14 Managing Mycorrhiza in Tropical Multispecies Agroecosystems

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Key questions

1. How do mycorrhizal associations function in the context of a multispecies agroecosystem?
2. What role do mycorrhizal networks play in multispecies agroecosystems, and how important are these networks?
3. Through what mechanisms do the beneficial effects of mycorrhizas in multispecies agroecosystems become manifest?
4. How can mycorrhizal associations be represented in models of nutrient uptake and nutrient cycling?
5. How can mycorrhizal associations be managed in multispecies agroecosystems? Specifically, how can we determine the conditions under which there is a need for mycorrhizal management, rather than mycorrhizal inoculation? Can specific management practices that have a negative impact on mycorrhizal functioning be avoided?

14.1 Introduction

'Plants do not have roots, they have mycorrhizas' (Begon *et al.*, 1996). This quoted here in order to stress the well-known, but often neglected fact that most plant species do not simply have roots – they have a mutually beneficial root–fungus association known as 'mycorrhiza'. In this arrangement, the fungus (just like the root system) receives carbohydrates from the above-ground part of the plant in exchange for mineral nutrients. Although mycorrhizas are generally accepted to be of importance, mycorrhizal research remains segregated into a niche, rather than being fully integrated into research on plant ecology and agronomy. However, on the basis of the near universal occurrence of mycorrhizas, we must subscribe to the warning given by Newsham *et al.* (1995):

> [b]oth ecologists and physiologists need to be aware that the results of experiments on non-mycorrhizal individuals of normally mycorrhizal plants are most probably artefacts.

In this chapter we will therefore consider plant–fungus interactions, placing particular emphasis on multispecies agroecosystems.

 Below-ground Interactions in Tropical Agroecosystems (M. van Noordwijk, G. Cadisch and C.K. Ong)

Mycorrhizal research has come a long way from its beginnings at the end of the 19th century. Research on tropical mycorrhizas effectively started in 1897, when Janse described some morphological 'curiosities' in the roots of many of the plants in the Bogor Botanical Garden, Indonesia (Janse, 1897). These curiosities turned out to be almost ubiquitous in plant roots. Discussion followed about the nature of such plant–fungus interaction, and it soon became clear that the fungus was (in most circumstances) not harming but, probably, benefiting the plant. Under conditions of high nutrient supply, however, the net benefit for the plant may be zero or even negative (whilst still being positive for the fungus).

As a number of recent reviews of the taxonomic (Cairney and Chambers, 1999; Hibbett *et al.*, 2000; Morton and Redecker, 2001; Schlüßler *et al.*, 2001), ecological (Allen, 1992; van der Heijden and Sanders, 2002), agronomic and silvicultural (Sieverding, 1991; Bethlenfalvay and Linderman, 1992; Gianinazzi and Schüepp, 1994; Pfleger and Linderman, 1994; Gianinazzi *et al.*, 2002) aspects of mycorrhiza are available, we will here focus on the specific challenges of understanding and managing mycorrhizal associations in tropical multispecies agroecosystems, whilst placing emphasis on agroforestry systems. The methods used in mycorrhizal research will not be discussed in detail here (although Box 14.1 provides a very brief overview) and the reader is, therefore, referred to books by Norris *et al.* (1994), Brundrett *et al.* (1996) and Varma (1998).

Much research has focused on the life cycle of annual plants and thus on the sequence of events that lead a developing (crop) seed to become a fully mycorrhizal plant that can be harvested. The terminology and methods used when studying mycorrhizas in annual cropping systems have been borrowed from plant pathology – in which discipline, fungi establishing themselves on plant roots are viewed in terms of the potentially strong negative effects they may have on plant performance. The 'borrowing' of terms is evident in that researchers still use the expressions 'mycorrhizal infection of' or 'infection sites' when referring to host plants. Part of this conceptual bias also continues in discussions of whether mycorrhizal fungi can be considered to behave as parasites in those cases where non-mycorrhizal plants outperform mycorrhizal plants (Johnson *et al.*, 1997).

Such a life cycle approach is also possible for perennial plants; but it should not be forgotten that the mycorrhizal fungus is an organism with a long lifespan. Lifespans of more than a hundred years have been reported for individuals of ectomycorrhizal fungi, whilst the lifespan of individual arbuscular mycorrhizal fungi (AMF) could, in principle, be almost indefinite – considering that they have exhibited an asexual life style for over 400 million years. A focus on short-cycle phenomena (such as annual spore formation and the establishment of new colonies as a result of germinating spores) is more relevant to a rotation of crop monocultures than it is to multispecies agroecosystems. This is especially true when long-lived woody plants are included in such systems. Persistence of the fungal mycelium should be the key interest in the latter case. In general ecological terms, management of mycorrhizal fungi in perennial, multispecies agroecosystems should be directed less at conditions where *r*-selected organisms and strategies (high growth rate, smaller-sized individuals, high reproductive output but smaller investment in survival) prevail, and more towards conditions where *K*-selected organisms and strategies (lower growth rate, larger-sized individuals, smaller investment in reproduction but larger investment in survival) prevail (Hart *et al.*, 2001).

The balance between plant and fungus is easily overlooked in such partnerships: most (applied) ecologists look at the symbiosis exclusively from a 'plant's-eye' (phytocentric) view, and forget that a 'fungus'-eye' (mycocentric) view is equally valid. The phytocentric view considers the association between plant and fungus as something that (almost inevitably) results in maximum plant fitness. However, a more realistic approach is to address the question of the extent to which the maximization of plant

Box 14.1. Methods for arbuscular mycorrhizal research.

Identifying mycorrhizal associations. Establishing the identity of an arbuscular mycorrhizal (AM) fungus is not easy. The microscopic structures of the mycorrhizal fungus in the plant root allow (to the experienced eye) identification to the level of fungal genus but not to species level, while the external mycelium is similar for almost all arbuscular mycorrhizal fungi (AMF). The taxonomy of AMF is, therefore, entirely based on spore structure. Although spores can be directly extracted from field samples (see below), their subsequent identification is often difficult. Therefore, field soils are often used as the basis for setting up pot cultures of different fungi (based on so-called trap plants). However, different trap plants and different cultivation conditions (temperature, soil pH, etc.) result in different species combinations. The other disadvantages of trap cultures are that: (i) they select for fungi that sporulate prolifically (which probably makes it necessary to have a sequence of traps, which takes a lot of time); and (ii) they sometimes miss fungi that are highly selective for certain plants. With the advent of modern molecular tools, it is now possible to identify the fungi in roots directly. Not all laboratories possess the necessary equipment, however. Another difficulty is that primers used to selectively amplify fungal DNA can be too restrictive (leaving out members of the *Paraglomaceae* and *Archaeosporaceae*) or too inclusive (also amplifying the DNA of fungi that do not belong to the *Glomales*).

Determining the abundance of mycorrhizal fungi. The abundance of mycorrhizal fungi can be based on estimates of the number of fungal spores, the length of the extraradical mycelium in soil, or the extent to which plant roots are colonized (Varma, 1998). Spores can be extracted from the soil by washing and sieving, followed by centrifugation in a sucrose gradient. Fungal hyphae can be extracted from soil and the hyphae of AMF identified, as they are non-septate. From such samples, hyphal lengths can be calculated. Root fragments can be cleared and stained, after which fractional colonization can be assessed. It can be helpful to separate fractional colonization by hyphae, arbuscules and vesicles. Such an assessment of root colonization is difficult for roots that possess dark pigments. An important question we must ask is 'to what extent do different methods yield comparable results?' Efficiency of spore extraction depends on soil texture (spores can stick to aggregates in clayey soils, necessitating the use of a dispersant). Also, the size of the smallest screen of the sieve ultimately determines how many spores will be extracted. For these reasons estimates of spore abundance in similar ecosystems still show a very wide range. Comparisons between different methods can also yield divergent results. Spore extraction could bias the sample towards those species that are prolific spore formers. Onguene (2000) assessed mycorrhizal inoculum potential using three different methods (spore abundance, colonization of a test plant grown in disturbed soil, and colonization of a test plant in intact soil columns). The three methods yielded very similar results (Fig. B14.1). However, such good correlation between methods does not always occur, and the literature on this subject also provides examples where results obtained with different methods were substantially different.

