Chapter 10

Structure, Dynamics, and Restoration of Plant Communities: Do Arbuscular Mycorrhizae Matter?

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Restoration of plant communities inevitably requires an understanding of the functioning of natural communities, which are the main ecological forces that produce patterns. Following the historical line of Clements, the classical ecological theory explains the patterns of species composition and diversity of plant communities through the impact of the abiotic environment, differential dispersal, and the outcome of local biotic interactions. Since the 1950s and 1960s, the emphasis in theory has shifted toward biotic interactions within communities, especially resource partitioning and (avoidance of) competition in spatiotemporally heterogeneous environments (MacArthur and Connell 1966, MacArthur 1972, Whittaker and Levin 1977). Later, both equilibrium theory, explaining species coexistence in spatially heterogeneous environments (Tilman 1982, 1986), and nonequilibrium theory, explaining species coexistence in temporally heterogeneous environments (Connell 1978, Huston 1979, Chesson 1986), were thoroughly elaborated. Resource competition was still considered to be the driving force behind community patterns, though the role of herbivory was also considered quite often (reviewed by Brown and Gange 1990, Huntly 1991, Brown 1994). The role of symbiotic relationships, such as mycorrhiza, in the functioning of plant populations and communities was seldom taken into account, except in a few papers discussing the impact of mycorrhiza on competition among species representing different stages of plant community succession (for example, Janos 1982, E. Allen and M. Allen 1984).

In addition to the functioning of whole plant communities, attention has been paid to the dynamics of populations of single species. Why are certain species absent in most communities while others are frequent everywhere? (See Chapters 13 and 14.) Possible causes of plant species rarity have been dis-

cussed extensively (Rabinowitz 1981, Kunin and Gaston 1993). In certain cases, causes are easily identified; for example, where species are restricted to scarce or isolated habitats, occur at the margins of their distribution areas, or are directly exposed to adverse human impact (see Karron 1987, Rosenzweig 1995, Kunin and Gaston 1997). More often, however, the study of morphological and functional characteristics of plant individuals—which allows assessments of their competitive and dispersal abilities, demographic characteristics, resistance to herbivory, relationships to the abiotic environment, and so forth—has shown that there is no simple answer to the question: What specific traits or combinations of traits are responsible for rarity? (Berg et al. 1994, Gustafsson 1994, Sætersdal 1994, Eriksson et al. 1995, Thompson et al. 1996, Witkowski and Lamont 1997, Webb and Peart 1999, Wolf et al. 1999; also see Chapter 6 for a discussion about the influences of filters in assembling communities.) Again, compared to other, more obvious negative impact factors, such as competition or herbivory, the role of positive supportive interactions in determining plant species abundance is investigated much less often.

Early life stages, from seed germination to plant establishment, are important phases when the critical screening of colonizing diaspores takes place (Weiher and Keddy 1995, Kitajima and Tilman 1996). Following seed germination and the subsequent exhaustion of seed reserves, successful seedling establishment requires that plants acquire soil nutrients efficiently. Establishment also requires protection against pathogens, which in the majority of plant families is controlled, or at least influenced, by root symbioses with mycorrhizal fungi (Smith and Read 1997). More information is needed about the role of symbiotic relationships, including arbuscular mycorrhizal fungi, in early stages of plant life, because this may be an important determinant of the distribution and abundance of plant species.

Arbuscular Mycorrhiza and Plant Communities: More Evidence about the Role of the Invisible World

Arbuscular mycorrhizal fungi (AMF) communities are usually considered to be species-poor. Although Luoma et al. (1997) detected more than 200 distinct morphotypes of ectomycorrhiza on one single field site in Oregon, most studies on community richness of AMF did not mention more than 15 species (for example, Clapp et al. 1995, Stutz et al. 2000, Hildebrandt et al. 2001, Franke-Snyder et al. 2001). Studies that found more species (up to 40) did so by frequent field observations or by trapping in pot cultures (E. Allen et al. 1995, Egerton-Warburton and E. Allen 2000, Bever et al. 2001).

Fungi that form arbuscular mycorrhizal associations with the majority of land plants were originally assigned to the order Glomales within the Zygomycota (Morton and Benny 1990). However, publication of Gerdemann and Trappe's (1974) treatise redescribing the systematics of AMF led to consideration of differing species of AMF. An important result was that many of the groupings that Gerdemann and Trappe organized as genera (*Gigaspora, Acaulospora, Glomus*) are now recognized as belonging to different families, having diverged more than 300 million years ago. There are at least three clades of importance to us here: the Gigasporaceae (*Gigaspora, Scutellospora*) clade, which is morphologically distinct; the *Acaulospora* clade; and the *Glomus* clade (Morton and Redecker 2001). Walker and Trappe (1993) have identified 167 epithetes within the Glomeromycota so far. (The term *epithete* is used instead of species because the species concept in AMF is very controversial; see also the "Problems in Handling Arbuscular Mycorrhizal Fungi" section later in this chapter.)

Recent works have shown that the AMF can be unequivocally separated from all other major fungal groups in a monophyletic clade, based on molecular, morphological, and ecological characteristics. Consequently, they were removed from the polyphyletic Zygomycota and placed into a new monophyletic phylum, the Glomeromycota (Schwarzott et al. 2001, Schüßler et al. 2001), within which two new families, the Archaeosporaceae and Paraglomeraceae, both basal groups, have been described (Morton and Redecker 2001).

Both fossil and molecular evidence supports the idea that AMF are as old as the land flora and probably coevolved with the first land plants (Pirozynski and Malloch 1975, Simon et al. 1993, Remy et al. 1994, Redecker et al. 2000, Heckman et al. 2001). The AMF apparently were essential for the early land plants to scavenge phosphorus from the soil. Moreover, individual AMF and their communities may differ in their functional character; for example, in efficiency of transporting phosphorus to plants (Jakobsen et al. 1992, 2001). Physiological and life cycle traits, too, are known to differ among AMF taxa (see Dodd et al. 2000).

