wild-type tomato showed either a neutral or negative response to colonization when grown alone.

The examples provided so far involve AM plants, which are presumed to access the same sources of inorganic nutrients as their fungal symbionts (see Chapter 5). Formation of ectomycorrhizas also allowed *Pinus elliottii* to take up more P from inorganic sources in competition with non-mycorrhizal plants of the grass *Panicum chamaelonche* than when competing with another pine (Pedersen *et al.*, 1999). The authors cautioned that, in nature, the outcome might be quite different because *P. chamaelonche* would then form arbuscular mycorrhizas which would improve its ability to access the available inorganic P.

A recent field experiment has demonstrated significant below-ground interactions between ECM pinyon pine (*Pinus edulis*) and co-occurring AM shrubs. During drought, field performance and root biomass of pine was lower in the presence of shrubs, suggesting below-ground competition for resources (Figure 16.4a). Furthermore, when shrubs were removed experimentally, pine growth both above and below ground was increased. At the same time, ECM colonization doubled (Figure 16.4b), although the diversity of the fungal assemblages was unaffected (McHugh and Gehring, 2006). These results support several previous observations of effects of such processes as trenching to remove competition on performance of target species. The authors suggested that effects were particularly marked in their experiments because of water-limitation at the site.

However, for many plant species, organic N and P are increasingly recognized as major sources of nutrients. This is especially the case in plants forming ECM or ERM (see Chapters 9, 10, 11 and 15), where the fungal symbionts increase the diversity of available resources to include both inorganic and organic forms. The versatility is of great importance in nutrient-poor habitats such as heathlands, allowing the plants to exploit nutrient pools that are unavailable to AM or non-mycorrhizal species, permitting them to coexist with them without direct competition for N or P. Aerts (1999) has highlighted the changes that may occur as inorganic N in soil rises as a consequence of atmospheric N deposition. The consequence could well be that AM grasses like *Molinia caerulea* and *Deschampsia flexuosa*, dependent on inorganic N, would increase in abundance at the expense of members of the Ericaceae which currently dominate the heaths (see also Chapter 15).

There have been rather few experimental investigations of the way ectomycorrhizas influence plant competition for natural sources of nutrients. Perry *et al.* (1989b) used a replacement series to investigate the way both specific and generalist ECM fungi influenced competitive interactions between *Pseudostuga menziesii* and *Pinus ponderosa*, which co-occur in forests of south-west Oregon. They used soil from the litter and humus layers of such forests, which was sterilized before plants, inoculated or not with particular ECM fungi, were transplanted into the

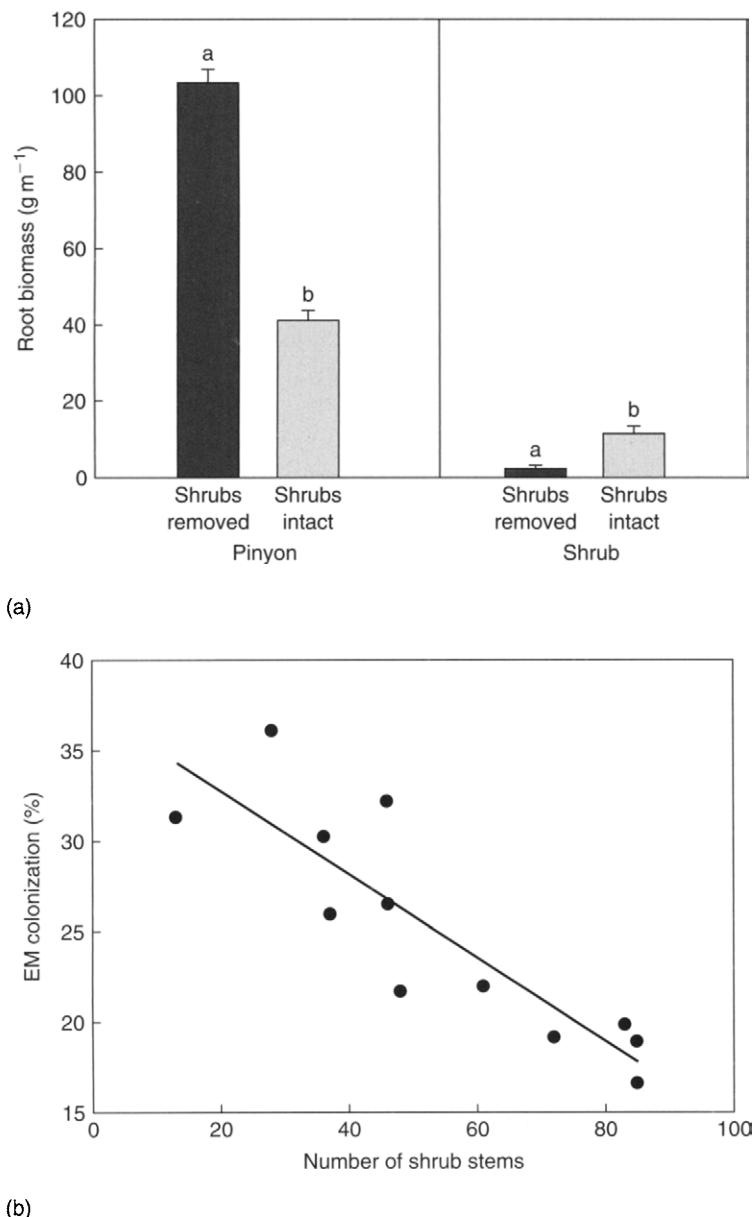


Figure 16.4 Effects of shrub removal on growth and ectomycorrhizal colonization of pinyon pine (*Pinus edulis*). (a) Above-ground shrub removal decreased shrub root biomass. In response, pinyon pines with associated shrubs removed had three times greater root biomass than pines with intact associated shrubs. Bars are means and standard errors. a and b indicate significant differences. (b) Relationship between ectomycorrhizal colonization (%) of mature pinyon pine trees and number of shrub stems remaining in the pinyon pine zone. Redrawn from McHugh and Gehring (2006).

pots. Without inoculation (but with mycorrhizas formed by contaminant *Thelephora terrestris*), the two tree species inhibited one another, but this inhibition disappeared when pots were inoculated with two ECM fungi specific for either *P. menziesii* or *P. ponderosa* (*Rhizopogon vinicolor* and *R. ochraceorubens*, respectively). When four fungi were inoculated (the two specific fungi plus two generalists), *P. menziesii* grew significantly better in mixtures than alone and growth of *P. ponderosa* did not decline. Results indicated that the generalist fungus *Laccaria laccata* enhanced N and P uptake by *P. menziesii* and reduced luxury consumption by *P. ponderosa*, but not sufficiently to reduce biomass production. The effect on growth appeared not to be the result of increasing numbers of fungi but rather depended on the presence of effective fungal symbionts. This point is important in relation to the discussion below.

Effects of AM on plant diversity

The influence of mycorrhizas on plant competition can be safely expected to feed through to changes in plant coexistence and biodiversity. However, the outcomes of experiments have been quite varied, so that it is important to analyse the reasons for the variations and, where possible, determine the mechanisms. Grime *et al.* (1987), using microcosms, reconstructed a plant assemblage representative of that occurring in nutrient-deficient calcareous soils in north-west Europe. The plants were a mixture of grasses and herbs, all but two of which (*Arabis hirsuta* and *Rumex acetosa*, from typically non-host families) were known to be heavily colonized by AM fungi in the field. The plants were grown from seed for one year in a sward of the dominant grass *Festuca ovina*, which had been pre-sown either in the non-mycorrhizal condition or as AM individuals which provided a natural source of inoculum for the subordinate plants. Survivorship was monitored throughout the year and the impact of colonization on biomass and on final structure of the community was determined at a single harvest. Among the grasses only *Holcus lanatus* showed a positive response to AM colonization in terms of dry weight; the others were either unaffected or reduced in growth (Table 16.1). Those forbs that are normally colonized by AM fungi showed significantly higher growth in the inoculated than the non-inoculated microcosms (Table 16.1). In contrast, *A. hirsuta* was more productive in the non-mycorrhizal community. Survivorship can provide a more direct index of fitness than productivity, especially in nutrient stressed habitats such as calcareous grasslands, as long as the survivors produce sufficient viable seed. Absence of AM inoculum led to major reductions of survivorship in the AM forbs (Table 16.2), but the reverse was observed with *A. hirsuta* and *R. acetosa*. Only small numbers of individuals of these plants were still alive after six months in AM microcosms. This study provides some insights into the roles of AM colonization in inhibiting the establishment of seedlings in closed turf. The non-host species, together with others, have been shown to be 'turf incompatible' and, in consequence, are relegated to ruderal situations (Grubb, 1976, 1977; Fenner, 1978). Similar negative effects of AM colonization on non-host species have been observed both in microcosms and in field situations (e.g. O'Connor *et al.*, 2002; van der Heijden *et al.*, 1998b).

It has been suggested that sensitivity to the presence of external AM mycelium is a factor determining growth and survivorship of non-hosts. *R. acetosa* and *A. hirsuta*, as well as a range of hosts and non-hosts, were grown in the same pots as AM 'nurse

Table 16.1 Effects of AM inoculation on shoot dry matter production per plant of species grown together in microcosms for 1 year.

| | Minus AM inoculation | Plus AM inoculation | Effects of infection |
|------------------------------------|-------------------------|------------------------|-------------------------|
| Grasses | | | |
| <i>Anthoxanthum odoratum</i> | 5.02 | 5.40 | — |
| <i>Briza media</i> | 13.55 | 54.26 | * |
| <i>Dactylis glomerata</i> | 160.19 | 286.84 | NS |
| <i>Festuca ovina</i> + | 922.68 | 609.43 | *** |
| <i>Festuca ovina</i> | 24.14 | 22.02 | * |
| <i>Festuca rubra</i> | 27.67 | 25.37 | * |
| <i>Poa pratensis</i> | 15.07 | 19.34 | NS |
| Forbs | | | |
| <i>Arabis hirsuta</i> ¹ | 0.26 | 0.13 | — |
| <i>Campanula rotundifolia</i> | 0.73 | 4.20 | ** |
| <i>Centaurea nigra</i> | 1.70 | 10.90 | *** |
| <i>Centaurium erythraea</i> | 0.23 | 7.08 | — |
| <i>Galium verum</i> | 1.87 | 9.39 | ** |
| <i>Hieracium pilosella</i> | 0.93 | 7.63 | *** |
| <i>Leontodon hispidus</i> | 0.83 | 3.72 | ** |
| <i>Plantago lanceolata</i> | 3.62 | 33.96 | *** |
| <i>Rumex acetosa</i> ¹ | 9.72 | 8.77 | NS |
| <i>Sanguisorba minor</i> | 5.06 | 17.14 | *** |
| <i>Scabiosa columbaria</i> | 2.46 | 10.19 | *** |
| <i>Silene nutans</i> | 16.85 | 44.89 | *** |

Data from Grime *et al.* (1987). ¹Non-host species; not colonized when inoculated. *Festuca ovina* was introduced to the microcosms as small plants (+) or as seed.