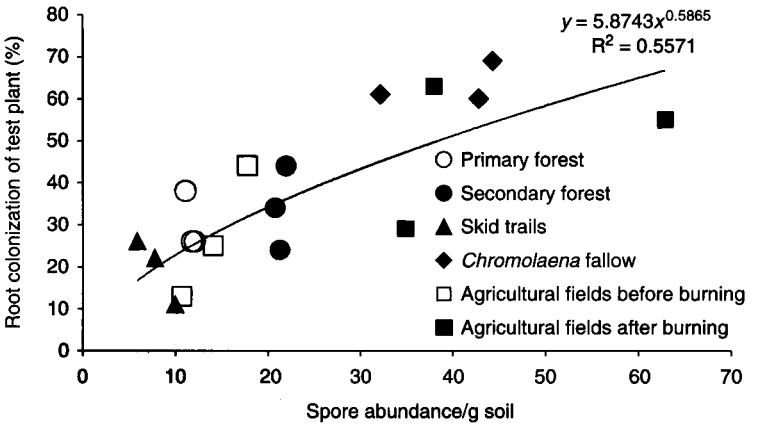

Fig. B14.1. Relationship between spore abundance and root colonization in a test tree (*Distemonanthus benthamianus, Caesalpiniaceae*) grown for 12 weeks, in six land-use types in three locations in southern Cameroon. After Onguene (2000).

fitness (or of plant primary production, as this is the farmer's target) is actually traded off against fungal fitness. Kiers *et al.* (2002) recently addressed the question of the extent to which farmers' management practices change the benefits of the mycorrhizal association. They suggested that agricultural practices would result in evolutionary changes in the fungus. More specifically, they predicted that increased use of fertilizer will lead to less effective mycorrhizal fungal genotypes, that crop rotation will prevent dominance of certain fungal genotypes, that tillage will have contrasting effects, and that inoculum addition will increase the chances of roots being colonized by more fungal genotypes, thereby allowing less beneficial ('parasitic') fungal strains to escape the defence mechanisms of the host.

'Superstrains' or 'superspecies' (single species or strains resulting in claimed, though often not proved, superior plant performance) may be insufficiently competitive or insufficiently able to reproduce. However, fungal species that maximize spore formation could do so at the expense of the plant, utilizing its carbon or the nutrients that could otherwise be transmitted to the plant. Strains could be 'superstrains' by virtue of a negative feedback mechanism, whereby the fungus demonstrates higher fitness on host plant *A* than on host plant *B*, yet plant *B* derives more benefit than plant *A* from that mycorrhizal fungus (Bever *et al.*, 2001). The occurrence of such negative feedback probably explains the observations made by Johnson *et al.* (1992), who noted that monocropping maize or soybean decreased diversity (evenness) of mycorrhizal fungi and led to a gradual decline in yield in these monocropping systems. Crops in a rotation that do not themselves depend on mycorrhiza, can give an important boost to the fungal population and, hence, have a positive effect on a subsequent crop in a rotation. If 'superstrains' are sufficiently competitive, they have the potential, under field conditions, to impoverish mycorrhizal fungal diversity. Plant species diversity and mycorrhizal fungal species diversity are often positively correlated (van der Heijden *et al.*, 1998). Low mycorrhizal species diversity is, in general, characteristic of highly fertilized or disturbed intensive agricultural systems (Johnson, 1993; Helgason *et al.*, 1998). By contrast, natural ecosystems and less intensively managed agroecosystems with a lower disturbance level are often characterized by high(er) diversity (see, however, Box 14.2).

Inoculating the trees at the nursery stage had a small, but statistically significant, positive effect on the fraction of trees that sur-

Box 14.2. Can mycorrhizal inoculation of trees help transform *Imperata cylindrica* grasslands into productive agroforestry systems?

Large areas of former rainforest in South-East Asia are covered in coarse grassland dominated by *Imperata cylindrica*. Fires tend to block succession to a woody secondary vegetation, and the systems are generally regarded as being degraded. Where such land is (or has become) accessible, it may be economically attractive to try to start an agroforestry land-use system. We must therefore ask: (i) will lands that previously supported mainly one species still host sufficient mycorrhizal inoculum to allow rapid tree growth?; and (ii) is it useful, or even necessary, to provide trees with suitable fungal partners at the nursery stage? Murniati (2002) tried to answer the latter question for four tree species (*Aleurites moluccana, Peronema canescens, Swietenia macrophylla* and *Artocarpus altilis*) using a series of experiments in East Kalimantan (Indonesia). A semicommercial product ('Mycofer') was used as an inoculum containing spores of four species of arbuscular mycorrhiza. Data collected included spore counts, spore identification (with 26 morphospecies of spores being identified in the survey as a whole), and records of the mycorrhization of roots and of the survival and growth of the trees.

In the grassland phase, the spore numbers of arbuscular mycorrhizal fungi were significantly lower in plots at the top of a ridge than they were on the midslope or in the valley. However, the number of morphospecies per 50 g of soil was only slightly lower in the ridge plots. In a total of nine samples, the cumulative number of morphospecies was 14, with an average of 5.6 species per sample.

Box 14.2. *Continued.*

During the first 2 years of the site's transformation to agroforestry (with samples being taken after 6 and 24 months; Fig. B14.2) the differences noted between sample positions disappeared, and overall spore numbers declined. In the samples taken after 6 months, the number of morphospecies was found to be slightly reduced in comparison with the number found in the grassland phase (36 samples now provided a total of 36 species, with an average of 3.8 per sample). However, at 24 months both spore numbers and the number of morphospecies identifiable in the samples were markedly reduced (12 samples contained seven species, with an average of 2.5 species per sample). Contrary to the expectations of the researcher, inoculating the trees with a mix of fungi had no positive or negative effect on spore numbers or diversity at 6 or 24 months. Of the four species introduced, only two were found in the soil at 24 months (and none at 6 months), with one dominating the spore numbers collected (the same being true in non-inoculated plots, despite trenching between plots), and one being found in small amounts. The fraction of tree roots that was mycorrhizal was 87 and 85% at 6 months, and 37 or 51% at 24 months, for inoculated and non-inoculated trees, respectively. These differences between treatments at a given sampling time were not statistically significant, but the hypothesis that inoculation would increase mycorrhization could be clearly rejected.

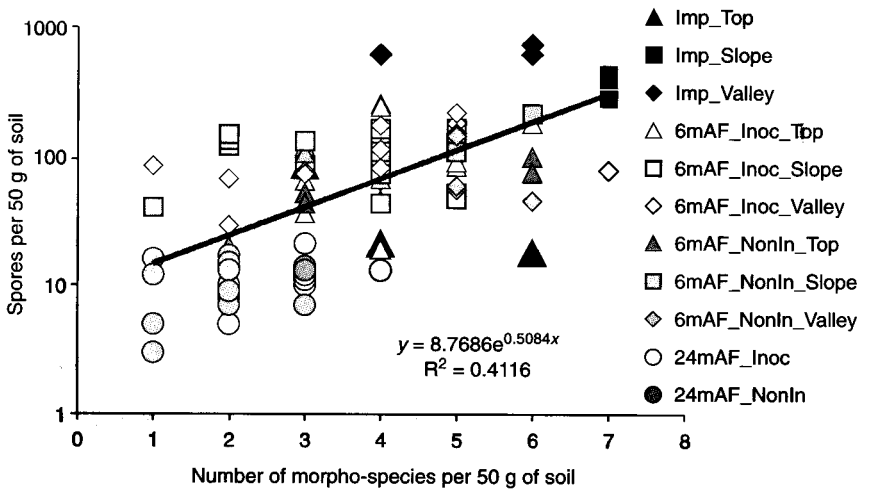

Fig. B14.2. Relationship between the number of morphospecies and total AM spore density in 50 g samples of soil taken in three landscape positions (Top = ridge top), in *Imperata cylindrica* grassland (Imp) before planting agroforestry tree species, and in the agroforestry plots (AF), 6 months after planting the trees, with or without inoculation with an AM spore mix (Inoc and NonIn, respectively); 24mAF represents a composite sample from AF plots at all slope positions, 24 months after tree planting. Based on Murniati (2002).

vived the transition from nursery to field (survival after 24 months was 80.0% and 86.5% for inoculated and non-inoculated trees, respectively ($P < 0.05$)). The overall conclusion of the research was that inoculation at the nursery stage is not essential for early tree growth, but it may have some positive effect on early survival. The shifts with time in spore densities and morphospecies composition were found to be substantial, so any observation on a single date may be difficult to interpret as an indication of whether or not inoculum potential is sufficient for a new site. In the grassland phase, the number of arbuscular mycorrhizas (AM) morphospecies clearly exceeded the number of plant species; an increase in plant diversity was accompanied by a reduction in AM spore diversity – but, of course, spore diversity is an incomplete indicator of AM fungal diversity.