The central role of symbiotic AMF in plant population dynamics—through their control of soil nutrient uptake, protection against root pathogens, and intra- and interplant species linkages—is now starting to be appreciated (Newsham et al. 1994, 1995a; Simard et al. 1997; Zobel et al. 1997; Watkinson 1998; van der Putten et al. 2001). Experiments conducted under greenhouse conditions have shown that the presence or absence of AMF may shift the competitive balance not only between mycorrhizal and a nonmycorrhizal species (E. Allen and M. Allen 1984) or between species with clearly different mycorrhizal dependency (Hetrick et al. 1989), but also between species of comparable mycorrhizal dependency, whether growing in even-

aged cohorts (Allsopp and Stock 1992, Hartnett et al. 1993, Marler et al. 1999) or in systems containing adults and seedlings (Eissenstat and Newman 1990, Moora and Zobel 1996, Marler et al. 1999). The presence or absence of AMF may also have a major effect on the population structure of plant species in the field (Wilson et al. 2001). The presence of mycorrhiza in one generation may increase seedling competitive ability in the subsequent generations (Heppell et al. 1998). The effect of AMF also depends on the presence of a particular plant genotype or genotypes (Ronsheim and Anderson 2001).

In addition, one can observe the effect of the presence or absence of AMF on the species composition and diversity of the plant community, both under microcosm conditions (Grime et al. 1987) and in the field (Gange et al. 1993, Newsham et al. 1995c, Hartnett and Wilson 1999). Tree species, which were thought to be mainly ectomycorrhizal, were found to benefit from AMF colonization. Even low colonization rates seemed to have a high impact on the trees' nutrient demands (van der Heijden and Vosátka 1999, van der Heijden 2001). Egerton-Warburton and M. Allen (2001) analyzed the cost-benefit relationship in roots of *Quercus agrifolia* Nee. occupied by arbuscular mycorrhizal and ectomycorrhizal fungi and demonstrated a shift from an arbuscular mycorrhizal–dominated stage in young seedlings to an ectomycorrhizal-dominated stage in saplings.

Plants in most plant communities, however, are able to form symbiotic associations with one or more AMF species; nonmycorrhizal plant communities are very rarely found in nature (Smith and Read 1997). For this reason, the approach of comparing the presence or absence of AMF is gradually being replaced by the more informative study of the distribution and impact of natural AMF taxa (Clapp et al. 1995, van der Heijden et al. 1998b, Helgason et al. 1999, Bever et al. 2001). Although AMF can colonize roots of a taxonomically diverse range of plants, ecological specificity does occur in arbuscular mycorrhizal associations (McGonigle and Fitter 1990), and certain specific plant-AMF combinations can be more beneficial than others for both partners (Sanders 1993, van der Heijden et al. 1998b). Van der Heijden et al. (1998a) showed experimentally that the coexistence of vascular plant species in a microcosm was dependent on the species composition of the AMF community. These results lead to the conclusion that the occurrence and abundance of a vascular plant species in a particular community may depend on the presence of certain AMF species or combinations of species (Read 1998).

Are Plant and Fungal Communities Patchy?

Habitat fragmentation and its impact on the distribution of plant and animal species has now been acknowledged (Young et al. 1996, Hanski and Gilpin

1997, Tilman and Kareiva 1997). The significance of dispersal limitation for plant populations (Turnbull et al. 2000) and communities (Austin 1999, Huston 1999, Grace 2001) has been the object of many recent case studies and theoretical discussions. Both ongoing fragmentation of natural ecosystems in landscapes under human impact and dispersal limitations under fully natural conditions are important forces in structuring plant communities. One may ask: Is the distribution of AMF in natural ecosystems patchy, and does it have any significant impact on distribution of vascular plant species?

The first question to be answered, then, is: How are AMF dispersed under natural conditions? Several studies have shown that wind and water play important roles, but even animals of various sizes, from large mammals to small grasshoppers, might be vectors (M. Allen 1987, Warner et al. 1987).

Potentially concomitant variation in the species composition of AMF communities has been identified on the basis of the presence of spores in natural soils or in trap cultures. Considerable variation in AMF communities has been observed between plant communities (Johnson 1993, Merryweather and Fitter 1998b, Stutz et al. 2000) and within them (Rosendahl et al. 1989, Eom et al. 2000). These studies suggest that, at the least, the infection potential of soil in different plant communities may differ. Even seasonal dynamics may be important (Šmilauer 2001). Information about the variability of functionally active AMF communities in plant roots, however, is extremely scarce. Helgason et al. (1998, 1999) showed that AMF communities in plant roots differed between two contrasting types of ecosystems: an agricultural field and a deciduous woodland. There was also a considerable within-stand variation in AMF communities. A loss of AMF diversity due to agricultural activities (Helgason et al. 1998, Daniell et al. 2001) strongly suggests that variation in natural AMF communities may be partly due to habitat fragmentation, since intensively managed landscapes around and between natural ecosystems contain considerably smaller numbers of AMF taxa and do not function as efficient inoculation sources.

Thus, if specific compatible relationships between certain AMF and plant taxa are required for mutual symbiont survival, the loss of compatible AMF species or individuals may limit the distribution of a particular plant species. As a result, the availability of AMF taxa may influence the composition and function of the plant community. The impact of symbiotic microbes as a determinant of plant species abundance has been recognized only quite recently. For example, Thrall et al. (2000) demonstrated the differential dependency of rare and common tree species on nitrogen-fixing bacteria. More specifically, the potential importance of coavailability of plant species and AMF taxa has been discussed by Barroetavena et al. (1998). Öpik et al. (unpublished data) found that early establishment of a rare plant

species, *Pulsatilla patens* (L.) Mill. (Ranunculaceae), and of a common species, *P. pratensis* (L.) Mill., depended upon different AMF taxa being unevenly distributed among fragments of natural landscapes. Thus, the establishment and performance of certain plant individuals in particular localities may really depend on the presence of the appropriate AMF taxa.