Table 16.2 Survivorship (%) of forbs after 6 months in mycorrhizal and non-mycorrhizal microcosms.

| Species | Mycorrhizal | Non-mycorrhizal |
|-----------------------------|-------------|-----------------|
| <i>Centaurium erythraea</i> | 64 | 2 |
| <i>Galium verum</i> | 58 | 11 |
| <i>Hieraceum pilosella</i> | 49 | 6 |
| <i>Leontodon hispidus</i> | 42 | 13 |
| <i>Plantago lanceolata</i> | 71 | 10 |
| <i>Sanguisorba minor</i> | 53 | 6 |
| <i>Scabiosa columbaria</i> | 84 | 16 |
| <i>Arabis hirsuta</i> | 8 | 42 |
| <i>Rumex acetosa</i> | 11 | 60 |

Significant increases of survivorship were obtained in most forbs grown in the mycorrhizal condition, the exceptions being the non-hosts *Arabis hirsuta* and *Rumex acetosa*, which show the reverse trend.

plants', with the roots separated by mesh that allowed passage of AM mycelium. The mycelium could therefore interact with the roots of the test species, which showed very different responses to its presence (Francis and Read, 1994, 1995). The 'non-hosts' (e.g. *A. hirsuta*) grew poorly and showed a reduction in survivorship,

but hosts such as *P. lanceolata* and *C. erythraea* responded positively. The basis of the antagonistic effect of AM fungi upon non-host ruderals remains to be elucidated. In some cases, adverse effects upon root development have been observed in the absence of colonization by the fungus, suggesting that there may be a chemical interaction (Allen *et al.*, 1989; Francis and Read, 1994), whereas in others, inhibition is associated with penetration of the root and prolific production of vesicles which might induce a significant C drain (Francis and Read, 1995). However, the explanation may be even simpler. Although the roots of the plants remained separate, it seems most likely that hyphal exploitation of the 'extra' soil within the mesh conferred a nutritional advantage on the hosts and reduced growth of the non-hosts was the result of competition. An interaction of this type has been observed in a similar experimental system, with the tomato mutant (*rmc*) growing with *Allium porrum* as the nurse plant (Cavagnaro *et al.*, 2004b). Regardless of the mechanisms of interaction, which certainly require more detailed cytological and physiological exploration, it is clear that AM fungi can be seen as major determinants both of the structure and biodiversity of plant communities.

The microcosm experiment of Grime and co-workers provided one of the first clear demonstrations that AM fungi are potentially major determinants both of the structure and diversity of plant assemblages. The effect of arbuscular mycorrhizas in increasing diversity was the result of marked reduction in growth of the dominant species, *F. ovina* and of the increased survival of some very AM responsive species, such as *C. erythraea*, although species richness was not actually changed. The relative performance of canopy dominants and subordinate species is crucial to the outcome. In this plant assemblage, *F. ovina* showed a negative growth response to AM inoculation despite high colonization.

Similar results have been obtained more recently, again using grassland or old-field plants grown in microcosms. van der Heijden *et al.* (1998b) showed increased diversity and, in one experiment, increased productivity which was not observed in the experiments of Grime *et al.* (1987). However, the two investigations were similar in that the response of the dominant grass species to AM inoculum was either neutral or negative. Thus in both these examples plant diversity was shown to increase with AM colonization and this was the result of improved growth and survivorship of AM subordinates, associated with dominants that actually had their own competitive ability reduced by AM colonization. Thus, there are two possible contributors to the effects: increased ability of the subordinates to access nutrients, by virtue of the nutrient scavenging ability of the AM mycelium growing from their roots, and reduced vigour and hence reduced competition by the dominants.

Two field investigations, backed by pot experiments, have given results that at first sight conflict with these microcosm experiments. As we have seen (above and Chapters 4 and 5), tall-grass prairie communities are dominated by warm-season, C4 grasses that are highly responsive to AM colonization. The plant assemblages also include cool-season, C3 grasses that are somewhat less responsive, and a wide range of forbs that show quite variable responses, at least in pot experiments. The interactions between the species were investigated in the field by selective removal of dominant C4 grasses and by suppression of AM fungi over two seasons by application of the fungicide benomyl (Smith *et al.*, 1999). Both treatments increased plant species richness and the benomyl treatment also increased Shannon diversity

index. The dominant and highly responsive *A. gerardii* and the subdominant and responsive *Bouteloua curtipendula* both declined when AM colonization was suppressed in the field, whereas the forbs and grasses (with variable responsiveness in pot experiments) increased in abundance. The similar effects of removal of dominants and benomyl application suggests that competition from the dominants is a major factor influencing floristic diversity in this system.

Similarly, the semi-arid hermland in South Australia investigated by O'Connor *et al.* (2002) was dominated by *Medicago minima*, an annual weed that was shown in pots to be highly responsive to AM colonization. Two other weeds, *Carrichtera annua* (Cruciferae, annual non-host) and *Salvia verbenaca* (Labiatae, short-lived perennial and non-responsive host), also made important contributions to overall biomass and there were minor contributions from a range of native species of variable responsiveness. Application of benomyl over one season effectively suppressed AM colonization compared with controls, either unwatered or receiving water only. Application of water as an additional control was necessary in this environment where rainfall is low, highly variable and unpredictable. Comparison of the two control treatments showed that watering increased productivity, but there were no differences from the unwatered control in terms of relative numbers of plants or biomass of each species. Suppression of AM activity markedly reduced the above-ground biomass and numbers of plants of the dominant, *M. minima*. *C. annua* and *S. verbenaca* both showed large increases in biomass, accompanied in the latter by greatly increased seedling survivorship. There were small differences in the other (native) species (*Velleia arguta*, *Erodium crinitum* and *Vittadinia gracilis*) between the benomyl and watered treatments (Figure 16.5). These alterations in biomass and abundance were not reflected in any net change in species richness, but diversity (+29%, Shannon H') and evenness (+32%, Shannon J) both increased in mycorrhiza-suppressed plots. The differences in diversity came from changes in contribution of the three major species (Figure 16.6). It appeared that when AM activity was suppressed, the competitive ability of the dominant *M. minima* was strongly reduced, with consequent advantages to *C. annua* and *S. verbenaca* that did not depend on their host status but rather on their lack of marked responsiveness. It is likely, however, that *S. verbenaca* utilizes the AM nutrient uptake pathway when colonized, most probably permitting this species to coexist successfully with *M. minima*.

We must conclude that the effects of AM colonization on plant diversity are not absolute and are strongly influenced by the responsiveness of the species in the community as predicted by Bergelson and Crawley (1988). Attempts have been made to construct conceptual models to aid understanding (Hartnett and Wilson, 2002; Urcelay and Diaz, 2003). It seems clear that in the investigations undertaken so far it is the responsiveness and hence competitiveness of the dominants that has the largest influence. When these are positively responsive then suppression of AM colonization is likely to increase diversity because the subordinates are released from competition. In contrast, when dominants are negatively responsive, AM suppression will decrease diversity because the subordinates will lose the competitive advantage conferred by AM symbioses. The balance of experimental evidence suggests that mycorrhizal responsiveness of the dominants, rather than subordinates, plays a very significant role (see Urcelay and Diaz, 2003), but investigations should be expanded to encompass a wider range of vegetation types.

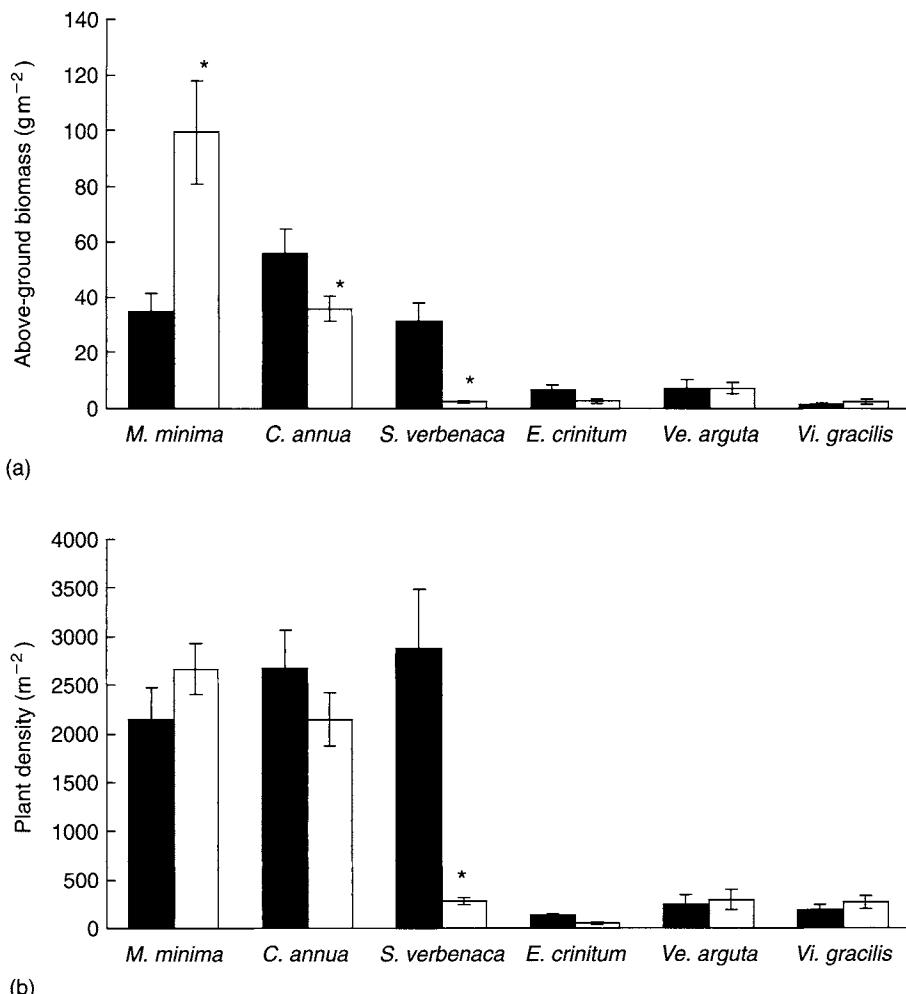


Figure 16.5 Response of major plant species in field plots in a semi-arid herland to suppression of AM colonization with benomyl (black bars) compared with watered plots (open bars). (a) Above-ground biomass; (b) plant density. An asterisk above the bar indicates that the control is significantly different ($P < 0.05$) from the fungicide-treated plots for that species, determined by Tukey's HSD test. Species shown accounted for $>90\%$ of plot biomass ($n = 7$). Species are: *Medicago minima*, *Carrichtera annua*, *Salvia verbenaca*, *Erodium crinitum*, *Vellea arguta*, *Vittadinia gracilis*. Reproduced from O'Connor et al. (2002), with permission of the *New Phytologist*.

Rather little work of this type has been carried out in ECM communities. However, observations in some tropical forest ecosystems suggest that ECM symbioses are of importance in promoting the ability of some tree species to develop monodominant stands, which contrast with highly diverse rainforest often present on similar soils in close proximity and associated with AM fungi (Connell and Lowman, 1989). This effect has been discussed in Chapter 15.