On the basis of their morphology (which correlates reasonably well with fungal taxonomy) mycorrhizal associations can be divided into four major types:

- AM – formerly known as vesicular–arbuscular mycorrhiza (VAM);
- Sheathing mycorrhizas – including ectomycorrhizas (ECM), ectendomycorrhizas, arbutoid mycorrhizas, and monotropoid mycorrhizas;
- Ericoid mycorrhizas;
- Orchid mycorrhizas.

The last two mycorrhizal types occur in specific plant families, as their names indicate. AM are formed in the roots or rhizoids of a wide range of plants (mosses and liverworts, ferns, gymnosperms and angiosperms). In fact, the arbuscular mycorrhizal condition can be said to be closest to that of what might be termed 'the original plant'. Several lines of evidence demonstrate that the first primeval plant was AM (Pirozynski and Malloch, 1975; Brundrett, 2002). It might therefore be concluded that non-mycorrhizal plants evolved from AM plants. From such an evolutionary perspective, the phenomenon of non-mycorrhizal plants (Tester *et al.*, 1987) needs to be explained, as it indicates that, under at least some conditions, plant fitness may be increased as a result of excluding the fungi from the roots.

An AM is formed by means of intracellular colonization by aseptate, obligatory symbiotic fungi belonging to the order *Glomales* (or even to an autonomous phylum *Glomeromycota*; see Schlüßler *et al.*, 2001). The other types of mycorrhiza (the sheathing, ericoid and orchid mycorrhizal types) are formed by septate fungi belonging to the *Ascomycota* and *Basidiomycota*, and include many species with above-ground fruiting bodies ('mushrooms'). These other types of mycorrhiza also form their association by means of intracellular colonization; the only exception is the ECM type, in which the colonization of healthy roots is always intercellular (Smith and Read, 1997).

ECM associations are of minor importance in many multispecies agroecosystems, as most ECM plants are woody perennials. The most important ECM food crop is the genus *Gnetum*, which is usually colonized by the highly host-specific fungus *Scleroderma sinnamariense*, easily recognized because of its yellow mycorrhizas and hyphal cords. ECM associations might be important in agroforestry systems in which ECM trees surround (or share) agricultural fields. However, members of only a few families of tropical trees form ECM (with *Caesalpiniaceae*, *Uapacaceae* and *Dipterocarpaceae* being the most important families). Agroforestry systems with a high diversity of trees are, therefore, unlikely to contain a large proportion of ECM trees. The relative importance of ECM in different vegetation types is not very well understood as yet. Wubet *et al.* (2003) reported the virtual absence of ECM trees in Afromontane forests. In contrast, in the miombo woodlands of southeastern Africa, ECM trees belonging to the *Caesalpiniaceae* (e.g. genera such as *Brachystegia*, *Julbernardia*, *Afzelia*) dominate and produce large amounts of fruiting bodies. These mushrooms are edible, and can contribute a substantial amount of protein to the diets of local people, especially at the start of the rainy season when food reserves run low. They can also become a meaningful source of income. However, introducing non-indigenous ECM trees into tropical countries is not without risk, as there are strong indications that introduced species of the ECM tree genera *Pinus* and *Eucalyptus* can increase the rate at which the areas' original soil organic matter is broken down (Chapela *et al.*, 2001). Introduced ECM trees could also harbour poisonous ECM mushrooms that local people are not familiar with. The remainder of this chapter will therefore only consider AM, as this association plays a very large role in seminatural ecosystems and in agroecosystems.

14.2 Arbuscular Mycorrhiza

An AM has three important components: the root itself, the fungal structures within the cells of the root (arbuscules, coils, vesicles, intraradical mycelium), and an extraradical mycelium that explores and

exploits the soil for nutrients and then transports those nutrients to the root. On the basis of mycorrhizal morphology, the following two types can be recognized (Smith and Smith, 1996): (i) the *Arum*-type, which has distinct intracellular arbuscules and an extensive intercellular phase in the root cortex; and (ii) the *Paris*-type, which has intracellular hyphal coils but lacks an intercellular phase. Intermediate forms also occur, so the distinction is not absolute. Most agricultural plants form mycorrhizas of the *Arum*-type, whereas many tree species form mycorrhizas of the *Paris*-type. Root morphology (and hence the taxonomic identity of the plant) determines which type of mycorrhiza is formed. The functional significance of both types is, however, hardly known. It is striking, though, that plants that lack chlorophyll (and thus which are parasites or saprophytes) and form arbuscular mycorrhizas, have mycorrhizas of the *Paris*-type. The only AM example of interplant carbon transport through a common mycelial network (see below) involves a tree seedling with *Paris*-type mycorrhizas receiving carbon from a plant with *Arum*-type mycorrhizas (Lerat *et al.*, 2002). One is therefore tempted to speculate that the *Paris*-type is correlated with parasitic behaviour on the part of the plant.

AM are ecologically obligate. AMF cannot complete their life cycle in the absence of a host plant and, for that reason, cannot be grown in pure culture. In fact, AM fungi lack the ability to take up and metabolize carbon through the extraradical mycelium (all carbon must hence go through the intraradical mycelium), and it is likely that genes relevant to the carbon metabolic pathway were lost during their long symbiosis with plants. AMF can, however, be grown in Petri dishes in monoxenic cultures with root cultures (Fortin *et al.*, 2002). Under field conditions many, if not most, AM plants are also unable to complete their life cycle in the absence of the fungi, although under specific conditions (absence of competition, addition of nutrients, etc.) they can grow without them. Janos (1996) argued that we should separate the concepts of mycorrhizal dependency (an intrinsic characteristic of plants that has evolved under certain environmental conditions) and mycorrhizal responsiveness (which depends not only on plant species, but also on the identity of the fungal isolate(s) and the abiotic conditions present). However, the concept of mycorrhizal responsiveness (Box 14.3) is known in the older literature as mycorrhizal dependency (Plenchette *et al.*, 1983).

AM fungi are symbionts of a very diverse set of herbaceous plants, shrubs, and trees of temperate and tropical habitats. In most tropical soils, very few woody species of tropical trees are non-mycorrhizal. In French Guyana, 75 species were investigated and were all found to be mycorrhizal. In Korup National Park (Cameroon) 55 out of 56 species investigated were found to be mycorrhizal, and in southern Cameroon this was true of all 97 woody species investigated (Onguene, 2000). Most tropical crops are also strongly dependent on and responsive to arbuscular mycorrhizas (Sieverding, 1991). Only a few families and genera of plants do not generally form arbuscular mycorrhizas; these include *Brassicaceae* (their root exudates are possibly even toxic to AM fungi), *Caryophyllaceae, Cyperaceae, Juncaceae, Chenopodiaceae* and *Amaranthaceae* (although each of these families has some representatives that are usually colonized by AM fungi).

The taxonomic structure of the *Glomales* is depicted in Table 14.1.