Occurrence and Function of AMF in Natural Ecosystems: A Case Study in the North American Sagebrush Steppe

Using assembly rules to facilitate restoration requires that we find such rules from observations of natural succession, coupled with experimental approaches including restoration research. Over the past three decades, the responses of sagebrush steppe communities to disturbance have been studied by Michael Allen and his research group (see corresponding articles cited in this section). The dominant species is an exotic introduced weed, *Salsola kali* L. This plant was introduced in the late 1800s from the Asian steppes and is considered a noxious weed. Many efforts have been made to control it in restoration situations. Grasses, including *Elymus smithii* (Rydb.) Gould, a C_3 species, and *Bouteloua gracilis* (H. B. K.) Lag. ex Steud., a C_4 species, are desirable outcomes (E. Allen 1982). These species are prime forage for rangelands for both cattle and native ungulates (for example, bison). The dominant shrub is *Artemisia tridentata* Nutt., or basin big sagebrush. This shrub is considered an increaser with grazing, and it is often removed in rangelands managed for cattle. However, it is important for wildlife including pronghorns, birds, and invertebrates.

In general, succession in sagebrush steppe communities proceeds from bare soil to weedy annuals to bunchgrasses to shrubs. The soils tend to be relatively nutrient-rich, but plant production is limited by drought and cold. An important characteristic is that, following disturbance, available nutrients increase and bound organic nutrients decline (M. Allen and MacMahon 1985). All late seral plants in these systems form arbuscular mycorrhiza relationships; ectomycorrhizae are limited by drought, being present only in riparian ribbon or higher-elevation conifer forests.

Possible Involvement Levels of Arbuscular Mycorrhizae in Plant Succession

In the initial sagebrush steppe research, it was postulated that *S. kali* could affect the rate of establishment of native plants. Further, because *S. kali* is a nonmycotrophic (never forming mycorrhizae) plant (Stahl 1900, Nicolson

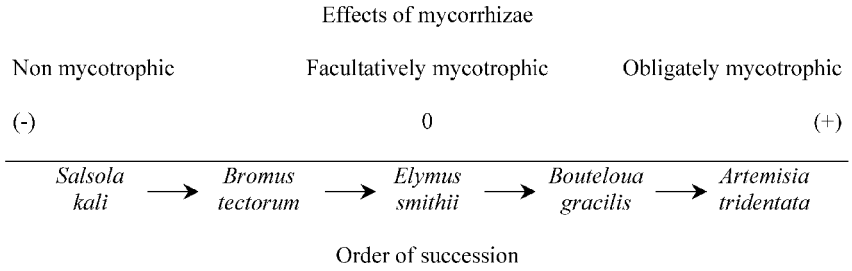

Figure 10.1 Plant successional model suggesting increasing dependence on mycorrhizal association in the sagebrush steppe. (After E. Allen 1984.)

1960), it has been suggested that mycorrhizae might play an important role in succession and thus could be used to enhance vegetation recovery to the detriment of the nonmycotrophic *S. kali*. In addition, *S. kali* is nitrophilous and preferentially establishes and grows in nutrient-rich soils (E. Allen and Knight 1984). The observation that *S. kali* does not form mycorrhizal relationships was confirmed, as well as the finding that following disturbance, mycorrhizal fungi needed to build up inoculum density just as plants must colonize and expand their range (E. Allen and M. Allen 1980). Further, it was possible to demonstrate that AMF actually were parasitic on *S. kali*, inhibiting growth and, in some cases, actually killing seedlings (M. Allen et al. 1989). Further work also demonstrated that there was a gradient in responsiveness of plants to mycorrhizae; herbaceous grasses were facultatively mycorrhizal, and woody species were more responsive, approaching obligately mycorrhizal status (E. Allen 1984b). Based on these observations, a model of succession was postulated in which the initial seral stage was dominated by nonmycotrophic plants or by facultatively mycorrhizal species. With succession, as the mycorrhizal fungi became more prevalent, more responsive plants could establish and persist (Figure 10.1).

For assembly rules, it is important that the different groups of AMF also appear to form distinct functional groups. Hart and Reader (2002) associated AMF taxonomic groupings with colonization strategies. Newsham et al. (1995b) reported differing ecological functions among the different fungal groups. For example, the smaller *Glomus* spp. form many individual infections, whereas the Gigasporaceae tend to form larger external mycelium (Wilson and Tommerup 1992). With few exceptions, smaller *Glomus* spores disperse readily, including by wind in the arid steppe regions (M. Allen et al. 1993). *Acaulospora, Scutellospora, Gigaspora,* and some large-spored *Glomus* species disperse slowly, largely by animal vectors (M. Allen 1988, M. Allen et al. 1993). Thus, it was postulated that just as different plants and

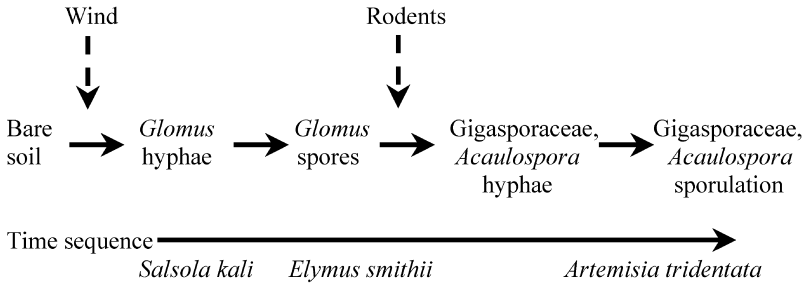

FIGURE 10.2 Fungal succession model with roles of animals and plants. Wind is the major vector for small *Glomus* spores and rodents are the major vector for the larger-spored species (M. Allen et al. 1993).

animals migrate using different vectors, and because invasion is related to successional status (for example, Diamond 1975), a similar process should occur for AMF. This led to the construction of a second model of succession based on the dispersal ability of the different AMF fungi (Figure 10.2). According to this model, AMF with small spores dispersed by wind would be prevalent in early-successional stages, whereas AMF with large spores dispersed by rodents would follow later.