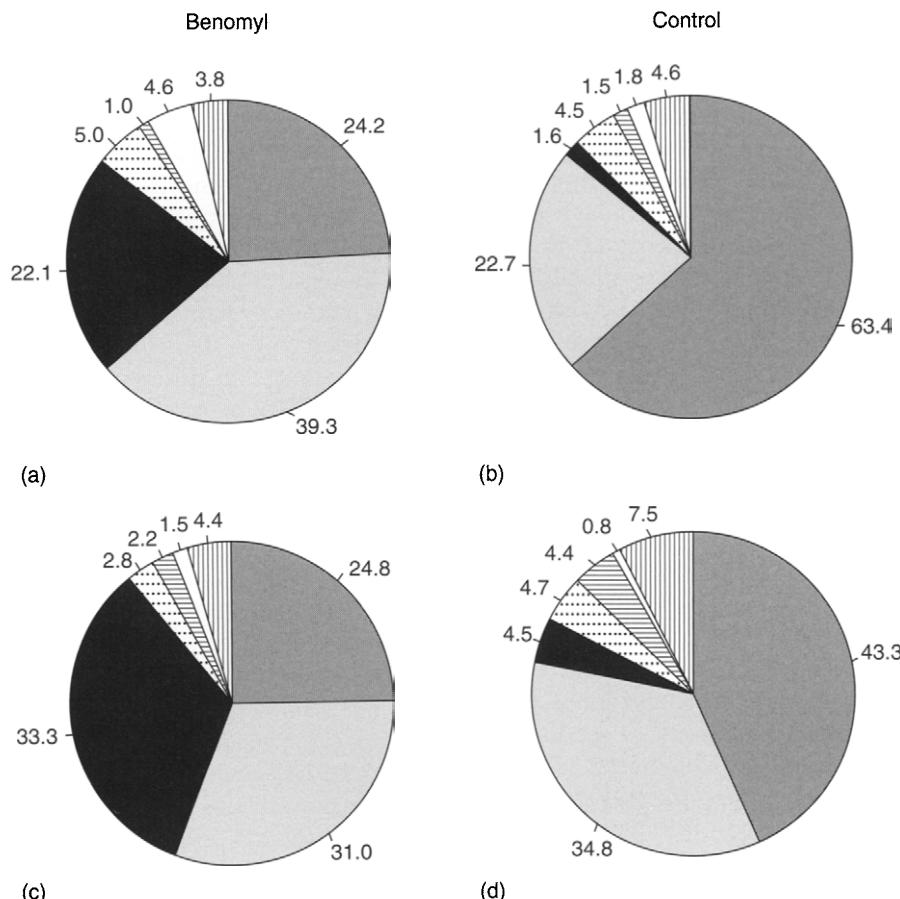


Figure 16.6 Species composition (%) of total aboveground biomass (a & b) and total number of individuals (c & d) in plots with AM fungi suppressed (Benomyl a, c) or AM fungi active (Control b, d). Species included are *M. minima* □; *C. annua* ■; *S. verbenaca* ■; *Ve. arguta* □; *Vi. gracilis* □; *E. crinitum* □; and Others ▨. Data represent the mean of seven replicates.

Effects of the fungal assemblage

In the discussion so far, the possible effects of differences in composition of the AM fungal assemblages to competitive outcomes and hence biodiversity and productivity have been ignored. However, the large diversity of function between different plant-fungus combinations, as well as selectivity in choice of partners, means that changes in the fungal community with respect to both numbers and identity of species could change plant interactions (see Chapters 1 and 4). In their microcosm experiment, van der Heijden *et al.* (1998b) compared the effects of adding four species of AM fungi separately and in combination, on the growth of the component plants and on outcomes in terms of measures of plant productivity and biodiversity. They found, not unexpectedly, that there were large differences in response of individual plants to the fungi inoculated separately and that the mix of four fungi had different effects again. Multiple inoculation was sometimes more effective in producing a response than single inoculation and the authors suggested that plant diversity and also productivity

would increase with increasing numbers of AM fungi in the soil assemblage, although in this experiment there were actually no changes in productivity.

Somewhat more persuasive evidence was provided by a second, field experiment which used up to 14 randomly selected AM fungal species inoculated onto a mix of 15 plant species. In this case, productivity in terms of plant biomass and hyphal length density did increase with increasing numbers of fungi and there was also an increase in diversity, all of which parameters reached an asymptote at about eight fungal species per microcosm. P in the plants increased and P in soil fell, indicating that increasing exploitation of soil P was one of the bases for the outcome. Johnson *et al.* (2004) provided some support for this finding, showing that increased P concentrations in the shoots of *Plantago lanceolata* were related to the diversity of AM fungi that developed in the roots, after culture in microcosms with different plant treatments. However, the interpretation that increased diversity of AM fungi is a driver for increased plant diversity and productivity has been criticized on the basis that the results of many experiments are confounded by 'sampling effect' (SE), where the chances of including a fungus conferring a large benefit to a plant increases as the number of AM fungi included in the test increases (Wardle, 1999). This effect was observed in the experiment of Perry *et al.* (1989b; see above), in which increases in productivity of the two tree seedlings when inoculated with four ECM fungi were due to the positive effects of *L. laccata* on *P. menziesii*, and not to the ECM fungal diversity.

Other investigations have failed to show a consistent link between mycorrhizal fungal species richness and plant productivity. The effects of ECM assemblage on growth and productivity of seedlings of two trees were examined in two soils of different nutrient availabilities (Jonsson *et al.*, 2001). Mycorrhizal effects on plant growth varied among the eight ECM fungi and between soils, when the plants were inoculated with one fungus alone. Only in one case (*Betula pendula* on low fertility soil) was there a clear positive relationship between fungal species richness and plant productivity that could be attributed to effects not related to SE. A negative relationship was observed between fungal species richness and productivity of *Pinus sylvestris* on high fertility soil; in the other two cases no effects related to fungal species richness were observed.

The observations that productivity is not necessarily related to fungal diversity should not come as a surprise. As with the effects of presence or absence of AM fungi (see above), the outcome will be strongly influenced not only by the ability of the fungi in the assemblage to colonize the roots, but also by the responsiveness of the plant species that make the largest contributions to the overall biomass. In the microcosm experiment of van der Heijden *et al.* (1998b), there was no change in productivity as the number of AM fungi increased, because the plant species that made the largest contributions to biomass were either non-responsive or negatively responsive and this did not change when they were inoculated with four species rather than one. Unfortunately, data for root colonization by AMF were not presented and, in any event, it would have been hard, using the techniques then available, to determine the contributions of the different fungi in mixtures to the overall colonization in the different species. Differences between fungi in type and infectivity of inoculum, as well as their competitive abilities, which are poorly understood (see Chapter 2), are likely to be significant in determining root occupancy and hence the ability to obtain resources for effective exploitation of the soil. In contrast to most investigations in which fungal species richness refers to the numbers of fungi

inoculated at the start of the experiment, Jonsson *et al.* (2001) showed that all inoculated ECM fungi did survive to the end of the experiment. Nevertheless, in some cases the proportions of root tips colonized by different fungi showed that they were not present in equal proportions, so that some competitive reductions had occurred.

The significant feedback in the way plants influence AM fungal communities, and *vice versa*, has mostly been studied at the level of spore production. Several investigations have shown positive relationships between numbers of AM fungal spores and numbers of plant species in a community (Burrows and Pfleger, 2002; Chen *et al.*, 2004). These findings support the observation that plant species-richness is sometimes positively correlated with AM fungal biomass (Hedlund *et al.*, 2003). Landis *et al.* (2004) analysed the species composition of AM spore assemblages in an oak wood, taking advantage of natural gradients in plant species composition. They showed a strong correlation between plant species-richness and AM fungal species-richness. These studies for the most part did not measure plant fitness or productivity in relation to the characteristics of the AM fungal species assemblage. However, Hedlund *et al.* (2003) did observe a negative correlation between AM biomass (determined from the signature fatty acid 16:1 ω 5) and plant biomass, suggesting that extensive root and soil colonization by the fungi was not a driver for high plant productivity.

Bever (2002a, 2003) examined the community dynamics of co-occurring plant and AM fungal species at a grassland site, in terms of growth and spore production as surrogates for 'fitness'. He provided considerable evidence for the existence of asymmetric fitness relationships and negative feedback between plants and AM fungi. He showed that, generally, a fungus delivers the greatest benefit to one plant species, but grows better (produces more spores) on another and expressed the relationships in terms of feedback models (Figure 16.7). He did not find evidence for positive feedback, in which a fungus that delivers the greatest growth benefit to a plant also receives the greatest benefit from it, a situation that would lead to the evolution of specificity and 'best friend partnerships' (see Chapter 1). These findings are highly significant, because they help to explain several of the general observations about the specificity, occurrence and function of AM symbioses in complex

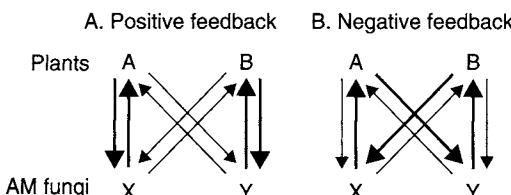


Figure 16.7 Representation of fitness sets that would produce positive and negative feedback within the interaction of plants and AM fungi. In these figures, the thickness and direction of the arrows represent the relative benefit that two plant types (A and B) and two AM fungal types (X and Y) receive from their association. (a) In the case of a highly symmetric fitness relationship between plants and AM fungi, an initial abundance of plant A will result in an increase in representation of AM fungus X, which will increase the growth rate of plant A and thereby generate a positive feedback that can lead to the loss of diversity. (b) Alternatively, in the case of a highly asymmetric fitness relationship, an initial abundance of plant A will increase the representation of AM fungus Y and thereby boost the performance of plant B, resulting in a negative feedback on plant A. Reproduced from Bever (2002), with permission.

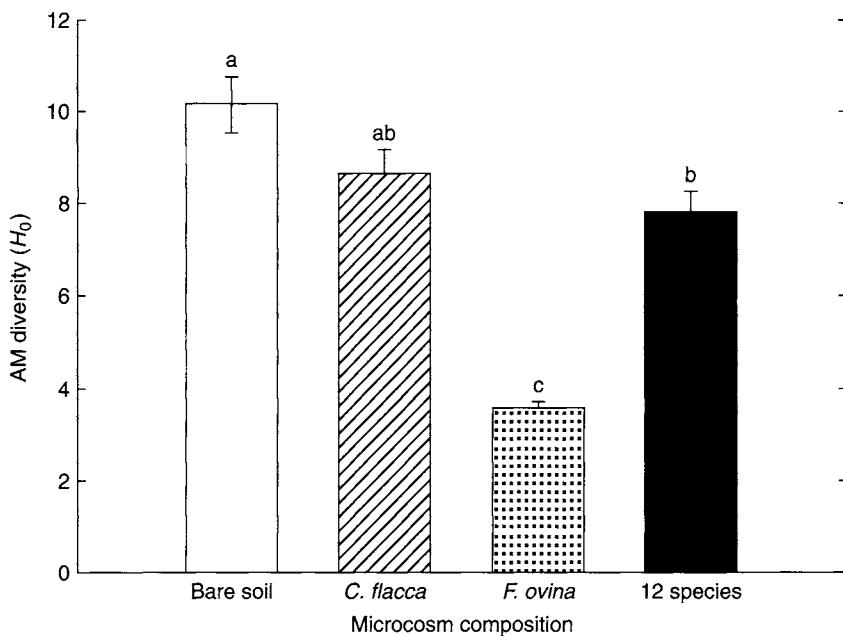


Figure 16.8 Mean diversity of AM fungi (H_0) colonizing the roots of bioassay seedlings of *Plantago lanceolata* removed from grassland microcosms after 12 weeks growth. The microcosms had bare soil (open bars), *Carex flacca* (hatched bars), *Festuca ovina* (stippled bars) or a mixture of 12 plant species (black bars). Means \pm standard errors of means, $n = 3$. Bars with the same letter are not significantly different ($P > 0.05$). From Johnson *et al.* (2004), with permission of the *New Phytologist*.

communities. Under negative feedback, specific advantageous symbioses will not be able to evolve, fluctuating dynamics in plant and fungal success will occur and co-occurrence of competing species will be maintained, leading to species-richness in both plant and fungal communities. As we have seen, this is the pattern that best fits our present knowledge of AM symbioses as in most cases non-specific associations with considerable functional diversity, that are characteristic of floristically rich ecosystems (Chapter 1).