The number of species of AM fungi discovered worldwide to date (159) is quite low, especially when we consider that there are probably more than 200,000 plant species that regularly form an arbuscular mycorrhizal association. Individual forest stands or grasslands can harbour between 30 and 50 AM fungal species (Bever *et al.*, 2001), whilst low-input or low-till agricultural systems can harbour up to 15 species (Franke-Snyder *et al.*, 2001; Jansa *et al.*, 2002). The obvious disparity between the number of AM plant species and the number of AM fungal species has traditionally been explained as being the result of a lack of specificity or selectivity on the part of the fungus. Such an explanation is based on the evolutionarily plausible scenario that, in mutualistic symbioses, there is no selection

Box 14.3. Mycorrhizal responsiveness.

Mycorrhizal responsiveness (MR) is defined as:

$$MR = (DW_{myc} - DW_{nonmyc}) / DW_{myc}$$ (Plenchette *et al.*, 1983)

MR is expressed on a dry weight (or C) basis (DW = dry weight). Instead of using carbon as the currency to measure plant response to mycorrhizas, plant phosphorus content of shoots and roots can also be used. This results in the mycorrhizal phosphorus responsiveness (MPR):

$$MPR = (\text{P-content}_{myc} - \text{P-content}_{nonmyc}) / \text{P-content}_{myc}$$

MPR is higher than MR if mycorrhizal plants have higher P concentrations than non-mycorrhizal plants. Use of MPR could give a biased view of plant response, because seed P reserves are often included in the P balance of the plant. Increased P concentrations can also be an artefact of experimental systems in which the non-mycorrhizal plants are P-limited, whereas the mycorrhizal plants are limited by another, unknown, nutrient, but not by P. This leads to luxury P-uptake (for details see Cardoso, 2002).

MR and MPR usually range from 0 to 1 (if the non-mycorrhizal plant fails to grow the MR is 1). If non-mycorrhizal plants outperform mycorrhizal plants, the MR is negative. The value of the MR is plant-species dependent, fungal-species dependent, and soil dependent, which means that comparisons are usually difficult to make. Large-seeded plants often rely on internal reserves for a prolonged time and often give a low MR, even when application of inoculum increases mycorrhizal colonization. MR can easily be measured under laboratory conditions, under which non-mycorrhizal controls can be obtained. Under field conditions, it is rare to find soils that are completely devoid of mycorrhizal inoculum. Application of the concept of MR under field conditions is therefore questionable. Instead, a mycorrhizal inoculation effect (MIE) can be used. This indicates the effect the introduced inoculum has as compared with the inherent field inoculum, and is defined as:

$$MIE = (DW_{inoc} - DW_{uninoc}) / DW_{inoc}$$ (Munyanziza *et al.*, 1997)

Again, MIE can be expressed on a C basis or on a P-uptake basis. MIE usually varies between 0 (if there is sufficient mycorrhizal inoculum and if field-inoculum quality is good enough) and 1 (if the amount of mycorrhizal inoculum is limiting under field conditions and/or the inoculum is not sufficiently effective). Negative values indicate either mycorrhizal redundancy (whereby costs for the mycorrhizal fungus in terms of carbon or nutrients are higher than mycorrhizal benefit) or that the applied inoculum is less beneficial than the field inoculum. In a comparison of five tree species in South Cameroon, Onguene (2000) noted MIEs of between 0.55 and 0.90 in skid trails, and values that were only slightly lower than these at landings. In an agricultural soil in which seedlings of *Terminalia superba* were grown, MIE was negative after the addition of inoculum collected in a pure stand of the grass *Paspalum conjugatum*. As mycorrhizal colonization increased after the addition of a grass inoculum, these data suggest that inoculum quality (related to selectivity of the inoculum) has an effect and should not be forgotten.

Usually, MIE data are compared after one growing season. However, in perennial agroecosystems, it is important to assess changes in MIE over time and then relate this to changes in the species composition of the inoculum.

Finally, different species of mycorrhizal fungi, or different mixed inocula, can be compared. Such comparisons can be useful for plants grown in pots but, for field conditions, persistence of those fungi is again as important as their initial effects.

for host specificity or selectivity. Consequently, a lack of specificity or selectivity was often taken for granted, and the issue not investigated. Until recently, there was also little empirical evidence for specificity between particular fungi and plants – at least at the fungal (morpho-) species level. During the last decade, however, more instances have been noted of selectivity in mycorrhizal associations, with specific combinations of plant and fungal species occurring more often than would be expected to result from chance alone. Previously, the fungi commonly used in experiments were

Table 14.1. Taxonomic structure of the *Glomales*. (After Morton and Redecker, 2001.)[a]

Order	Family	Genus	No. species[d]
Glomales[b]	*Archaeosporaceae*	*Archaeospora*	3
	Paraglomaceae	*Paraglomus*	2
	Glomaceae	*Glomus*[c]	85
	Acaulosporaceae	*Acaulospora*	31
		Entrophospora	4
	Gigasporaceae	*Gigaspora*	5
		Scutellospora	29

[a] Schüßler *et al.* (2001) proposed a somewhat different classification with a strong inflation in taxonomic rank (one phylum (*Glomeromycota*), four orders, and eight families).
[b] Although *Glomerales* would be grammatically correct, we prefer the use of the well-known name *Glomales*.
[c] *Glomus* is not monophyletic and needs to be split in three groups.
[d] Species number taken from the INVAM website (http://invam.caf.wvu.edu/), but many undescribed species still await formal recognition.

those most amenable to culture conditions (i.e. mostly generalist, *r*-selected species). It is possible that because of this choice of species, selectivity was underestimated and also that the potential for mycorrhizal networks (see below) was overestimated. Many recent studies have found unknown spore types (which researchers have so far not been able to cultivate) or unknown molecular types, based on unique sequence differences obvious between them and known species. Such findings support the idea that some degree of selectivity exists.

14.3 Mycorrhizal Functioning in (Multispecies) Agroecosystems

The essential beneficial effects that mycorrhizal fungi have on plants are a result of their ability to absorb nutrients in their inorganic (mineral) form more efficiently than a plant could alone (i.e. in a less costly manner). Therefore, the role that mycorrhizal fungi play in absorbing nutrients is most relevant under conditions of low nutrient availability, such as those commonly found in (sub)tropical (agro)ecosystems (Smith and Read, 1997). Mycorrhizal fungi extend the depletion zones around roots (see Chapter 10, this volume) of elements such as phosphorus (P) and zinc (Zn), which are relatively immobile as a result of their low diffusion rates. Under dry conditions, such as those occurring in semiarid climates, mycorrhizas may also be important in that they enhance nitrate uptake. Due to the much smaller diameter of hyphae (on average 5–10 μm, compared with 10–20 μm for root hairs and 100–500 μm for plant roots) and the large amounts of hyphae in soil, the total absorptive area is greatly increased in comparison with that of roots alone. If, for instance, the length of hyphae (hyphal diameter 10 μm) is 20 times that of roots (root diameter 200 μm) per unit soil volume, the contribution to nutrient uptake made by mycorrhizal hyphae could be similar to that made by roots if the surface area (length × diameter × π) were assumed to be an appropriate basis of comparison (see below).

For AMF, the ratio of hyphal length to root length generally varies between 25 and 250; but much larger ratios (well over 1000) have been found. However, various other mechanisms have been proposed to explain the effects that hyphae have in terms of extending depletion zones. For example, it has been proposed that the hyphae of mycorrhizal fungi may colonize soil pores that are too small for plant roots. It has also been suggested that the kinetic properties of the uptake systems of plants and mycorrhizal fungi may also differ, potentially allowing a

closer approximation to 'zero sink' uptake at the primary absorptive surface (see Chapter 10, this volume). However, such differences have not been clearly demonstrated. Finally, it has been claimed that mycorrhizal fungi may be able to access sources of nutrients that are not available to plants (organic phosphorus or nitrogen and sparingly soluble phosphorus). Although it is unlikely that large differences exist in the organic nitrogen and organic phosphorus uptake capacities of plants and mycorrhizal fungi, mycorrhizal fungi might have an advantage over plants insofar as they can better explore the soil, by which means they can more effectively compete with saprotrophic microorganisms. The suggestion that mycorrhizal fungi have access to forms of phosphorus that are (biochemically) inaccessible to plants has not been confirmed under field conditions; however, it has been confirmed in monoxenic cultures, pot systems with well-defined sparingly soluble P-sources, and pot experiments using natural soil containing sparingly soluble P-sources (Cardoso, 2002). The role of mycorrhizas in nutrient uptake models is elaborated below.