Finally, we must consider some basic competitive outcomes. E. Allen and Knight (1984) demonstrated that *S. kali* competed with grasses for water and nutrients, reducing grass establishment. However, grass density and mass increased under *S. kali* when the soils contained AMF (E. Allen and Knight 1984). The presence of *Glomus* AMF aided the competitive ability and subsequent establishment of the grasses (E. Allen and M. Allen 1984, 1986). If *S. kali* was removed, however, grasses readily established but were replaced partially by *A. tridentata* over time (E. Allen 1988).

The mechanisms were not limited simply to competition. AMF were actually found to infect *S. kali*. These fungi would colonize and obtain enough carbon from the plant for sporulation, but they did not appear to provide nutrients in exchange (M. Allen and E. Allen 1990). Further, the fungi appeared to trigger an immune response that resulted in growth depressions and seedling mortality (M. Allen et al. 1989).

Conceptual Synthesis

Survey results showed that all major AMF groups can be found in association with sagebrush and grasses in the sage steppe region (E. Allen et al. 1995). Thus, the question becomes whether there are differential fungus-

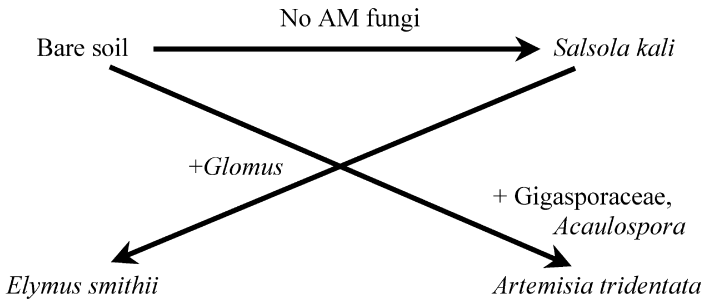

Figure 10.3 Postulated synthesized succession model in the sagebrush steppe showing different outcome of vegetation structure depending on availability and type of arbuscular mycorrhizal (AM) fungi.

plant responses leading to the patterns of succession observed and whether these responses can be used in restoration. It was postulated that *S. kali* would compete with grasses and that without AMF, *S. kali* would do better, but with AMF, the grasses would do best. It was expected that *Glomus* spp. would initially invade and facilitate the grasses, which in turn would support greater AMF biomass, including the larger *Acaulospora* and Gigasporaceae spores as they invaded. These fungi, in turn, would enhance growth of shrubs such as *A. tridentata* more than the growth of the smaller, more ephemeral *Glomus* spp. (Figure 10.3).

If this model is accurate, one could begin to manipulate the resulting community in several ways. First, by adding AMF, one could reduce the cover and persistence of *S. kali*. Second, a diversity of AMF could favor a complex mix of grasses and shrubs (as well as other species). Finally, by creating a patchy distribution of inoculum types, one could reconstruct a patchy but species-rich plant community capable of sustaining a higher diversity of animals. Alternatively, if the goal was to achieve range grasses in some sections and a mixture of predominantly shrub cover for wildlife in another, initial inoculations could facilitate such a pattern.

Experimental Validation and Added Complexities

Several studies in this vegetation type and in others support such a model. E. Allen (1984a, 1984b) noted that the addition of AMF increased plant diversity by promoting the establishment of later seral forbs and grasses in the matrix of an early seral environment. Grime et al. (1987) reported that adding AMF increased plant diversity by facilitating arbuscular mycorrhizal plants in the competitive mix of arbuscular mycorrhizal and nonmy-