Bever's analyses are based on spore assemblages, which do not provide information on the AM associations developed with roots and therefore potentially significant for plant growth and nutrition. However, advances in techniques to identify AM sequence variants have allowed the diversity of the AM communities to be followed in roots of *Plantago lanceolata* growing in microcosms containing 12 species of plants from limestone grassland, compared with those in microcosms containing bare soil or monocultures of the non-host *Carex flacca* or the highly mycorrhizal *Festuca ovina* (Johnson *et al.*, 2004, 2005). AM fungal diversity was significantly influenced by floristic composition (Figure 16.8) but, surprisingly, the greatest species richness (Shannon H_0) was found in the bare soil and *C. flacca* microcosms and the least with *F. ovina*. The authors suggested that colonization from soil without plants or supporting a non-host was initiated from spores of a large number of species that had survived the 3-year pretreatment without plants. In contrast, active mycelium

in soil was most likely to have initiated colonization in those microcosms supporting host species. If this explanation is correct, colonization in *P. lanceolata* would then reflect the assemblage of fungi that occupied the roots of these host plants and, in consequence, any pre-existing plant-fungus selectivity. Even if spores were present in these soils, colonization from them would most likely have been slower and result in lower root occupancy than that from the mycelium. Multifaceted investigations of this type will increasingly be needed to understand the highly complex interactions and feedbacks between assemblages of plants and fungi that are gradually being revealed. Moreover, the length of the experiments will be an important consideration, especially with respect to determining any temporal differences in colonization from different sources of inoculum and changes in fungal assemblages which might possibly be related to the occurrence of *r*- and *K*-selected species.

The role of mycelial networks in distributing nutrients among members of a plant assemblage

As outlined in earlier chapters, there is considerable evidence that plants may be linked into common mycelial networks (CMNs), one for each of the mycorrhizal fungi present at a particular site. There seems little doubt that CMNs are an important means by which seedlings become mycorrhizal when establishing in the neighbourhood of colonized plants. The roles of the networks in redistributing nutrients among the linked plants has been extensively discussed, but there is still no general consensus on their quantitative significance or indeed on mechanisms underlying any transfers. It seems quite likely that the processes facilitated by CMNs vary among different mycorrhizal types. Available data have been thoroughly summarized and reviewed in recent years (Robinson and Fitter, 1999; Simard *et al.*, 2002; Simard and Durall, 2004).

One of the first demonstrations of movement of ^{14}C -labelled compounds between AM species was obtained in the microcosm experiment of Grime *et al.* (1987), discussed above. Immediately prior to harvesting the microcosms, $^{14}\text{CO}_2$ was fed to shoots of *Festuca* and the pattern of distribution of label was determined. The transfer of radioactivity occurred almost exclusively in the microcosms containing AM fungi and only the plant species which were actually colonized contained high amounts of ^{14}C . These observations provide evidence that plants in mixed assemblages are interconnected by their AM fungi. It was also suggested that net movement of organic C between plants was an important contributor to the outcomes in the microcosms in terms of plant growth. However, Bergelson and Crawley (1988) pointed out that a more likely explanation was that subordinates were released from competition. Numerous studies have followed up this observation, some of them investigating possible source-sink effects by experimental manipulations such as shading. In many cases, ^{14}C was certainly transferred from the shoots of one plant to the AM roots of another, but this does not demonstrate transfer to the plant cells of the receiver nor the quantitative contribution of transfers to overall C budgets. It does show that the plants are linked by a common AM fungus and it does indicate that there is the potential for plants to share the expense of maintaining the fungal network and its nutrient uptake activities, which might have important consequences for the relative benefits of AM colonization to the plants involved (see Chapter 4). The extent of contributions to the CMN by different plants might very well be influenced by changes in source-sink relationships.

In a study of the possible roles of AM colonization in mediating interactions between the invasive weed *Centaurea maculosa* and the native grass *Festuca idahoensis*, Marler *et al.* (1999) showed that neither species responded positively to AM inoculation when grown singly in pots. However, when grown together, *C. maculosa* exerted much greater competitive effects in inhibiting the growth of *F. idahoensis* when the plants were inoculated with AM fungi than when they were not. The researchers then used ^{13}C labelling and pots compartmented with mesh, to explore the possibility that C transfer from *F. idahoensis* to *C. maculata* was the basis for successful competition (Zabinski *et al.*, 2002; Carey *et al.*, 2004). The experiments of Zabinski *et al.* (2002) found no evidence for C transfer in either direction between the plants, despite the fact that growth differences were the same as those previously reported. They also demonstrated that *C. maculosa* had significantly higher P concentrations when grown in pots with a grass, with their root systems separated by mesh, and concluded that the weed was more effective at exploiting its AM symbiosis for P acquisition than the native grasses. Carey *et al.* (2004) re-examined the possibility of net C transfer but, despite obtaining some evidence that it occurred, on the basis of distribution of ^{13}C , they were not able completely to discount the involvement of differences in water-use efficiency in altering ^{13}C distribution patterns. Differences in obtaining P from a common mycelial network, or indeed from separate networks formed by different AM fungal species, remain the most parsimonious explanation for competitive outcomes in this case.

The two investigations outlined above studied plants growing at the same time, in pots. Lerat *et al.* (2002) explored the possibility of seasonal differences in C transfer between the deciduous tree, *Acer saccharum* (sugar maple) and the geophyte *Erythronium americanum* (trout lily), with the plants grown in fibre pots located in a maple forest. *E. americanum* produces its leaves in spring, around the time of maple bud-burst, but before the maple leaves are fully photosynthetic. Later, as the maple canopy closes, *E. americanum* dies back and survives as a corm, which produces new roots in early autumn. The potential for these two species, both of which form arbuscular mycorrhizas, to provide ^{14}C to each other is therefore offset between seasons. Labelling with $^{14}\text{CO}_2$ was carried out in both spring and autumn. The results were suggestive of some transfer from *E. americanum* to *A. saccharum* in spring, including to the expanding leaves, which implies transfer from fungus to plant and redistribution to the shoot. No label was found in adjacent ECM *Betula alleghaniensis*, arguing for transfer through the AM mycelial network. In autumn there was also a suggestion that ^{14}C moved from *A. saccharum* to roots of some of the *E. americanum* plants. There was no labelling in the corms, so in this case it is possible that ^{14}C was retained in the fungal structures within the *E. americanum* roots.

Despite some intriguing findings, it is probably fair to say that there is still little unequivocal evidence for transfer of ^{14}C -labelled compounds in nutritionally relevant amounts from one plant species to another based on transfer in an AM mycelial network. The mechanistic bases for the transfers have not been explored and, as outlined in Chapter 5, it appears unlikely that C moves from fungus to plant as the C skeletons of amino acids or other organic N molecules (Pfeffer *et al.*, 2004; Govindarajulu *et al.*, 2005; Jin *et al.*, 2005).

Evidence for transfer of organic nutrients between plants linked by an ECM network is much more persuasive, but significance in terms of outcomes of competitive interactions between plants has not been established either in pots or in the field.

However, the very existence of mycoheterotrophic species such as *Monotropa hypopitys* depends on C transfer between autotroph and heterotroph via common mycelial links (see Chapter 13). The ability of ECM mycelial links to function as potential conduits for the transfer of organic C was demonstrated by autoradiography after feeding $^{14}\text{CO}_2$ to the shoots of 'donor' plants (Finlay and Read, 1986a; see Chapter 8). Label was transported through the interconnecting mycelium and accumulated in the colonized roots of 'receiver' plants. The fragility of these hyphal links is such that it is difficult to trace them in natural soil. However, indirect evidence for the existence of such links in the field was provided by the observation that $^{14}\text{CO}_2$ fed to an adult plant of *Pinus contorta* was subsequently detected in roots of neighbouring plants of the same or of different species, providing they were also ectomycorrhizal (Read *et al.*, 1985). Only small amounts of activity were found in neighbouring AM species. The greatest amounts of radioactivity were detected in a number of 'receiver' plants that had been subjected to artificial shading during and after the period of isotope feeding to the 'donor'. This suggested that carbon transfer might be influenced by sink strength.

Experiments of this type have, together with similar investigations of AM transfer, been justifiably criticized on the grounds that they are only capable of revealing unidirectional transport and hence do not take into account the possibility of C transfer in the opposite direction (Newman, 1988; Jakobsen, 1991; Robinson and Fitter, 1999). In order to establish unequivocally that net transfer is occurring, it is necessary to demonstrate that one of the interconnected plants gains more material than the other in an exchange. In an enlightening study using double labelling methods, Simard *et al.* (1997a, 1997c) demonstrated that significant net transfer of carbon can occur in interspecific combinations of plants colonized by shared ECM symbionts. The two ECM plant species used in the study, birch (*Betula papyrifera*) and Douglas fir (*Pseudotsuga menziesii*), co-occur naturally in mixed wet forests of British Columbia, where they share seven of 11 ECM morphotypes, which occupy 90% of the root tips of both species (Simard *et al.*, 1997b). The likelihood of the occurrence of interconnection between the species was therefore great. For comparative purposes a further co-occurring species, *Thuja occidentalis* forming arbuscular mycorrhizas, was included in the study. Individual one-year-old plants of each species were planted 50 cm apart in triangular arrangements. The triangular groups were allowed to grow for either one or two years before experimental procedures were imposed. Four to 6 weeks prior to labelling, *P. menziesii* plants were subjected to three shading treatments; deep shade, partial shade and ambient light. *P. menziesii* or *B. papyrifera* in each group were then pulse-labelled (2 h) with either $^{13}\text{CO}_2$ or $^{14}\text{CO}_2$. The labelling pulse was followed by a 9-day chase under the same shading regimes.

Transfer of organic C occurred in both directions, with significant net transfer from *B. papyrifera* to *P. menziesii* (Figure 16.9a, b). Redistribution from roots to shoots in amounts that were much higher than previously observed in ECM systems was also found (Finlay and Read, 1986a). This is an important point of difference from experiments with plants linked by AM fungi and confirms that transfer of C, possibly as the C skeletons of N compounds, occurs from fungus to plant (see Chapter 9). Nevertheless, the recent suggestion that N transfer occurs as inorganic ammonium/ammonia throws some doubt on this mechanism (Chalot *et al.*, 2006).

In the experiment carried out one year after planting, net transfer to the conifer was found only when it was grown in full sun. The amount transferred represented 2% of the total isotope fixed by both species, 4% of the isotope assimilated by

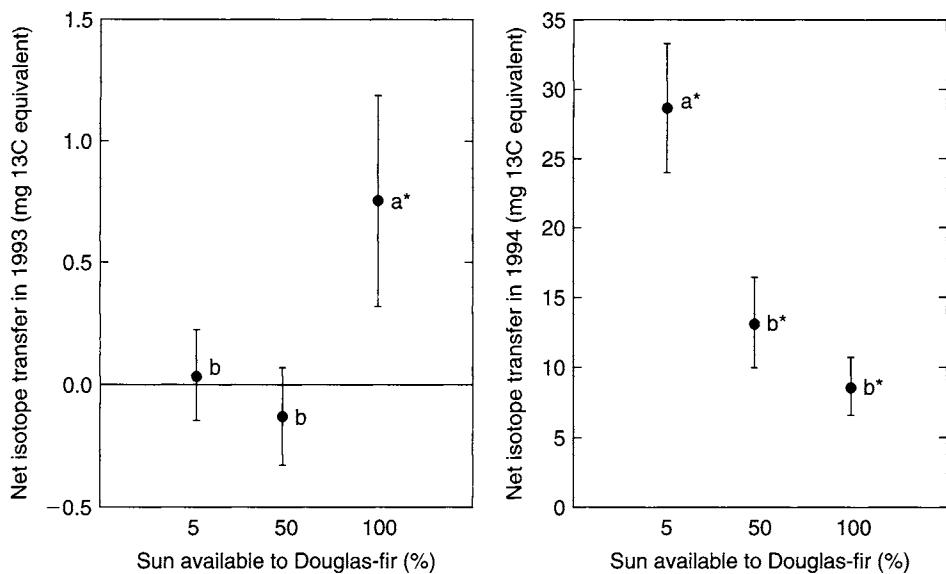


Figure 16.9 Net isotope transfer between *Betula* and *Pseudotsuga*, in full sun (100%) or in 50% or 5% of full sun. (a) 1993; (b) 1994. Means denoted by the same letter do not differ significantly ($P = 0.01$). See text for explanation. Data from Simard (1995), reproduced with permission. See also Simard *et al.* (1997c).