The beneficial effects of the AM symbiosis have been attributed to improved phosphorus nutrition. It is also important to identify whether the mycorrhizal symbiosis has other effects that might benefit the plant (e.g. protection against pathogenic fungi, heavy metals or aluminium; better drought resistance; improved soil aggregation). Such beneficial effects have been reported previously; however, in those instances, it was not clear whether said effects were genuine mycorrhizal effects or a result of the improved phosphorus status of the plant. A comparison of mycorrhizal plants and non-mycorrhizal plants with the same P-status has now made clear that said beneficial effects are genuine, independent of P-status, and that AM symbiosis should therefore be considered to be multifunctional (Newsham *et al.*, 1995). As different species of mycorrhizal fungi forage at different distances from the root surface, there is also functional diversity within the role of P-uptake (Jakobsen *et al.*, 2001). It is possible that functional diversity is linked to taxonomic diversity within the *Glomales*. Boddington and Dodd (1999) have suggested that members of the genus *Glomus* could be more important to the plant in terms of the provision of P, whereas members of the genus *Gigaspora* might be more important in terms of the contribution made to soil structure. Our database is, at present, insufficient to address the question of the relationship between the taxonomy and ecological function of the *Glomales* in more detail.

14.4 The Importance of the Mycorrhizal Network

As the mycelium (the network of hyphae) of mycorrhizal fungi is perennial, and grows away from its centre, several plants can be connected by the same fungal individual in a common mycorrhizal network. Such interconnections occur both between plants of the same species and between plants of different species. Interconnecting plants of different species is a logical consequence of the limited selectivity exhibited by (several) AMF. However, as indicated above, lack of selectivity could have been overestimated, and may not be universally true for all species of AMF.

In networks involving more than one plant both nutrients and carbon could be moved from one plant to another. Interplant movement of carbon (a concept known as the 'wood wide web') has attracted a lot of interest and heated debate. The existence of such networks has raised a number of questions:

- Is there gross transport of carbon only (with the benefits being equal to the costs in the case of both partners), or are net quantities of carbon transferred? Carbon labelling one plant (e.g. with $^{14}CO_2$) and then finding that isotope label in another plant only demonstrates gross transport. However, it does leave open the possibility that similar amounts of unlabelled carbon could move in the opposite direction. Double labelling (e.g. using $^{14}CO_2$ to label one plant and $^{13}CO_2$ to label the other) could help resolve this issue.

- If net transport occurs, is it taking place in quantities that are ecologically relevant? Isotope labelling is an extremely sensitive method, whereby even trace amounts of carbon transported can be measured.
- What is the fate of the carbon subsequent to transportation? Does it remain in the fungus (optimizing fungal fitness), or does it end up in the plant (contributing to plant fitness)?

In the case of AMF, data based on double labelling indicate that net transport can occur, though in most cases the quantities involved are small. It also indicates, however, that the magnitude of the flux depends on sink–source relationships. Therefore, in the case of plants whose photosynthetic rates differ because one plant is shaded, transport could be larger. In most cases, however, the carbon transferred remained in the roots, and was not transported to the shoots, suggesting that the carbon is rigorously controlled by the fungus. Recently, Lerat *et al.* (2002) provided the first evidence that carbon transported through a common AM network ends up in the shoots of the receiver plant. Such reversed carbon transport (from fungus to plant) must also occur in the case of 'saprotrophic' plants that are without chlorophyll and so depend on fungi to obtain their energy for them (Bidartondo *et al.*, 2002).

In light of the above, it is unlikely that a strongly phytocentric version of the concept of mycorrhizal networks (whereby each plant gives as far as it is able and receives according to its needs) can be maintained. However, less plant-oriented, more mycocentric versions of the concept of mycorrhizal networks may instead be applied. Such a concept has been introduced by Fitter (2001):

> To the plants, therefore, the common mycelial network is a club with a variable subscription fee and a range of potential membership benefits; to the fungus, the plants are the potential club members whose subscriptions keep the club afloat.

It remains to be investigated to what extent these mycorrhizal networks are important in multispecies agroecosystems.

14.5 Benefits of a Perennial Mycorrhizal System in Multispecies Agroecosystems

The following mechanisms, which all result in a perennial mycorrhizal system, have been proposed to explain how diverse and beneficial mycorrhizal communities are maintained in multispecies agroecosystems.

- Mixtures of plants generally allow a larger diversity of mycorrhizal fungal species to flourish. As the mycorrhizal symbiosis is multifunctional, and different fungal species are likely to fulfil (partly) different functional roles, a mixed-species system gives rise to a larger range of potential benefits for individual plants. Increased mycorrhizal diversity as such (or increased numbers of species of mycorrhizal fungi) has also been shown to increase primary productivity (van der Heijden *et al.*, 1998). In mixtures of plants it may, in principle, be possible to maintain fungal species that greatly benefit a certain plant species but are unable to reproduce on it, as they depend on another plant species for reproduction (see the concept of 'negative feedback', as described by Bever *et al.*, 2001). However, direct evidence of the latter mechanism is, at present, lacking.
- A mixture of plants, especially a mixture of crops and trees such as occurs in agroforestry systems, may exhibit deep rooting, resulting in higher levels of mycorrhizal inoculum at greater depths. This increases the volume of soil in which nutrients can be efficiently taken up (Cardoso, 2002; see Fig. 14.1). In several countries in the West African savannah zone, it has been claimed that a specific cultivar of cassava (*Manihot esculenta*) can restore soil fertility after nutrient depletion as a result of continuous cropping. This cultivar is a slow-growing and deep-rooting landrace, and it would be interesting to investigate whether the claimed beneficial effect is due to mycorrhizal activity in deeper soil layers.
- A mixture of plants, especially a mixture of plants with different growth phenologies, could result in a continuity of hosts over time, thereby allowing mycorrhizal fungi to differentially take carbon from

different plants depending on their photosynthetic activity. Mycorrhizal continuity can also be maintained if weeds or cover crops are established on a field after the major crop has been harvested. Bare fallows, or long periods during which land is kept free from any (mycorrhizal) plant growth in order to conserve soil water, have been related to a plant nutritional disorder in dry areas of Australia. A decline in the levels of the mycorrhizal inoculum involved leads to P and Zn deficiency, causing poor growth in oilseed crops (especially linseed), pulses and cereals grown on clayey soils that are otherwise considered quite fertile. The problem (known as 'long-fallow disorder') can be remedied by applying P and Zn fertilizers. However, it can also be addressed through the use of agricultural practices that provide mycorrhizal continuity (Thompson, 1996).

- A mixture of plants, especially one that includes perennial plants, prevents mycorrhizal mycelium being regularly disturbed. In regularly disturbed agroecosystems dominated by annual crops, where an annual life cycle is imposed on the mycorrhizal fungi, the 'late' establishment of mycorrhizas could be a factor that limits seedling growth. This could result in limited phosphorus uptake by the seedling, which feeds back into a lower growth rate. The important role that mycorrhizal networks play in the early growth of maize has been convincingly demonstrated by Miller (2000). Moreover, early establishment and nodulation of legumes is enhanced in the presence of a mycorrhizal network (Goss and de Varennes, 2002). Mycorrhizal sufficiency in agricultural fields could help young sorghum plants escape, or compensate for, the detrimental effects of witchweed (*Striga hermonthica*; Lendzemo and Kuyper, 2001). However, the exact mechanisms still require further study under field conditions.
- Mixtures of plants allow a continuity of carbon flow and, hence, mycorrhizal activity. This contributes to improved soil carbon sequestration and soil aggregation, and helps prevent soil erosion. Both fungal hyphae and glomalin, a specific glycoprotein produced by AMF, play a very important role in these respects (Rillig and Steinberg, 2002).