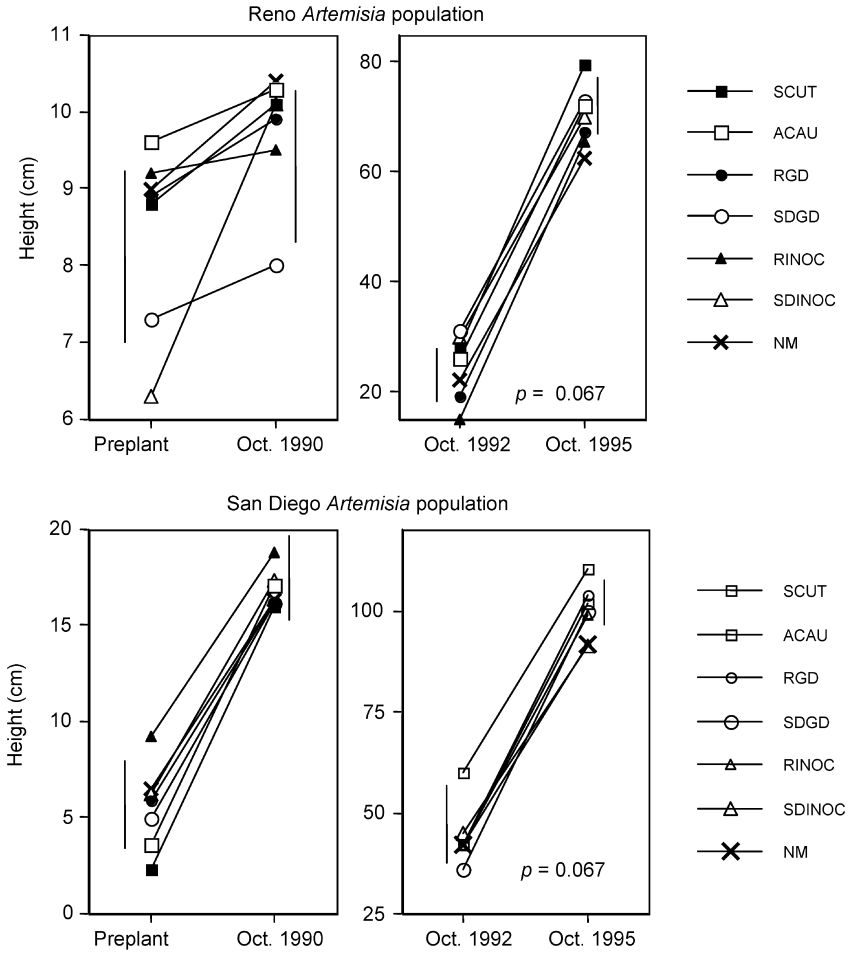

Figure 10.4 Growth responses of *Artemisia tridentata* to AM fungi using a reciprocal transplant experiment. The experimental design is described in Weinbaum et al. 1996. Two sites, Sky Oaks Biological Station, 120 km from San Diego, and Beddell Flats, 40 km from Reno, Nevada, were used, along with their populations of *A. tridentata* and AM fungi including *Scutellospora calospora* (found at the Reno site; SCUT), *Acaulospora elegans* (found at the San Diego site, ACAU), *Glomus deserticola* (found at Reno, RGD, as well as in San Diego, SDGD), and whole soil inoculum (a mixed species inoculum from Reno, RINOC, and San Diego, SDINOC; NM: nonmycorrhizal soil). Data are from M. Allen et al. 1992; Hickson 1993; E. Allen and M. Allen, unpublished. The two sites were analyzed separately. In both cases, using a repeated measures ANOVA, both plant populations ($p < .0001$ for both plant populations and sites) and inoculum sources (p: .0677 for Reno, $p = .012$ for San Diego) were different (see relevant power analyzes in Klironomos et al. 1999).

cotrophic plants. Van der Heijden et al. (1998) found that a mix of AMF promoted diversity of plants, especially forbs. Klironomos et al. (1999) looked at the spatial structure of AMF in soil. There were distinctive patterns separating *Glomus*, *Acaulospora*, and Gigasporaceae, related to the presence of shrubs versus forbs in a shrubland patch recovering from fire.

On a practical note, direct replacement or short-term storage and replacement of topsoil ensures the reapplication of AMF to a site and facilitates recovery of mycorrhizae and mycotrophic plants (E. Allen and M. Allen 1980, Miller and Jastrow 1992). Most restoration plans now require the use of topsoil, and some are beginning to require mycorrhizae (see also the section "Occurrence and Function of AMF in Degraded Ecosystems" later in this chapter).

The addition of *Glomus* inoculum to soils with very low AMF (caused by long-term storage of the respread topsoil) enhanced the competitive ability of the native grass *E. smithii* in the presence of *S. kali*. This pattern became especially noticeable as drought stress became important (E. Allen and M. Allen 1986).

We still know little about how important species composition of AMF is to restoration. In a 5-year study of AMF and the establishment and growth of *A. tridentata*, important differences among species of fungi were found. Two populations of *A. tridentata*were monitored on the edges of the Great Basin in the western United States at two sites, one near San Diego, California, and one near Reno, Nevada. Soils had been tilled and AMF removed using benomyl (Weinbaum et al. 1996). The worst condition for the shrub's survival and growth was to be planted with no mycorrhizae. *Glomus desert*i*cola* Trappe, Bloss and J.A. Menge initially stimulated plant growth, but the larger-spored AMF actually inhibited some growth initially. However, as *Scutellospora calospora* (T.H. Nicolson and Gerd.) C. Walker and F.E. Sanders and *Acaulospora elegans* Trappe and Gerd. expanded the hyphal network, these fungi subsequently stimulated the growth of *A. tridentata* more than *G. deserticola* (Figure 10.4).

This study also showed the importance of ecotypic differentiation. There were significant differences between the *G. deserticola* collected from the San Diego and Reno sites. For example, the *A. tridentata* at the San Diego site grew best with the San Diego *G. deserticola*, whereas it grew at intermediate rates (fifth) with the Reno *G. deserticola*. Almost the reverse was true for the Reno plants. Exotic fungi did have lower survival rates than local inoculum (Weinbaum et al. 1996), but the exotic inoculum did not disappear during the study period.

Although these community interactions show a level of organization that can be useful in restoration studies, the environment often plays havoc with any potential assembly rules we derive. This affects the outcomes, often in unpredictable ways.

A second example of this unpredictability was found in the Kemmerer, Wyoming, inoculation studies. It was predicted that adding inoculum would increase the competitive ability of *E. smithii* over that of *S. kali*. However, the AMF directly parasitized the *S. kali*, taking carbon (M. Allen and E. Allen 1990) and killing individual root tips (M. Allen et al. 1989). This reduced the growth of *S. kali* and, incidentally, also reduced the ability of the plant remains to trap snow during the winter. Soil moisture was thus reduced, and *E. smithii* was subject to early drought stress (E. Allen and M. Allen 1988). In this way, AMF actually were detrimental to the plants, whereas normally the symbiosis would be mutualistic.

Lessons for Using Arbuscular Mycorrhizae in Restoration Management

The role of mycorrhizae as symbionts in succession is relatively well documented (for example, Janos 1980, M. Allen 1987). The presence or absence of the mutualism has remarkably consistent effects, shifting the initial seral vegetation states to later ones. Further, because the hyphae bind soil particles and immobilize nutrients (in both plant and fungal tissue), these associations have dramatic effects on nutrient cycling. Some of these effects have already been incorporated into restoration practices such as carefully managing soils; providing source areas for immigration; and, in extreme cases, inoculating seedlings or soils. In essence, these practices are incorporating the assembly rules at the functional level.

However, the assembly of mycorrhizal fungal communities, like the assembly of all communities, contains a large element of chance. Dispersals of plants and fungi are independent events that become interdependent. Some plants prefer specific fungi, but the preferences are not absolute, and so there is elasticity in the system.