B. papyrifera and 7% of that assimilated by *P. menziesii*. In the 50% and 5% of full sun treatments, isotope transfer to *P. menziesii* was balanced by transfer to *B. papyrifera* (i.e. there was zero net transfer). In the second year after planting, net isotope transfer represented on average 6% of the total isotope fixed, averaged over all shading treatments, and in the deepest shaded it was double that in the 50% or full sun treatments. This amount of transfer represents a substantial carbon gain by *P. menziesii* and is similar to that which has been considered sufficient to improve growth and survival of connected ramets in some clonal plants (Hutchings and Bradbury, 1986; Alpert *et al.*, 1991). No net transfer to the AM *Thuja* occurred, implicating the ECM networks in the transfers between plants.

Both spatial and temporal factors were considered to have contributed to the increased net transfer and to the effect of shading upon C transfers in the second year. In the spatial context, below-ground proximity of roots of the potentially interacting species, as well as the numbers of fungal interconnections between them, were likely to be larger two years after planting than one year. Of perhaps greater importance was the fact that the application of isotope was carried out in August of the second year, fully one month after cessation of shoot elongation in *P. menziesii*, whereas the first year feeding experiment took place in July when shoot activity was ongoing. The likely importance of seasonal effects upon carbon allocation below ground has been stressed earlier (see Chapter 8).

Net transfer from *B. papyrifera* to *P. menziesii* coincided with whole seedling net photosynthetic rates which were 1.5 and 4.3 times greater for *B. papyrifera* than *P. menziesii* in full sun and full shade, respectively, and occurred where foliar N concentrations were 1.2–6.7 times higher in *B. papyrifera* than *P. menziesii*. It is therefore possible that transfer was influenced by gradients of assimilate and/or nutrient concentration

between the two species. Since rates of photosynthesis of *B. papyrifera* in full sun were so much greater than those of *P. menziesii* in shade, it is likely that carbon supply to the colonized roots of *Betula* would be greater than to those of *Pseudotsuga*. This, combined with the fact that the *Betula* plants contained significantly more N than those of *P. menziesii*, may have contributed to the sink effect observed.

Simard *et al.* (1997a) examined the roles of the CMN in more detail in a pot experiment in which the same two plant species were grown in separate mesh pouches that allowed the development of extensive mycelial links between them. Direct transfer through the mycelium was prevented in half the microcosms by severing the links just before the labelling pulse was applied. As in the field experiments, both *B. papifera* and *P. menziesii* received isotope from their neighbours and there was an indication that, again, *P. menziesii* received more C from *B. papifera* than *vice versa*, although differences were not significant. Surprisingly, severing mycelial links between the plants had no significant effect on transfer between the plants, so that the relative importance of mycelial links, *vis à vis* transfer through soil must remain in doubt.

In the experiments described by Simard, transfer of C in some form of combination with N, perhaps in the form of amino compounds, is a possibility. This was examined by Arnebrant *et al.* (1993), who investigated transfer of the products of $^{14}\text{CO}_2$ and $^{15}\text{N}_2$ fixation by ECM and actinorhizal *Alnus glutinosa* to neighbouring ECM *Pinus contorta* in laboratory microcosms. They showed in two out of three experiments that material labelled with both isotopes moved between the plants and appeared not only in the ECM roots of the *Pinus* receivers, but also in the shoots. Transfer in the reverse direction was not investigated, so that net transfer could not be calculated. The plants were not separated by mesh and there were no non-mycorrhizal or AM control plants, so it remains possible that the transfers observed took place indirectly through soil, bypassing the mycelial network. Evidence for the operation of both transfer pathways was found in a field study by He *et al.* (2006), following labelling of shoots of donor plants of *P. sabiniana* with K^{15}NO_3 . ^{15}N appeared most rapidly in the roots of AM annuals growing nearby (*Cynosurus echinatus*, *Torilis arvensis* and *Trifolium hirsutum*) but, after 4 weeks, was also detectable in their shoots as well as in the roots and shoots of woody species which formed ecto- and/or arbuscular mycorrhizas (*Quercus douglasii*, *Ceanothus cuneatus* and *P. sabiniana*). These results indicate that the CMN is not the only pathway through which N transfers between plants occur in the field and confirm, as shown earlier for AM systems (McNeill and Wood, 1990), that an indirect pathway involving N cycling in soil can result in considerable N movement.

All in all, the studies with C and N isotopes indicate that there is a much greater likelihood of net movement via CMNs formed between ECM species than AM species and that movement through the soil must not be ignored in some environments. There is still much to be learned about the biochemical pathways associated with transfer of C compounds between symbionts but, where gradients of C and N co-occur, there is a strong possibility that organic N compounds may be involved, at least in ECM systems. There remains a major gap between demonstration of nutrient transfers between plants via CMNs and elucidation of their significance both in plant growth and in competitive interactions between individuals and species. The whole plant and community level effects will be even harder to unravel, but of great importance in understanding vegetation dynamics.

Multitrophic interactions

The mycorrhizosphere

The recognition of stimulatory effects of some classes of bacteria on the processes of ECM colonization of roots has heightened awareness of the complexities of microbial interactions in soil. The mantles of ECM roots have long been known to provide a significant habitat for bacteria (Foster and Marks, 1966; Nurmiaho-Lassila *et al.*, 1997) (Figure 16.10), with assemblages distinct from those associated with uncolonized roots (Rambelli, 1973). As techniques enabling more detailed examination have been developed, a great deal more about the bacterial associates of ectomycorrhizas has been revealed. Although we are still a very long way from understanding the diversity of interactions, it is now certain that there can be considerable differences in bacterial populations influenced by identity of plants and ECM fungi, as well as by soils. Interactions of ECM roots and mycelium with saprotrophic fungi has received much less attention, probably in part because of the long-held view that bacteria dominate activities in the rhizosphere (de Boer *et al.*, 2006). Nevertheless, recent work is beginning to demonstrate both that saprotrophic fungi have important roles in rhizosphere processes (Mougel *et al.*, 2006) and that inter-actions between them and mycorrhizal fungal symbionts should not be ignored.

Scanning and transmission electron microscopy by Nurmiaho-Lassila *et al.* (1997) revealed a large number of morphologically different types of bacteria in habitats provided by intact ectomycorrhizas formed between *Pinus sylvestris* and *Suillus bovinus* or *Paxillus involutus* grown in natural forest soil. With *S. bovinus*, not only were bacteria found on the mantle surfaces, but also in inter- and intracellular locations

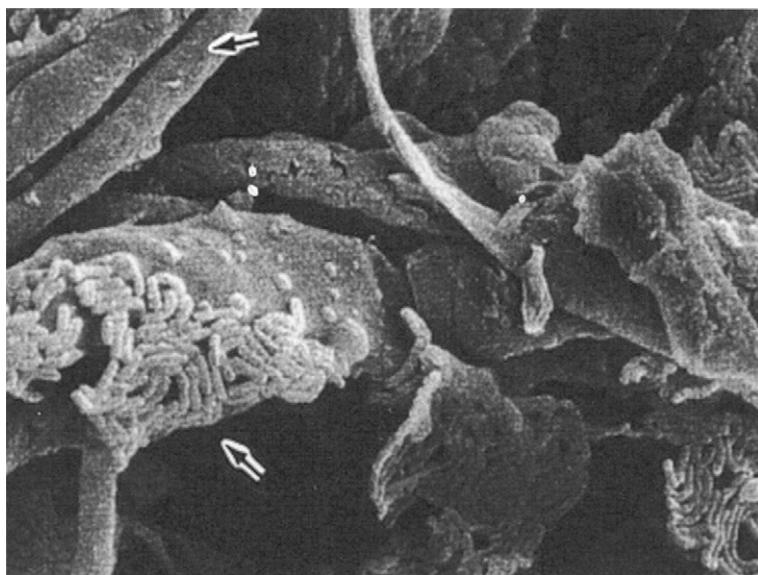
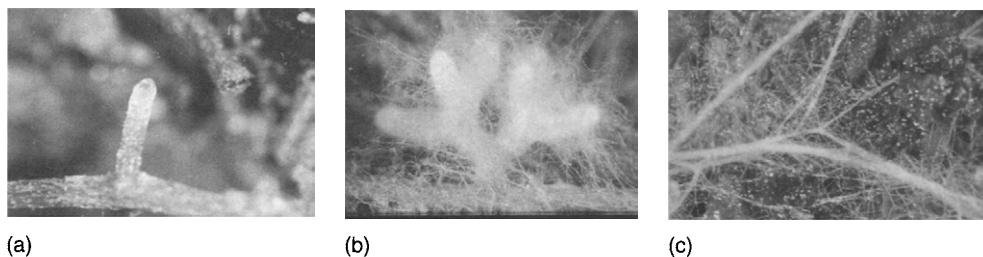


Figure 16.10 Monolayer of rod-shaped bacteria on the external hyphae of *Suillus bovinus*. Bar = 5 µm. Reproduced from Nurmiaho-Lassila *et al.* (1997), with permission.

in the mantle and Hartig net. Mycelial strands of the fungi had relatively sparse bacterial populations, whereas extensive monolayers of bacteria developed on the fine hyphal branches. In contrast, ECM root tips of *P. involutus* harboured few bacteria whether on the surface or internally, but the intact external mycelium supported both bacterial colonies and solitary bacteria. In a subsequent investigation, Timonen *et al.* (1998) showed differences in metabolic activity between the bacteria associated with the different fungal symbionts, although these were not as great as differences associated with the two soils used. Use of PCR and RFLPs enabled the detection of sequences belonging to the Archaea associated with external mycelium of both ECM fungi and uncolonized humus, but not from non-mycorrhizal short roots (Bomberg *et al.*, 2003). Such differences in microbial populations were carried through to the populations of protozoans dependent on them, with numbers of protozoa higher in *S. bovinus* mycorrhizospheres than in those of *P. involutus* (Figures 16.11 and 16.12; see also Colour Plate 16.1) (Timonen *et al.*, 2004).

The roles of bacterial communities in association with ECM fungi have not received much concerted attention, but could include activities that increase nutrient availability in various ways. One such possibility is that bacteria may enhance the production of low molecular weight organic acids and that this may increase the capacity of ECM mycelium to weather minerals (Leyval and Berthelin, 1991), with consequent release



(a)

(b)

(c)

Figure 16.11 Roots of *Pinus sylvestris* used in studies of the protozoan populations associated with (a) non-mycorrhizal short roots (NM), (b) ectomycorrhizal short roots (MR) and (c) extraradical mycelium (EH) of *Paxillus involutus* (see Figure 16.12). Reproduced from Timonen *et al.* (2004) with permission. See also Colour Plate 16.1.