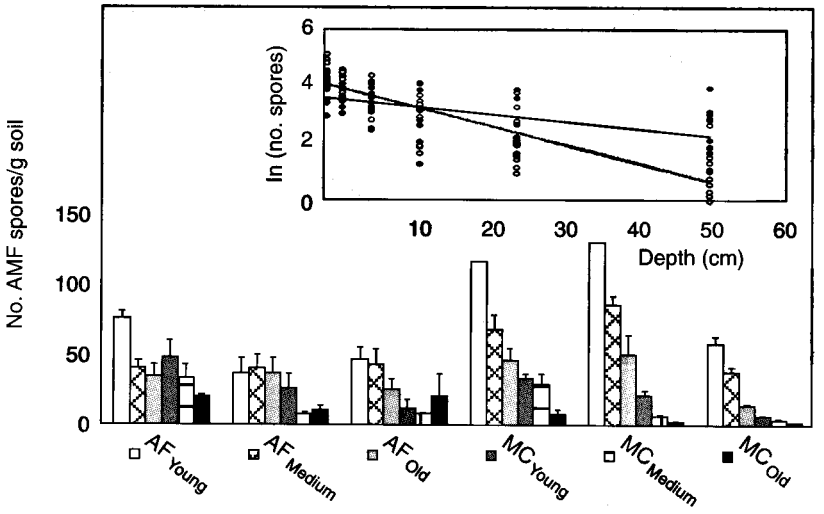

Fig. 14.1. The numbers of spores of arbuscular mycorrhizal fungi (AMF)/g soil in agroforestry and conventional coffee systems of different ages. The figure in the top right-hand corner is the regression analysis of the natural logarithm of the number of spores of AMF as a function of soil depth in the agroforestry (AF) system (●) and monocultural (conventional) (MC) system (○). Both intercepts and slope differ significantly between both systems. After Cardoso (2002).

14.6 Mycorrhizas in Models of Nutrient Uptake

Most nutrient uptake models for plants are still based on roots rather than on mycorrhizas. The simplest way of incorporating mycorrhizal hyphae into such models is to treat them as 'thin roots', and describe nutrient transport as occurring by diffusion to a small cylindrical sink. In the modelling tradition followed by Nye and Tinker (1977) and Barber (1984), the physiological uptake parameters at the root (or hyphal) surface have to be known to estimate the likely rate of uptake. In the approach taken by de Willigen and van Noordwijk (1987; see also Chapter 10, this volume) the uptake potential is derived on the basis of high estimates of the physiological parameters, in which the root can (approximately) act as a 'zero sink' (i.e. a sink of infinite strength that is able to maintain a concentration of zero at its surface, regardless of the rate of external supply). Actual uptake is taken to be the minimum of the current 'demand' and this uptake potential. Mycorrhizal hyphae will, in this approach, increase uptake potential. However, they will only increase uptake if plant demand cannot be met by the roots alone. The WaNuLCAS model (van Noordwijk and Lusiana, 1999, 2000) of agroforestry systems or other mixed-plant communities includes a representation of mycorrhizal hyphae as part of the 'effective root length' that determines the uptake potential of all plant components in each time step.

In order to understand this approach, we first have to consider how we can best deal with variation in root diameter in a tree or crop root system. As root diameter affects potential uptake rate in a cylindrical zero-sink model, an appropriately derived average root diameter in each layer and zone is needed for the uptake function. Also required is a way to estimate the equivalent effective root length of each component at such a diameter. A number of options exist for making this comparison between roots and/or hyphae of different diameter, and involve the use of the relationship $L \times D^x$ (where L is length, D is diameter and x a parameter to be defined). If we sum roots (of variable D) on the basis of root length (so effectively use $x = 0$) we will probably underestimate the potential contribution of high-diameter roots (de Willigen and van Noordwijk, 1987). If root surface area is used ($x = 1$) the potential contribution made by high-diameter roots will be overestimated in a zero-sink uptake process, where diffusion through soil is the rate-limiting step. If biomass is used ($x = 2$) the result will be even more biased towards high-diameter roots. A comparison of the product of root length and the square root of root diameter (so $x = 0.5$) appears to give the best results (van Noordwijk and Brouwer, 1997), in the sense that, when comparing roots of different diameter on the basis of an equal $L \times D^{0.5}$, the predicted uptake potential is least sensitive to D (Fig. 14.2).

In equation form, the average root diameter for a mix of crops and tree roots of different diameters, as used in the WaNuLCAS model, is: (see bottom of page) where *CLrv* and *TLrv* refer to root length densities (cm/cm^3) of crop and tree, respectively, and *CDiam* and *TDiam* to root diameters.

Based on the above rule for adding roots of different diameter on the basis of the square root of their diameter, we can also get a first approximation of the effects of mycorrhizal hyphae. The total length of hyphae can be derived from the fraction of crop or tree roots that is mycorrhizal and the length of hyphae per unit length of mycorrhizal root. The effective root length (*EffLrv*) can therefore be derived from:

$$EffLrvC_{ij} = LrvC_{ij}\left[1+\frac{Inffrac.HypLeng.\sqrt{HypDiam}}{\sqrt{RtDiamC}}\right] \quad (14.2)$$

where the *Inffrac* parameter indicates the fraction of roots that is mycorrhizal, *HypLeng* gives hyphal length per unit mycorrhizal root and *HypDiam* the average diameter of hyphae.

$$RtDiamAV_{ij} = \left[\frac{Rt\_CLrv_{ij}\sqrt{Rt\_CDiam}+Rt\_TLrv_{ij}\sqrt{Rt\_TDiam}}{Rt\_CLrv_{ij}+Rt\_TLrv}\right]^2 \quad (14.1)$$

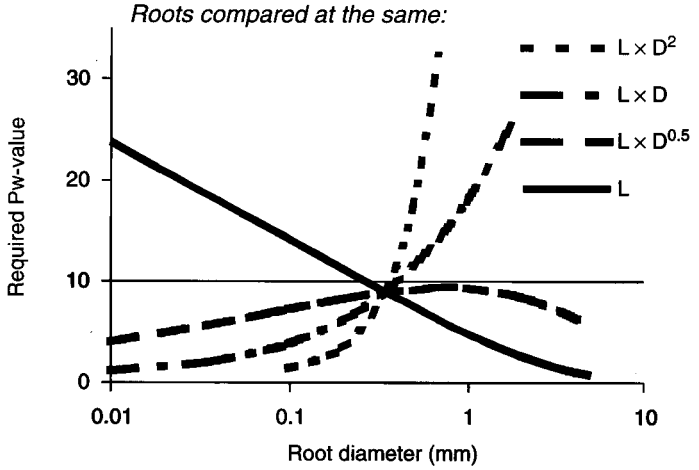

Fig. 14.2. Effect of root diameter D on the soil P supply (as expressed in the Pw-value) that is needed to meet the demand of a crop such as wheat when root systems of different diameter are compared at equal length L, root surface area π L×D or volume 0.25 π L×D^2 ; the smallest effect of root diameter (the flattest line) exists when root length times the square root of the root diameter (L×D$^{0.5}$) is used (Van Noordwijk and Brouwer, 1997).

This equation effectively converts the mycorrhizal hyphae into an equivalent length at the diameter of the roots. This option is provided in WaNuLCAS for both crop and tree.

An alternative way of calculating hyphae length entails calculating the total length of mycorrhizal hyphae in the soil (for various depth and spatial zones). These are then assigned, based on proportions, to the various plants sharing the space. Either way, a total length of hyphae plus roots could be estimated for each plant component in each soil compartment.

The inclusion of mycorrhizas in models of the Barber–Cushman type, such as that of Yanai *et al.* (1995), which considers mycorrhizal hyphae as very thin roots, tends to greatly overestimate actual uptake. It is difficult to incorporate into such models the negative feedback that occurs between saturation of plant demand and a reduction in net uptake. In reality such feedback may include time lags, and the transfer of nutrients from hyphae to roots, and the receipt of a 'feedback signal' in the case of saturation of demand, may take more time than in a situation with roots only.