As noted earlier, the environment plays a major role in determining the outcomes of interactions among organisms. Since all rules are subject to the chaotic system that comprises our environment, caution is necessary. However, that does not prevent us from adopting some rules to enhance restoration. From the study of mycorrhizae, these rules include

- Manage soil for mycorrhizae and, if possible, retain existing inoculum.

- Use local mycorrhizae whenever possible.
- Understand the environment where restoration efforts will occur.
- Develop inoculation approaches as a last, but often essential, resort.
- Manage for AMF diversity, recognizing that the sequence of fungal additions may depend on plant composition.

Occurrence, Function, and Practical Use of Arbuscular Mycorrhizal Fungi in Degraded Ecosystems

Industrial activities increase the numbers of waste sites and degrade natural ecosystems. Such sites are open to succession, but their dynamics are generally slow because of a degraded soil fertility. Soil microflora is involved in fertility degradation in two ways. On the one hand, fertility is lost partially because of the direct detrimental effects of industrial activities on populations of soil microorganisms normally involved in cycling and mobilization of trophic resources. On the other hand, once soil microorganisms are established, edaphic stresses negatively influence them (Esher et al. 1992, Langer and Günther 2001). Populations of AMF are essential for soil development and successful plant establishment, as we showed in the previous section of this chapter. The presence of AMF as obligate plant symbionts in the majority of plant species may reduce the negative effects of stress caused by a lack of nutrients or organic matter resulting from adverse soil structure or extreme pH (Sylvia and Williams 1992). Other specific roles of the AMF symbiosis on degraded sites are pathogen defense (Filion et al. 1999), increased drought and heavy metal resistance (Galli et al. 1994, Hildebrandt et al. 1999, Kaldorf et al. 1999, Augé et al. 2001), and enhanced tolerance of high salinity because AMF enlarge the absorption zone of roots (Hardie 1985). In this context, the elimination of multifunctional AMF populations could hamper plant establishment and survival considerably (Visser 1985, Pfleger et al. 1994).

Succession of Vegetation and AMF Populations in Degraded Ecosystems

Recent research has shown that plant community structure is actually determined by the suitability of plant rhizosphere microorganism systems to specific site conditions (Smith and Smith 1996). Succession patterns and the involvement of AMF observed on highly disturbed sites such as spoil banks in central Europe resulting from industrial or mining activities are highly consistent with those of the case study and the proposed models presented in the "Occurrence and Function of AMF in Natural Ecosystems" section

earlier in this chapter. A spontaneous plant succession is relatively slow, and primarily annual ruderal plants, mostly nonmycotrophic species, invade these sites at the beginning of succession. Even these first invaders (for example, Chenopodiaceae) can influence fungal populations indirectly and positively by providing a niche for sporulation in the cavity of dead seeds (Rydlová and Vosátka 2000). Various dominant species colonizing spoil banks during primary succession exhibit various levels of mycorrhizal dependence, from strictly nonmycotrophic *Chenopodium* and *Sisymbrium* to facultatively mycotrophic grasses such as *Elymus*, *Arrhenatherum*, and *Calamagrostis* (Rydlová and Vosátka 2001). Nonmycotrophic plant species can also be colonized, but arbuscules are usually not found and the function of mycorrhiza is thus questionable (Janos 1980, Rydlová and Vosátka 2001). Miller (1979) concluded that the occurrence of mycorrhizal colonization in the Chenopodiaceae is relevant to the life strategy of the plant. For example, shrubby members of the Chenopodiaceae with a stress-tolerant life strategy associate with AMF, whereas ruderal annuals of the family are nonmycorrhizal.

Secondary colonizers of degraded sites, particularly grasses, belong to facultative mycotrophs as classified by Janos (1980). Generally, temperate grasses are less mycotrophic, as found by Hetrick et al. (1989), but some grasses have been found to be highly mycorrhizal on degraded soils (Vosátka et al. 1995). Newsham et al. (1995a) concluded from a series of field experiments that the reason grasses benefit from AMF might be increased resistance to root pathogens rather than enhancement of nutrient uptake. It could be assumed that mycotrophy of plants in all succession stages is highly dependent not only on the plant species but also on many abiotic environmental factors.

The succession of AMF communities is closely interrelated with the succession of plant communities, changes in nutrient availability, and the distribution of mycorrhizal propagules on the site. The question remains whether native AMF or nonindigenous isolates are better adapted to edaphic conditions of soils from degraded sites. Some results indicate that native AMF can develop and function better in the soils of their origin (Enkhtuya et al. 2000). In soils from the first succession stages of vegetation on degraded sites, however, the composition of the native AMF population should differ from that of latter stages; in addition, there could be marked differences in the respective effectiveness of these AMF populations.

Potentials for Application of AMF Inocula in Restoration

As mentioned earlier, regeneration succession on highly degraded sites mostly results in a low diversity of plant communities and associated micro-

bial populations. To promote and accelerate succession in restoration processes and to develop sustainable revegetation practices, it is necessary to study the implementation possibilities of phytomicrobial complexes (plants–symbiotic fungi–beneficial rhizosphere bacteria) that are tolerant to various stresses. To accomplish this objective, it is essential to restore or reintroduce functional populations of beneficial AMF in the soil. Mycorrhizal symbiosis develops when sufficient numbers of AMF propagules are in the soil; however, the number of spores and root colonization are often reduced by soil disturbance (Waaland and E. Allen 1987, Helgason et al. 1998). Therefore, if AMF have deteriorated or been eliminated, they should be reintroduced by inoculation of soils. Different populations or geographical isolates of AMF have been found to show a high variability in their tolerance to edaphic stress, represented by the amount of contamination present at a site (Weissenhorn et al. 1993, Bartolome-Esteban and Schenck 1994). In most cases, AMF isolates adapted to specific local soil conditions are better able to survive and have the potential to stimulate plant growth. It is probable that such AMF ecotypes result from a long-term adaptation to soils with extreme properties (Sylvia and Williams 1992). Such isolates should be selected and used to produce inoculum for field applications. A collection of stress-adapted AMF isolated from soils of different types of degraded ecosystems in central Europe has been established. Some of the isolates are registered in an international type collection—the European Bank of Glomeromycota (Dodd et al. 1994; see "Sequence Analysis" later)—and can be used in future revegetation of polluted sites.