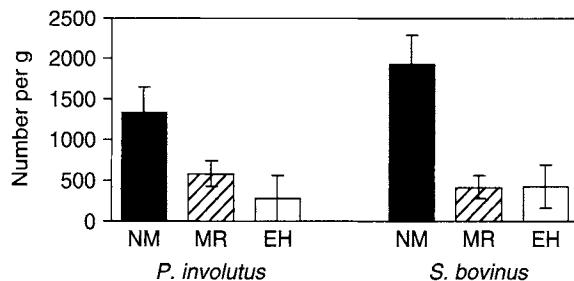


Figure 16.12 Numbers of culturable protozoa on uncolonized short roots (NM), mycorrhizal root tips (MR) and extraradical mycelium (EH) of roots of *Pinus sylvestris* mycorrhizal with either *Paxillus involutus* or *Suillus bovinus* (see Figure 16.11). Reproduced from Timonen *et al.* (2004), with permission.

of a range of nutrients such as P, K and Ca which could then be transferred to the plant symbionts (Griffiths *et al.*, 1991, 1994; Wallander, 2000; Landeweert *et al.*, 2001; Hoffland *et al.*, 2004; see Chapters 10 and 15). The quantitative contribution of mineral-weathering to nutrient acquisition may be rather low, but could become increasingly significant as increased anthropogenic N depositions change the nutrient balance of some forest ecosystems. Another possibility is that bacteria in the ectomycorrhizosphere or more closely associated with ECM mantles might have sufficient N₂ fixing capabilities to make significant contributions to forest N balance. This, as pointed out by Izumi *et al.* (2006) and Timonen *et al.* (1998) requires further investigation.

The suggestion that bacteria in ECM rhizospheres might be directly involved in the dynamics of mycorrhiza-formation was made by Bowen and Theodorou (1979). They showed, *in vitro*, that the ability of *Rhizophagus luteolus* to colonize roots of *Pinus radiata* was enhanced by the presence of some bacterial isolates, but inhibited by others. Subsequently, Garbaye and Bowen (1987) examined the process of mycorrhiza formation by three fungal symbionts in different steam-sterilized soils, inoculated with microbial populations from one of the soils. There were differences in responses, dependent on both soil and fungus and positive effects outnumbered negative ones. Subsequent isolation of bacteria from surface-sterilized *P. radiata*-*R. luteolus* mycorrhizas showed that most were fluorescent pseudomonads and that 80% of them had positive effects on ECM formation by this plant-fungus combination (Garbaye and Bowen, 1989a, 1989b). The term mycorrhiza-helper-bacterium (MHB) was coined to describe these microorganisms. The populations of fluorescent pseudomonads have been further characterized for different ectomycorrhizas. In one case, functional characterization *in vitro*, including a range of attributes such as production of HCN and antibiotics, N₂-fixing and phosphate-solubilizing activity, led Frey-Klett *et al.* (2005) to suggest that the ectomycorrhizosphere controls *P. fluorescens* populations in such a way as to select strains potentially beneficial to the symbiosis. In reviewing the topic, Garbaye (1994) suggested five hypotheses to explain the beneficial effects of MHBs. These include facilitation of colonization either by production of cell wall softening enzymes or enhancement of the plant-fungus recognition processes. More work is needed to establish clear relationships between effectiveness of MHBs, enzyme production and wall softening using a functionally relevant test system. Nutritional enhancement of fungal growth by MHBs is also a possibility but, in view of the apparent specificity of the effect, it must be of a highly specialized type. Detoxification of compounds present in soil by MHBs could, indirectly, provide a nutritional effect and it is noteworthy that many of the responses to MHBs have been observed in artificial media or nursery soils which have been subjected to sterilization treatments. These have the potential to release toxins and so influence subsequent microbial interactions. Changes in the rhizosphere, possibly leading to the production of chelating agents such as siderophores or stimulants that enhance germination of fungal propagules, might also contribute to the effectiveness of MHBs. There remains considerable scope for experimental analysis of the roles of these bacteria. New molecular methods will help to reveal details of the rather specific interactions and subsequently their underlying mechanisms.

The significance of interactions between ECM fungi and fast growing and destructive wood and litter decomposing saprotrophs, which together dominate the microbial communities in highly organic forest soils, has been highlighted by Leake *et al.*

(2002). They point out how few investigations in ecologically relevant situations have been carried out, despite the potential for interactions between the two fungal groups to have major consequences for nutrient cycling. The early field investigations of Gadgil and Gadgil (1971, 1975), which showed that exclusion of ECM fungi by trenching increased litter decomposition by saprotrophs, was found to be difficult to repeat. Recently, the sharp demarcation between ECM, monospecific stands of *Dicymbe corymbosum* and AM-dominated, species-rich rainforest provided a useful system in which to investigate the influences of ECM on litter decomposition (Mayor and Henkel, 2006). The ECM stands naturally have much higher litter accumulation than the AM forest. Trenching in these stands showed no effect whatever of exclusion of ECM fungi on either the fraction of litter remaining after 12 months or in concentrations of major nutrients, N, P and Mg. There were, however, significant effects on potassium and calcium concentrations. Likewise, there were no marked differences in decomposition of litter samples transferred between the two forest types, although again Ca concentrations were higher when ECM fungi had no access to the litter bags. The results of this study strongly argue against suppression of saprotrophs by ECM fungi in this environment (see Chapter 15).

Despite these negative findings, evidence for direct interactions has been forthcoming from experiments using microcosms containing natural soil. It appears that spatial redistribution of nutrients such as P in the mycelial systems of saprotrophs such as *Hypholoma fasciculare* can lead to increased nutrient availability to ECM fungi, either via natural turnover of the mycelium or even via antagonistic effects in the zones where two mycelia interact (Lindahl *et al.*, 1999, 2001). The review by Leake *et al.* (2002) presents data on the interactions between two ECM fungi and the wood-destroying fungus *Phanaerochaete velutina*. Using a combination of $^{14}\text{CO}_2$ feeding to the shoots of *Betula pendula* to follow C allocation in the mycelium of associated *Paxillus involutus* or *Suillus bovinus*, and measurements of the extent of mycelial development of *P. velutina* growing from wood blocks, they were able to show that growth of both types of fungi was inhibited in the zones where the mycelia interacted. Although this work did not extend to providing information on redistribution of N or P, it did reveal the fierce competition between the two types of fungi, which is almost certain to feed back into changes in nutrient availability and litter decomposition shown by the 'Gadgil effect'. These effects now deserve re-examination, taking into account the potential changes in below-ground litter as a result of ECM formation itself (Langley and Hungate, 2003). Modification of litter quality and abundance will influence decomposition, nutrient cycling and, hence, plant establishment and succession (Facelli and Facelli, 1993; see Chapter 15).

Interest in the rhizospheres of AM plants has increased in recent years (Azcón-Aguilar and Barea, 1992) and it is clear that AM development can have important effects on soil microflora and fauna, although results are very variable (see Wamberg *et al.*, 2003). Formation of arbuscular mycorrhizas influences both the quality and quantity of root exudation and rhizodeposition (Graham *et al.*, 1981; Marschner *et al.*, 1997; Johnson *et al.*, 2002; Jones *et al.*, 2004). Marschner and Crowley (1996) used *Pseudomonas fluorescens* strain 2-79RL to follow effects of AM formation in *Capsicum* on bacterial activity. This *P. fluorescens* strain harbours a ribosomal promoter coupled to a lux gene cassette and it therefore emits light when in exponential growth phase. Results showed reduced bacterial activity in the rhizospheres of plants colonized by two *Glomus* species. Analysis of the bacterial rhizosphere communities of

maize using PCR-Denaturing Gradient Gel Electrophoresis (DGGE) led to the conclusion that AM colonization changed the bacterial community structure both on the root surface and in the soil at greater distances from the root (Marschner and Baumann, 2003). The latter had presumably been invaded by external AM mycelium. Not unexpectedly, differences have also been revealed between the rhizospheres of plants inoculated with different AM fungi (Marschner and Timonen, 2005). In two of these investigations, some effects appeared to be transmitted via the plant between separate compartments of split pots. Negative effects of AM fungi on growth of saprotrophic fungi in the rhizospheres of three plant species were suggested by some data of Smith SE *et al.* (2004a), but have not yet been substantiated. Despite these findings, the functional relevance of changes in bacterial and fungal inhabitants of rhizospheres remains to be revealed.

Grazing by protozoans, collembolans and mites significantly influences bacterial and fungal numbers in density- and time-dependent ways, so that much of the variation in bacterial communities between different experiments may be explained on these bases. For example, Rønn *et al.* (2002) found no effect of mycorrhiza-formation by *Pisum sativum* on bacterial numbers in the rhizosphere (determined from plate counts), but did show marked increases in protozoa that were grazing on and presumably controlling the bacterial numbers. In a more detailed analysis, Wamberg *et al.* (2003), using both plate counts and DGGE, showed that bacterial numbers were unaffected by AM colonization, but specific bacterial groups grew preferentially in the rhizospheres of non-mycorrhizal *P. sativum*. The effects were mediated by changes in rhizodeposition and grazing by protozoans. Moreover, fungivorous members of the soil fauna have the potential to damage the external mycorrhizal mycelium with consequent effects on function, as well as on nutrient cycling (see below).

Interactions between mycorrhizas, pathogens and endophytes

There are many reports of the interactions between mycorrhizal colonization of plants and the incidence and severity of diseases caused by plant pathogens. The effects are variable and influenced by plant nutrition, relative density of the inoculum of the pathogen and mycorrhizal fungus, and whether or not the plants were mycorrhizal before being challenged with propagules of the pathogen (Harley and Smith, 1983; Graham, 1988; Linderman, 1992; Fitter and Garbaye, 1994). The main instances of arbuscular mycorrhizas reducing disease are for root-infecting fungi and nematodes. Shoot pathogens are usually unaffected or their effects increased in AM plants. A number of mechanisms to explain lower disease losses in AM plants have been suggested. These include: competition for colonization sites, so that prior occupancy by a mycorrhizal fungus reduces the opportunities for colonization by pathogens; mobilization of plant defence mechanisms during AM colonization, discussed in Chapters 3 and 6; and improved nutrient status, which increases the resistance of the plants to attack by disease organisms and increased tolerance of disease, particularly root damage. Indeed, direct negative effects of AM hyphae on *Fusarium* within mycorrhizal transformed carrot roots have been observed (Benhamou *et al.*, 1994) and there are reports of decreased development of the pathogen *Phytophthora parasitica* as a result of both localized and systemic induced resistance in mycorrhizal and non-mycorrhizal parts of AM root systems of tomato (Cordier *et al.*, 1998).

Although these interactions have been most closely studied in relation to crops, they are highly relevant to the influence of mycorrhizas in natural ecosystems where pests and pathogens may exert considerable influence on community structure. This was shown to be the explanation for the apparently beneficial impact of AM fungal colonization upon the annual grass *Vulpia ciliata* (Newsham *et al.*, 1994, 1995). Using benomyl to reduce AM colonization of the roots in the field, West *et al.* (1993a) showed that there was no relationship between extent of AM colonization and the rates of P uptake. However, the fungicide also controlled weakly pathogenic fungi such as the cosmopolitan *Fusarium oxysporum*, which was known to reduce fecundity of the plant. The benefits of AM fungi were suggested to be the result of their ability to protect the plant from pathogens (Newsham *et al.*, 1994). This possibility was examined further in field-grown populations of *V. ciliata* exposed to different concentrations of benomyl so as to control the extent of colonization by both AM and pathogenic fungi (Newsham *et al.*, 1995). Fecundity was shown to be largely unchanged by fungicide application, despite the fact that benomyl significantly reduced the abundance of all fungi in roots. However, the abundance of root pathogenic fungi, especially *F. oxysporum*, was negatively correlated with fecundity, even though plants displayed no disease symptoms. The poor relationship between fecundity and benomyl application contrasted markedly with the effects of benomyl on AM and pathogenic fungi and with the negative effects of root pathogens on fecundity. These effects could be explained if the two groups of fungi interacted, so that when both were reduced in abundance by fungicides the positive and negative effects on the plants cancelled each other out, so that the net effect on fecundity was slight.