Whatever the case, root-uptake models that include mycorrhizas on the basis of total hyphal length and transport to cylindrical sinks suggest that the amount of mycorrhizal mycelium found under normal conditions greatly exceeds that needed for plant growth. Such results from models could well be correct, as any consideration of (excess) mycelium outside plant roots should not approach the issue from a phytocentric perspective. Rather, they should be considered from a mycocentric perspective, whereby the large fungal biomass reflects the maximization of the fungus' fitness. A large fungal biomass immobilizes substantial amounts of nutrients that are not accounted for in the plant.

Finally, it should be noted that models such as those above are based on the assumption that nutrient uptake occurs over the whole hyphal surface, analogous to plants where nutrients and water are taken up over the whole surface of the fine roots. Insufficient consideration has been given to the possibility that mineral nutrient acquisition in mycorrhizal fungi occurs only at the hyphal tip or only through specialized structures. It has, however, been suggested that mineral uptake occurs (preferentially)

through specific structures, called Branched Absorbing Structures (BAS). These small, bushy structures (resembling arbuscules) form on runner hyphae at regular intervals. They have a small diameter (1.5–3.5 μm) and a relatively short lifespan (approximately 5 weeks), after which they senesce and are closed off by septa (Bago *et al.*, 1998; Bago, 2000).

An important challenge faced by those modelling nutrient uptake through fungal hyphae at the single plant level is, therefore, the need to assess: (i) whether or not preferential sites for nutrient uptake exist; and (ii) how much of the nutrients taken up by the fungus are immobilized in the microbial biomass and, hence, not made available to the plant. It should be borne in mind, however, that phosphorus immobilized in mycorrhizal fungal biomass should, in the long term, be considered to be more available to plants than would be the case if the mineral phosphorus had been fixed to iron and aluminium (hydr)oxides (cf. Cardoso, 2002). When applying these ideas to mixed-plant communities or agroecosystems, attention needs to be directed at the 'rules' that govern the sharing of access to a mycorrhizal network (Box 14.4).

Quantitative simulation models can be used as a tool to test whether, through their interaction with relatively well-known aspects of plant–soil interactions, relatively simple mechanisms are sufficient to explain observed phenomena. Thus far, most models are phytocentric (a result of their agronomic focus), and do not include a perspective that considers the long-term survival of the fungal partner. A number of general principles can be formulated, however, to help manage actual agroecosystems for better 'mycorrhization'.

Box 14.4. Model approach to sharing access to a mycorrhizal network.

Version 2.2 of the WANULCAS model includes a simple option to describe root parasitism. This was inspired by the parasitic trees of the sandalwood family (*Santalaceae*), which provide high-value wood through important forms of agroforestry practised in the drier, eastern parts of Indonesia.

The conventional idea of root parasitism is one in which the 'parasite' steals water and nutrients from the host. In fact, however, it may be more accurate to say that a parasite such as sandalwood 'steals' or 'takes control of' the roots of other plants. The roots of the 'host' then start to function as though they belong to the sandalwood, and will take up water and nutrients as needed by that tree. By 'stealing roots', the sandalwood saves the energy associated with the making of fine roots. However, it is not at all clear who 'pays' the energy costs associated with the maintenance of these fine roots. Probably such roots are not adequately maintained by the sandalwood tree. The parasitic plant, on an evolutionary timescale, faces the following dilemma: if it does not maintain the 'capital stocks' it has 'taken over', the benefit received is short lived; however, if it does maintain said 'capital', the benefit of parasitism compared with a plant that makes and maintains all its fine roots itself may be small.

In a recent survey of sandalwood roots, Wawo (2002) only found parasitic contact in the case of relatively small roots. This suggests two things:

1. Sandalwood is not able to make contact with thicker, woody roots, and thus cannot take over major parts of a root system in one go.
2. After sandalwood has parasitized a root, the further growth of the fine roots is limited (in terms of both length and girth). Such limiting occurs because the host stops investing resources in the parasitized roots, whilst the sandalwood itself makes no investment.

Looking at the parasitic process in this way enables us to perceive that sandalwood must still need a fairly elaborate root system, in order to constantly find new roots belonging to other plants that it can parasitize.

These concepts can also be applied to multiple access, by various plants, to a network of mycorrhizal fungi in the soil.

14.7 Managing Arbuscular Mycorrhizal Associations

Applied mycorrhizal research has often been aimed at the production of inoculum. From a management perspective, however, attention should focus on the identification of: (i) the conditions under which the management of indigenous inoculum is the better approach; and (ii) the conditions under which the application of externally produced inoculum is either desirable or imperative. Decision trees (Fig. 14.3) to allow an answer to that question have been published by Brundrett *et al.* (1996) and Dodd and Thompson (1994). Such decision trees suggest implicitly what is actually confirmed by agricultural and forestry practices: most sites still contain sufficient inoculum. However, if insufficient inoculum is present, judicious management will allow sufficient inoculum to be created and maintained.

In order to manage an indigenous inoculum, it is imperative to know which factors are beneficial or inimical to the inoculum potential, and to the diversity or functioning of mycorrhizal fungi. Beneficial and adverse practices are listed in Table 14.2.

In agroecosystems, plant species selection is very important. In rotational systems, the sequence in which plant species with different mycorrhizal responsiveness are planted could affect the productivity of any one specific crop. Failure is likely to result if a highly responsive species, such as linseed (*Linum usitatissimum*), is planted after either a bare fallow or a crop that negatively affects mycorrhizal inoculum potential, such as the non-mycorrhizal *Brassicaceae*, which can even poison mycorrhizal fungi (Schreiner and Koide, 1993). Species selection in multispecies agroecosystems is important too, although the question of matching species in terms of fungal selectivity has not been explicitly addressed.

Forest disturbance as a result of commercial logging may reduce or even eliminate mycorrhizal fungi from forest sites. Alexander *et al.* (1992) noted a severe decrease in the levels of AM fungal spores found in a Malaysian forest following heavy logging. Selective logging, however, was found to have had a slightly positive effect. Sites of forest operations (skid trails and landings) in Cameroon also showed a strong decrease in spore numbers and mycorrhizal

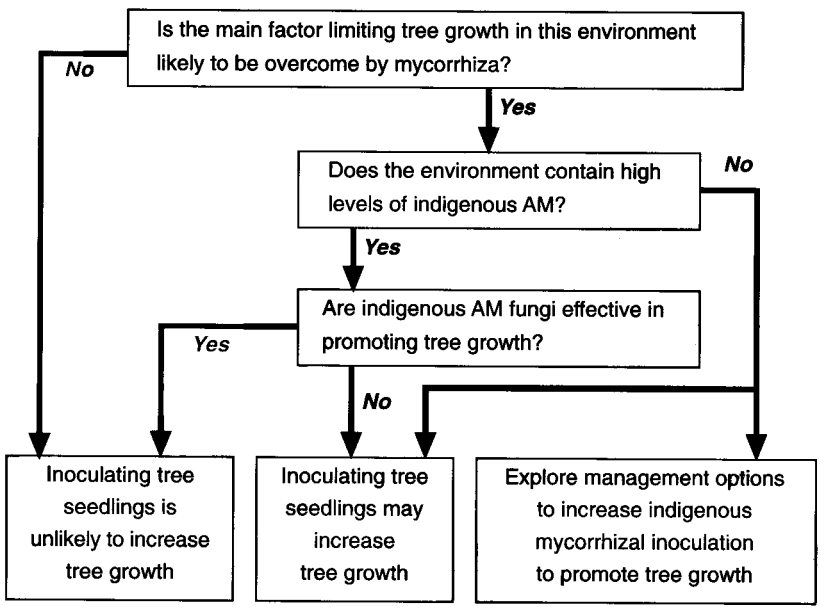

Fig. 14.3. Decision tree for use in determining under which conditions inoculation with mycorrhizal fungi or management of mycorrhizal associations is likely to be most successful. After Dodd and Thompson (1994).