Inoculation biotechnology can be used to support restoration and reestablishment of AMF populations, where selected efficient strains of AMF and/or indigenous strains are multiplied and introduced to the soil either directly or by transplanting preinoculated plants. For example, inoculation technology was used for the restoration of a *Leymus arenarius* (L.) Hochst. (= *Elymus arenarius* L.) grass cover on a spoil bank at Samphire Hoe (UK) comprising material recovered from Eurotunnel excavations. Grasses preinoculated with native AMF from the site showed better development throughout two vegetation seasons than did noninoculated plants. It is interesting that precultivation of plants in highly fertilized substrate did not help to ensure sustainability of revegetation at this harsh site with high salinity, low fertility, and erosion problems (Dodd et al. 2002). Most of the pot and field experiments have shown the potential of mycorrhizal inoculations to facilitate plant growth on sites with unfavorable conditions resulting either from industrial activities or from naturally harsh environments. However, as noted, inoculations can be limited or even fail when they are conducted in highly con-

taminated soils or under extremely harsh conditions in degraded ecosystems. This is illustrated by the following examples of field applications in degraded ecosystems.

Experiment 1: Inoculation of Lotus corniculatus *on Colliery Spoil Bank (Betteshanger, UK)*

A field trial was conducted to investigate the effects of inoculation with AMF on the establishment and growth of *Lotus corniculatus* L. plants on colliery spoil bank in Betteshanger, Kent, UK. There was a noninoculated treatment and a treatment inoculated with a mixture of five AMF species, using the commercial mycorrhizal product TerraVital-D (PlantWorks Ltd., UK). Seeds of *Lotus corniculatus* were sown, and 20 mL of inoculum was placed under each seed patch. Survival of plants and mycorrhizal colonization were registered after 6 months of growth.

No plant survived the 6-month period on the noninoculated plots, whereas about 90 percent of the plants survived in the mycorrhizal treatment. Mycorrhizal colonization of plants was 52 percent (Vosátka 2000).

Experiment 2: Inoculation of Leymus arenarius *on Volcanic Field (Hekla, Iceland)*

Ecosystem degradation and desertification are by far the most serious environmental problems in Iceland. Erosion is accelerated by volcanic activity and harsh weather conditions. Efforts are constantly being made to reclaim eroded lands by distributing seeds and fertilizers. However, the results of these efforts are quite variable, since it usually takes years of fertilization, followed by reseeding, to obtain permanent vegetation cover. For a field trial conducted on the volcanic fields of the Hekla volcano, seeds of *Leymus arenarius* (L.) Hochst. were sown (200 per m^2) onto plots that were noninoculated or inoculated with 1 L of soil from either established adjacent *L. arenarius* stands or the commercial mycorrhizal product TerraVital-D (PlantWorks Ltd., UK) consisting of five AMF isolates, including some adapted to arctic conditions and eroded soils.

Inoculation with TerraVital-D substantially increased survival of *L. arenarius* plants after sowing. In control plots, only 8 plants per plot emerged, whereas on two plots treated with soil inoculum from adjacent vegetated sites or with TerraVital-D, 26 and 48 plants emerged, respectively (Figure 10.5a). The plants from the inoculated plots were significantly taller and more developed (Figures 10.5b, and 10.5c). These preliminary results indi-

cate that reclamation work in naturally degraded and disturbed volcanic fields of cold deserts in Iceland can benefit from the addition of appropriate AMF. Further research is needed, however, to determine the long-term effects of inoculation and to select the most effective inoculum types and modes of application.

Experiment 3: Field Trial on Spoil Bank (Cottbus, Germany)

Two tree species, *Sorbus aucuparia* L. and *Robinia pseudoacacia* L., and the leguminous shrub *Cytisus scoparius* (L.) Link were planted on lignite spoil banks near Cottbus, Germany. The trial was conducted by the Brandenburgische Technical University in Cottbus. Seeds were sown to randomized blocks with four replications of 4-m rows. Four AMF species—*Glomus claroideum* N.C. Schenck and G.S. Sm., *G. geosporum* (T.H. Nicolson and Gerd.) C. Walker, *G. mosseae* (T.H. Nicolson and Gerd.) Gerd. and Trappe, and *G. intraradices* N.C. Schenck and G.S. Sm.—were used, with two isolates of each species, one from polluted soils and one from unpolluted soils.

Neither mortality nor height of *C. scoparius* was affected by mycorrhizal inoculation (data shown in Vosátka et al. 1999). Furthermore, *S. aucuparia* and *R. pseudoacacia* died during the summer and autumn of 1997. There were extremely adverse soil conditions in the field; that is, soil pH fluctuating around 3 and an unfavorable sandy soil structure. The results showed that the mycorrhizal inoculation alone could not compensate for these conditions. An additional reason for the plant dieback could be that in areas of AMF inoculation, the phosphorus supply was adequate but the nitrogen supply was not (Weber, personal communication). This may explain why the leguminous plants survived longer at the site. However, they also became nitrogen-limited, were not able to store enough resources to survive the winter, and finally died. This field study illustrates that for restoring extreme sites, further soil management in addition to the use of properly selected AMF may be essential.