The interaction between AM fungi and root pathogens was further investigated using a transplant approach (Newsham *et al.*, 1995). Seedlings of *V. ciliata* were grown in a growth chamber with a factorial combination of inoculum of *F. oxysporum* or a *Glomus* sp., both isolated from *V. ciliata* at the field site. They were planted into a natural population of the grass in the field. After 62 days of growth, clear evidence was obtained that colonization by *Glomus* gave a protective effect. Plants inoculated with *Glomus* performed as well as control plants, even when simultaneously inoculated with *F. oxysporum*, whereas those inoculated with *F. oxysporum* alone grew less well (Table 16.3). The *Glomus* sp. had no net effect on the performance of the plants in the absence of the pathogen. Analysis of P status of the tissues showed that there was no correlation between shoot P concentration and the abundance of either type of fungus in roots. Rather, the differences between treatments

Table 16.3 Effects of a factorial combination of *Fusarium oxysporum* (F) and a *Glomus* spp. (G) on shoot biomass and root length of *Vulpia ciliata* plants grown in the laboratory, transplanted into the field and sampled from the field after 62 days' growth.

| Variable | Treatment | | | | Main effects | | Interaction $G \times F$ |
|-----------------------------|-----------|------|------|------|--------------|------------------|-----------------------------|
| | -G | +G | -G | +G | G | F | |
| | -F | -F | +F | +F | | | |
| Log (ln) shoot biomass (mg) | 2.4a | 2.2a | 1.4b | 2.2a | $F = 3.5$ | $F = 17.3^{***}$ | $F = 4.8^*$ |
| Root length (cm) | 217a | 203a | 111b | 228a | $F = 9.4^*$ | $F = 9.0^{**}$ | $F = 9.0^{**}$ |

Data from Newsham *et al.* (1995). Means are of 16 replicates; where followed by different letters they differ at $P < 0.05$. Significant main and interaction effects in ANOVA are indicated by: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

seem to have been due to a reduction in the frequency of pathogenic hyphae within roots brought about by AM colonization. Of course, we now know that an additional contributor to the interaction could have been P uptake via the AM pathway, leading to enhanced competitive acquisition of P by AM plants despite the fact that they showed no net benefits compared with those in which AM colonization was suppressed (see above and Chapter 5).

Suppression of other organisms by AM fungi has been implicated in increased plant survival in nature. The root-feeding nematode, *Pratelenchus penetrans*, plays a significant role in decline of the dune grass *Ammophila arenaria* as dune succession proceeds. Results of de la Peña *et al.* (2006) demonstrate that colonization of *A. arenaria* roots by a suite of AM fungi isolated from dune systems significantly reduced nematode numbers and multiplication rates. The effects were not dependent on pre-establishment of the mutualists in the roots and operated locally. It was suggested that AM colonization was important in facilitating the persistence of *A. arenaria* in stable parts of the dunes, where burial of the roots no longer afforded protection from the nematodes.

Effects of ECM fungi in reducing disease have been known for some time. Both *Pisolithus tinctorius* and *Thelephora terrestris* have been shown to reduce the impacts of the root pathogen *Phytophthora cinnamomi* on *Pinus* spp. (Marx, 1969, 1973), whereas inoculation with *Laccaria laccata* reduced disease caused by *Fusarium oxysporum* in *Pseudotsuga menziesii* (Sylvia and Sinclair, 1983a), *Picea abies* (Sampangi and Perrin, 1985) and *P. sylvestris* (Chakravarty and Unestam, 1987a, 1987b). Mechanisms proposed to explain such effects include protection of the root by the physical barrier imposed by the fungal mantle, production of phenolic compounds in the plant tissues induced by the ECM fungus (Sylvia and Sinclair, 1983b) and antibiotic production by ECM fungi themselves. The last effect may operate before any root colonization has occurred for Duchesne *et al.* (1988a, 1988b) observed that inoculation of *Pinus resinosa* with *Paxillus involutus* reduced pathogenicity of *Fusarium oxysporum* and that increases in seedling survival were associated with a sixfold reduction in sporulation of the pathogen in the *Pinus* rhizosphere. Ethanol-soluble compounds in the rhizosphere exerted fungi-toxic effects within 3 days of ECM inoculation. Such suppressive effects may be of considerable importance in seedling survival, both in natural environments and in forestry plantations. Little is known about the chemical bases for the antibiotic activity, although Duchesne *et al.* (1989a) showed that production of oxalic acid was related to suppression and Kope *et al.* (1991) isolated two antifungal compounds (benzoylformic and mandelic acids) from liquid medium in which *Pisolithus tinctorius* had been growing. It is regrettable that most of the experiments on disease suppressive and antibiotic effects of ECM fungi have been carried out under rather unrealistic conditions. Epidemiological studies under natural conditions are necessary to determine if, or at what stages, colonization by ECM fungi can reduce disease impacts and potentially influence plant communities.

A group of interactions that has received rather little attention is those between mycorrhizas and fungal shoot endophytes. Many plants, including a number of common grasses, harbour seed-transmitted, fungal endophytes from the Clavicipitaceae (e.g. *Neotyphodium* and *Epichloë*). The infections are either symptomless or cause suppression of flowering (Clay and Schardl, 2002). The fungi inhabit the intercellular spaces of the shoots and derive organic C from their photosynthetic partners; the latter receive benefits in terms of reduced herbivory. Several investiga-

tions have shown that the presence of fungal endophytes reduces the extent of AM colonization (Chu-Chou *et al.*, 1992; Müller, 2003; Omacini *et al.*, 2006), so that any mycorrhizal influences on the plants are potentially moderated by the endophytes. The mechanism of reduction has not been explored, but might be attributable to competition for organic C or production of toxic metabolites.

Although experiments have only been carried out on grasses, the widespread occurrence of shoot endophytes and their interactions with mycorrhizal colonization mean that it is risky to ignore them in investigations of the effects of either on plant communities. Significantly, benomyl reduces or eliminates shoot endophytes as well as mycorrhizal fungi, so that application to create non-mycorrhizal controls will also remove these symbionts and hence their modifying effects (Omacini *et al.*, 2006).

Interactions with soil fauna and above- and below-ground herbivores

Interactions of mycorrhizal fungi with the vast diversity of animals that inhabit the soil has received rather limited attention, probably because of the enormous difficulties of studying the highly complex interactions, effects of which can only be detected by combining sensitive experimental design with careful data analysis. Pot experiments involving additions of the organisms under investigation risk being too simplistic, whereas field experiments utilizing biocides are difficult to interpret because of the lack of specificity of the chemicals applied to suppress different groups. Recent reviews have addressed some of these complexities in relation to herbivores (Gehring and Whitham, 2002) and soil invertebrates (Gange and Brown, 2002).

It is well established that soil animals, such as collembolans, mites and nematodes play very significant roles in nutrient turnover, due to their effects in fragmenting litter and grazing on decomposer organisms such as fungi and bacteria. The grazing may also stimulate growth and reproduction of the fungi (Lussenhop, 1992). Analysis of effects of below-ground grazing on ectomycorrhizas has concentrated on fungivory, because most of the youngest root tissue is enveloped in mycelium. Ek *et al.* (1994a) examined the effects of different densities of the collembolan *Onychiurus armatus* on ecotymycorrhizas of *Pinus contorta* formed by *Paxillus involutus*. Impacts of fungivory on nutrient uptake by the extramatrical mycelium were examined by placing cups, containing $^{15}\text{NH}_4$ or phytin, to which the fungus alone had access, in the soil. Low densities of the collembolan induced greater development of ECM mycelium and increased uptake of and transfer of ^{15}N to the plants. Mycelial growth was reduced only at high densities of *O. armatus*. Collembolan populations did not increase in ECM treatments compared with non-mycorrhizal ones, possibly suggesting that alternative, preferred, food sources were present. In another investigation, ECM birch and pine plants were exposed to either a naturally complex microfaunal assemblage or a highly simplified one. The complex animal assemblage reduced colonization after 57 weeks, but actually increased shoot growth and N and P uptake compared to the plants that had received the simple assemblage (Setala, 1995).

In the case of AM fungi, considerable emphasis has been placed on the possibility that the grazing fauna may damage the fungal networks and hence reduce their capacity to deliver nutrients to associated plants (Fitter and Garbaye, 1994). It was thought that such effects might partially explain the apparent lack of AM responses under field situations. McGonigle and Fitter (1988a) applied the broad-spectrum

insecticide chlорfenvinphos to a semi-natural, species-rich grassland in northern Britain and monitored the response of a constituent grass of the plant assemblage, *Holcus lanatus*. In the presence of the insecticide, which reduced density of collembolans to one third of the original population, there was a large increase in P uptake by the grass and a significantly greater shoot biomass. Clearly, the lack of specificity of the insecticide makes interpretation of these responses difficult, but increases of P uptake per unit root length or area is hard to explain by any mechanism other than one involving increased AM function when insects were suppressed.

Pot experiments with collembolans have sometimes suggested that the insects damage AM mycelial networks and consequently reduce the potential for enhanced P delivery to the plants (Warnock *et al.*, 1982). This finding was essentially confirmed by Finlay (1985) whose results did, however, indicate that the density of collembolans was important (as shown also for ectomycorrhizas). At low densities, AM responses were actually increased, possibly because increased nutrient mobilization was more important than mycelial damage. Increase in collembolan numbers did result in increased damage to AM hyphal networks of *Glomus intraradices* in an agar culture experiment (Klironomos and Ursic, 1998), but these authors also showed that AM fungal hyphae were not the preferred food source of *Folsomia candida*. This collembolan selectively grazed on *Alternaria alternata* or *Trichoderma harzianum*, in preference to *G. intraradices* and was more fecund when feeding on the conidial fungi, regardless of density. Using compartmented pots to separate roots from AM hyphae and collembolans, Larsen and Jakobsen (1996) again showed that *F. candida* probably did not graze on hyphae of *G. caledonium* or *G. intraradices*. Furthermore, at densities of the collembolan similar to those found in the field, there were no significant effects on the delivery of ^{32}P to plants of *Trifolium subterraneum* via the mycorrhizal pathway. Later investigations have confirmed that experimental outcomes can be highly variable, emphasizing the complexities of the interactions and their underlying mechanisms (Harris and Boerner, 1990; Kaiser and Lussenhop, 1991; Gange, 2000; Lussenhop and BassiriRad, 2005). Lussenhop and BassiriRad (2005) again confirmed that hyphae of *G. intraradices* were a minor food source for *F. candida*, as only about 5% of gut contents were fungal hyphae and there were no effects of the collembolan on length density of the external mycelium. This work also showed that the greatest N uptake by seedlings of *Fraxinus pennsylvanica* was in AM plants with moderate collembolan numbers. The effects of collembolans in damaging AM fungal mycelium and hence reducing nutrient uptake may well have been given undue emphasis, but the interactions and their outcomes in realistic field situations deserve more consideration. Recently, Johnson *et al.* (2005) demonstrated in a natural grassland that the collembolan *Protaphorura armata* at natural densities decreased C flux through soil measured after pulse labelling the sward with $^{13}\text{CO}_2$. Other data presented were strongly indicative of disruption of AM mycelium in soil by the collembolans.