Table 14.2. Positive and negative influences on arbuscular mycorrhizas by different agricultural management practices. (After Smith and Read, 1997.)

Management factor	Positive influence	Negative influence
Plant species	Host species	Non-host species
	High colonization	
	High spore production	
	High mycorrhizal root length density	
Bare fallow	None	Reduces populations
Pasture	Increased propagule densities	
Disturbance	Minimum tillage	Conventional tillage
		Compaction
Management	Organic–biodynamic	Conventional
Fertilizer	Drip feeding	High applications of soluble P and N
	Slow release	
	Rock phosphate	
Fumigation	None	Reduces propagules
Fungicides	Variable effects	Variable effects
Low light (glasshouse)	None	Colonization or growth decreased

inoculum potential (MIP), as assessed by baiting (Onguene, 2000). There was no sign of a recovery over time. Alexander *et al.* (1992) showed that heavy logging decreased MIP by 75%, whereas heavy logging coupled with soil compaction caused a 90% decrease, and heavy logging with subsequent erosion a 95% decrease. Even when followed by a slight recovery, a strong decline in the level of mycorrhizal inoculum may retard secondary succession. Such retardation of secondary succession was reported by Cuenca and Lovera (1992) after bulldozers were used to clear the topsoil of a Venezuelan savannah. If vegetation develops on such sites, it often consists of plants that are not dependent on mycorrhizas. Succession can therefore be arrested by the lack, or the slow build-up, of sufficient inoculum. Under such conditions, successful revegetation may well depend on inoculum addition.

Sieverding (1991) stated that slash-and-burn agriculture has little negative impact on mycorrhizal inoculum. Data from Cameroon (Onguene, 2000) are consistent with this observation, as mycorrhizal inoculum levels were found to be somewhat higher in both agricultural fields and in young fallow, as compared with secondary or primary rain forest. Higher inoculum levels after the onset of shifting cultivation could be due to increased soil surface temperatures and decreased soil moisture after canopy opening (both of which act as triggers for spore formation) as well as to the shift in species composition. Both climatic factors probably force an annual life cycle on mycorrhizal fungi and select for mycorrhizal fungi that have an *r*-strategy that depends less on the mycelium and more on spore production. The importance of higher surface soil temperature and/or lower soil moisture has also been demonstrated by Cardoso (2002; see Fig. 14.1), who found that spore numbers in the topsoil of a conventional coffee field were significantly higher than those in an agroforestry coffee system, whereas the opposite pattern was evident for the deeper soil layers.

Agricultural intensification does not only cause mycorrhizal inoculum to decline through regular disturbance, but also selects for mycorrhizal fungi that are less beneficial to the plant. In The Netherlands, Dekkers and van der Werff (2001) demonstrated that phosphorus fertilizers negatively affect the functioning of mycorrhizal communities. Fifteen years after P-fertilizer treatment ended, mycorrhizal communities in soils that had been loaded with phosphorus fertilizers were still functioning less efficiently than those in unfertilized soil. Corkidi *et al.* (2002) demonstrated, in two semiarid grass-

lands in North America, that N-fertilization not only altered the balance between the costs and benefits of the mycorrhizal symbiosis, but also shifted the community towards less beneficial mutualists.

Only if mycorrhizal inoculum quantity or quality at a certain site is limiting productivity in agroecosystems should inoculation become an option. Inoculation treatments consist either of a single species inoculum (containing a so-called 'superstrain') or a generic, mixed inoculum ('biofertilizer' in general), based on the truism that any mycorrhizal inoculum is always better than no inoculum. Judicious management or application of such an inoculum for agricultural purposes potentially reduces the need for a phosphate fertilizer. However, it should be emphasized that the comparison between rhizobia (as biofertilizers that deliver nitrogen) and mycorrhizal fungi (as biofertilizers that deliver phosphorus) falls short. Mycorrhizal fungi do not add phosphorus to ecosystems, they only increase a plant's access to this often scarce resource. This distinction has important implications for long-term nutrient balances in agroecosystems. In the short term, however, phosphorus depletion is not likely to become problematic, as the total phosphorus pool in P-fixing tropical soils up to 1 m in depth can easily amount to 6000 kg/ha, a volume that will not be quickly depleted by the annual P removal rate of 5 kg/ha (assuming two harvests per year, of 2.5 t biomass per cropping season, and a biomass P-content of 0.1%). Although, from the plant's perspective, it is immaterial whether the P taken up is derived from a relatively inaccessible pool through the activity of the mycorrhizal mycelium or from a soluble pool after (excessive) fertilizer use, we should not forget that it could make a difference with regard to micronutrients such as Cu and Zn. Fertilizer use could decrease mycorrhizal activity and, hence, result in micronutrient deficiencies (Lambert *et al.*, 1979).

Several single isolates of AMF have been shown to promote the growth of fast-growing tree species in low-nutrient soils (Prematuri, 1995; Setiadi, 1996; Prematuri and Dodd, 1997). Isolates of *Gigaspora rosea, Glomus etunicatum, Acaulospora scrobiculata* and *Acaulospora* sp. significantly promoted the growth of *Paraserianthes falcataria* and *Acacia mangium* at degraded nickel-mine sites (Setiadi, 1996). Such mine sites are often characterized by surface erosion and, as a consequence, by a low mycorrhizal inoculum potential. Isolates of *Scutellospora weresubiae, Glomus manihotis* and *Glomus mosseae* significantly boosted growth of *Pterocarpus indicus, P. vidalianus* and *Albizia saman* to levels that were 1.5–3 times higher than the control (Prematuri, 1995; Prematuri and Dodd, 1997). However, the performance of the inoculum over time is as important as initial plant response to inoculum addition. It is therefore necessary to study changes in species composition in terms of the way it is affected by competition with the indigenous species. A commercially acquired inoculum, which results in a high initial benefit but the effect of which does not persist over time, forces the buyer to acquire this inoculum regularly. This creates buyer dependency on the supplier of the mycorrhizal inoculum.

This chapter began by considering the discovery of mycorrhiza a century ago. Though observational techniques, concepts and methods have now greatly improved, many crop plants throughout the world still suffer serious nutrient deficiencies. Better mycorrhization would, at least in the short or medium term, help this situation by allowing more efficient 'mining' of the soil. Finally, it should be stressed that, in the context of multispecies agroecosystems, the concept that better management of the fungal partner in mycorrhization can improve the overall nutrient-use efficiency of the agricultural sector is one that retains potential. However, that potential remains, thus far, unproven.

Conclusions

1. The biology of mycorrhizal associations should be understood from both a 'plant-centric' and a 'fungus-centric' perspective.
2. Many crop plants around the world suffer serious nutrient deficiencies: better mycorrhization would, at least in the short or medium term, help them 'mine' the soil more efficiently.
3. Mycorrhizal fungi do not add phosphorus to (agro)ecosystems – they only increase a plant's access to this often scarce resource. They therefore differ from rhizobia, which can act as true biofertilizers by delivering nitrogen to the (agro)ecosystem.
4. Applied mycorrhizal research has often been aimed at the production of inoculum; however, management of indigenous inoculum may provide more direct benefits.
5. Agricultural intensification can not only result in a decline in mycorrhizal inoculum due to regular disturbance, but it can also select for mycorrhizal fungi that are less beneficial to the plant.
6. Forest disturbance as a result of commercial logging may reduce or even eliminate mycorrhizal fungi from forest sites.
7. Isolates of AMF can promote the growth of fast-growing tree species in low-nutrient soils, especially where these have been severely disturbed (e.g. mine spoils); grasslands, however, may contain a healthy inoculum potential for trees.

Future research needs

1. Better indicators, applicable at the farmer level, of situations where inoculation with mycorrhizal fungi is opportune.
2. Better understanding of the persistence of diverse mycorrhizal networks in multispecies agroecosystems as the basis for inoculation, to balance current understanding, which is based on spore counts and identification.
3. Mycorrhization of models of plant nutrient and water uptake: comparison of existing quantitative approaches and development of new algorithms.