Interactions between AM plants and the fungal-feeding nematode *Aphelenchus avenae* provide an interesting comparison with the work with collembolans (Bakhtiar *et al.*, 2001). This organism is also fungivorous and AM fungi do appear to support its growth. Populations declined when *A. avenae* was inoculated onto non-mycorrhizal plants of *T. subterraneum*, but increased markedly when the plants were colonized by either *G. coronatum* or *Gi. margarita*, with final populations much higher with *G. coronatum*. Presence of the nematode decreased root colonization by both fungi to

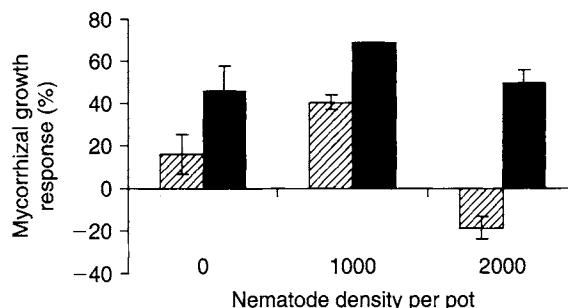


Figure 16.13 Mycorrhizal growth response (%), relative to non-mycorrhizal controls, of *Trifolium subterraneum* colonized by *Gigaspora margarita* (shaded bars) or *Glomus coronatum* (black bars) grown with different densities of the fungivorous nematode *Aphelenchus avenae*. Values are means, \pm standard errors of means ($n = 3$). Data of Bakhtiar *et al.* (2001).

the same extent and decreased the percentage of spores with contents, markedly so for *G. coronatum*. It therefore appeared that *G. coronatum* was either more palatable or more accessible to the nematode. Despite this damage, mycorrhizal growth responses were increased at moderate nematode densities (Figure 16.13), again suggesting that increased hyphal turnover as a result of nematode grazing had beneficial effects that offset damage to the hyphae if nematode populations were not exceptionally high. Effects on the two fungi were again different, highlighting the diversity of interactions to be expected in complex soil environments.

Interactions between mycorrhizal fungi and above-ground herbivores are at least as complex as those with soil animals, but our knowledge is even more fragmentary. By surveying published literature, Gehring and Whitham (1994, 2002), have shown that both ECM and AM colonization is reduced by herbivory in about two-thirds of the investigations and either unaffected or very infrequently increased in the remainder. The predominance of negative effects has been attributed to a reduced capacity of the plants to support their fungal symbionts with organic C. The authors highlighted the facts that again most work has been carried out on AM plants and that investigations focused mainly on the extent of colonization and rarely explored outcomes in terms of functioning of the symbioses or consequences for community interactions.

Effects of mycorrhizal colonization on the herbivores has also been investigated, again with a predominance of AM investigations. In these, the generalist herbivores were more often negatively affected by AM colonization than the specialists, an effect attributed to the greater sensitivity of generalists to defensive compounds produced by their hosts and by the possibility that production of these compounds is likely to be enhanced in mycorrhizal plants.

AM hyphae and soil structure

It has long been recognized that hyphae of AM fungi are important binding agents in soil (Miller and Jastrow, 2002). This was implicit in the use of weight of adhering soil to estimate the length of external hyphae (Graham *et al.*, 1982b) and anyone who has washed roots out of soil knows that the mycorrhizal treatments are much

harder work than uninoculated ones! Aggregation of sand grains by AM hyphae in dunes has been repeatedly confirmed (Koske, 1975; Koske *et al.*, 1975; Clough and Sutton, 1976; Forster, 1979; Forster and Nicolson, 1979; Graham *et al.*, 1982b). In these ecosystems, AM fungi are particularly important in stabilization, because they use recent photosynthate and, unlike soil saprotrophs, do not depend on readily available organic substrates from soil, which may be in relatively short supply. In many soils, it is also evident that roots and hyphae play a major role, together with other organic components in stabilization of aggregates.

Oades (1993) reviewed the contributions of biological processes to the development and stabilization of soil structure and showed that they vary with soil texture. Aggregate formation and stability depend more on organic matter and the activities of organisms in soils with relatively low clay contents. In many soils, there is evidence for the existence of a hierarchy of aggregates of different sizes, with the smaller ones progressively packaged together to form the larger ones. This hierarchy covers a range of sizes over many orders of magnitude (<2 to >2000 µm), with the smaller aggregates held together by stronger forces than the larger aggregates. Roots and fungal hyphae certainly stabilize macroaggregates (>250 µm) acting as temporary binding agents which hold together smaller particles (Tisdall and Oades, 1979, 1982; Tisdall, 1994, 1995). Hyphae are also probably important in stabilizing microaggregates. Clay particles (<2 µm) adhere to mucilage on the surface of hyphae. The importance of AM hyphae as part of this complex binding network was demonstrated by Tisdall and Oades (1979), who showed that the effectiveness of roots of *Lolium perenne* (ryegrass) in stabilizing aggregates >2000 µm was related to the length of hyphae in those aggregates. Roots of *Trifolium repens* had considerably less effect than those of *L. perenne*, despite the fact that AM colonization of roots was 50% and 13% respectively after 14 weeks' growth. This illustrates that it is the root and hyphal length densities in soil and not the per cent colonization of the roots that are important in this context.

The involvement of roots and hyphae in aggregate formation and stabilization has also been followed in a chronosequence of tall grass prairie restoration (Cook *et al.*, 1988; Miller and Jastrow, 1990; Jastrow *et al.*, 1998). In this case, the development of mycorrhizas was related to different root size classes. The analysis showed that fine roots and hyphae had significant direct effects on the geometric mean diameter (GMD) of water stable aggregates, while very fine roots had no direct effects. The indirect effects of both types of root were assumed to be related to their colonized lengths and hence to production of extraradical hyphae. Of the plant species in the communities, prairie grasses and members of the Compositae, both of which produced extensive fine roots, were the best predictors of high GMD, whereas other species were apparently less important.

Undoubtedly, both roots and hyphae play a part in the stabilization processes (Miller and Jastrow, 2002). Thomas *et al.* (1986) showed that the contribution of roots of *Allium cepa* to the process was stronger than that of the associated AM hyphae but, unfortunately, their results were confounded by the difference in sizes of AM and non-mycorrhizal root systems. In following up this work, they used a single plant of *Glycine max*, with a split root system and mesh dividers to produce four treatments in a single pot: no roots plus saprotrophic hyphae; hyphae alone; roots plus hyphae (mycorrhizal roots); and roots plus saprotrophic fungi (non-mycorrhizal roots). Although experimental procedures resulted in lower water-stable aggregates

at the end of the experiment than in the initial soil, the differences between treatments were significant, in descending order of aggregation: mycorrhizal roots plus hyphae > mycorrhizal hyphae = non-mycorrhizal roots > control.

The contribution of roots and hyphae to aggregate stability increases as the concentration of organic matter in soils increases under pasture. The organic binding agents are relatively transient compared with the more persistent agents that cement the smaller particles. Consequently, the larger aggregates, which are so important in determining the occurrence of free-draining pores, are not only relatively temporary but also fragile and subject to disruption by tillage. Miller and Jastrow (1990) emphasize that, because of the transitory nature of the bonds, stabilization by roots and hyphae depends on their continued production. These points are obviously relevant to the management of plant–mycorrhizal populations to increase and maintain the structural stability of soil and may, as outlined in Chapter 5, have significant consequences for plant water relations as well as sequestration of organic C in soils.

Exudates from hyphae of quite specialized types have been suggested to be important contributors to stabilization of soil structure by AM fungi. There is an increasing body of work investigating the role of glycoproteins produced by AM fungi (termed glomalin or glomalin-related soil protein – GRSP) in stabilizing soil structure. The proteins are poorly characterized biochemically, with difficulties emanating at least partly because they are very stable and extraction from soil requires such treatments as autoclaving in 50 mM citrate buffer (Wright and Jawson, 2001). A recent investigation has shown that 'glomalin' is almost certainly a heat-shock protein which is retained in the AM fungal mycelium (Driver *et al.*, 2005; Gadkar and Rillig, 2006; see Chapter 5). Although it was initially thought that AM fungi secreted the GRSPs, it now appears more likely that accumulation in soil is a result of hyphal turnover, linked to the recalcitrant nature of the glycoprotein. More work is required both to determine the roles of GRSPs in AM fungal biology, as well as quantitative relationships between GRSPs, hyphal length density and turnover and soil structural stability and organic C content (Rillig and Mumme, 2006). Until such work has been carried out it will be advisable to adopt a critical approach to use of GRSPs either as measures of AM activity in soil or as major contributors to soil stabilization and C sequestration.

Conclusions

It is clear that mycorrhizas markedly influence plant interactions in nature and the extent to which they do so is beginning to be revealed. Ecologists are now focusing consistently on the importance of below-ground processes in plant coexistence and competitive interactions, as well as invasiveness, productivity and biodiversity. Consideration of the mechanisms that underlie the effects must in future be informed by experiments and, importantly, take the most parsimonious interpretation of results.

The difficulties associated with experimentation in the field are large and, in most cases, involve application of relatively unspecific biocides, so that outcomes need to be interpreted with caution and conclusions backed up by careful observations and controlled experiments. The modifying effects of plant pathogens, shoot endophytes and soil animals of various types are unlikely to be characterized and understood

until we have more effective and specific methods to create effective control treatments. Carefully designed experiments that exclude confounding problems such as sampling effect are also required to unravel mechanisms underlying demonstrations that mycorrhizas can influence productivity and also plant coexistence and diversity. The ways in which they do so appear to be partly dependent on the extent to which the members of the plant assemblages rely on their fungal symbionts for effective nutrient acquisition. Sufficient information is now available to show that broad generalizations are unwise; mycorrhizas do not always increase plant diversity, nor do increasing numbers of fungal symbionts always increase productivity. Likewise, the outcomes of multitrophic interactions are variable and mechanisms even less well understood. They are, however, likely to play significant roles in nature.

Plant ecologists necessarily take a phytocentric view of mycorrhizal symbioses. It is, however, critical to remember that the fungi are also engaged in a multiplicity of interactions, the outcomes of which will influence the composition of fungal assemblages and hence colonization of co-occurring plants. Now that it is clearly appreciated that there is enormous diversity in symbiotic outcome of interactions between different plants and fungi, it should be obvious that the mycorrhizal condition cannot be treated as a 'black box'. A much more 'fungicentric' view of mycorrhizal symbioses and their contribution to vegetation dynamics will be warranted in future.

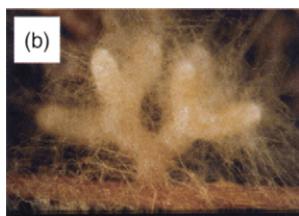


Plate 16.1 Roots of *Pinus sylvestris* used in studies of the protozoan populations associated with (a) non-mycorrhizal short roots (NM), (b) ectomycorrhizal short roots (MR) and (c) extraradical mycelium of *Paxillus involutus* (see Figure 16.11).



Plate 17.1 Edible mushroom production in Mexico. (a) Girl selling *Boletus edulis* in a market. (b) and (c) Baskets of ECM fungi of which the main ones are: *Gomphus flocculosus*, *Lactarius salmonicolor*, *Helvella crispa* and *Amanita aspera* var *franchetii*. Photographs courtesy Jesus Perez-Moreno.