Restoration Ecology Seen Through the Eyes of a Mycologist

AMF are present even in soils with very harsh physical and chemical properties, and at least certain AMF taxa already recolonize soils of degraded or anthropogenic ecosystems at early stages of plant succession. It is feasible to inoculate mycorrhizal fungi alone or together with bacteria, to increase the health, growth, and quality of plants used for revegetation. Before starting revegetation, soil conditions should be checked and, if necessary, sparse

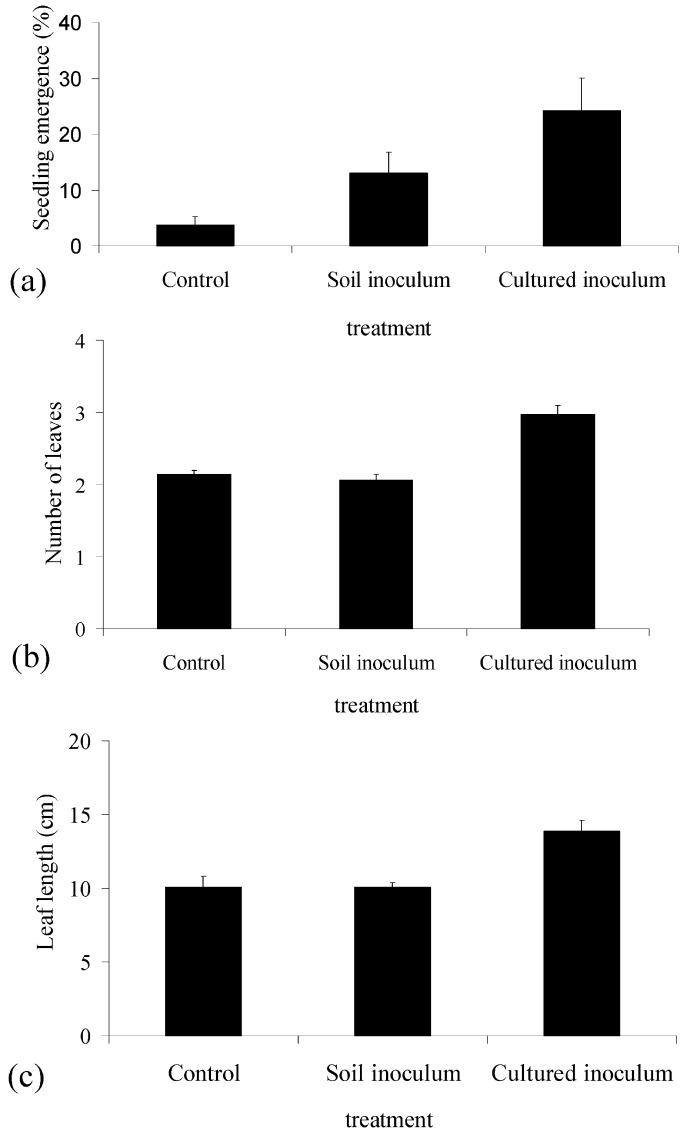

Figure 10.5 Survival rate (a), leaf length, (b) and number of leaves (c) of *Leymus arenarius* plants sown on 1-m_2 plots on a volcanic field in Iceland and inoculated with either soil inoculum from adjacent grass dune or commercial mycorrhizal product TerraVital-D (PlantWorks Ltd., UK).

nutrient pools should be compensated by fertilization to ensure basic essential conditions for plant establishment.

One of the strategies for the restoration of AMF populations could be based on an increase of native AMF by precropping with highly mycotrophic species. The resulting increased inoculum potential on such precultivated sites could be advantageous for a successful and consequent mycorrhization of plants used for revegetation. An alternative strategy is to use native fungi isolated from the site as inoculum. Further research should result in the development of a novel biotechnology for the inoculation of plant material for revegetation of low-grade industrial land. Application of appropriate phytomicrobial complexes, including stress-tolerant plants and beneficial plant-associated microbial consortia, bears the potential for a substantial increase in the effectiveness and sustainability of revegetation practices.

Distribution of AMF Communities in Nature: A Fungal Geobotany Is Needed

The examples presented illustrate the ecological importance of the structure and composition of native AMF communities associated with given plant species. The possibility of analyzing and using these structures constitutes a key to understanding spontaneously occurring ecosystem regeneration or to controlling managed restoration. In this context, poor basic knowledge of the distribution of AMF taxa under natural conditions is the main bottleneck to specifying their role in the functioning and dynamics of plant communities.

Limitations of Using Spores to Analyze AMF Communities

Traditionally, the taxonomy of AMF has been based on the morphology of their asexual soil-borne spores (Gerdemann and Trappe 1974, Morton and Benny 1990); these spores, however, cannot be traced to their host plant. Moreover, AMF communities *in planta* and spore communities in soil often differ in their species composition (for example, Clapp et al. 1995, Merryweather and Fitter 1998a, Turnau et al. 2001), due to sporulation and colonization differences between AMF taxa. Therefore, ecological studies of AMF based on analyses of spore diversity must be interpreted with caution. Most studies in the past were performed on spores, because their extraction from soil was relatively easy (Gerdemann and Nicolson 1963) and the only way to determine the different species was based on spore morphology.

In most cases, analyses of spore diversity that requires abundant quantities of vital spores were not performed directly on the spores collected in the field but were carried out after establishment of trap cultures under greenhouse conditions to gather pure isolates of the different spore types. This method, in which entrapping plants are inoculated with AMF structures from the field, is time- and space-consuming. Also, depending on the start-up method used (single spores, multiple spores, roots), the success rates can be quite variable. Additionally, the most effective mycorrhizal strains in the field might have a low tolerance to greenhouse conditions, and these strains might never be obtained in trap cultures. This phenomenon was shown in a phosphorus-polluted grassland in the vicinity of a former fertilizer plant in the central Saale Valley (Thuringia, Germany; for a detailed description, see Langer and Günther 2001). Although direct identification of AMF in roots from the field revealed dominance of a *Glomus* sp. related to *Glomus clarum* T.H. Nicolson and N.C. Schenck (AJ243275; 66–71 percent identity), identification by trapping maize plants under greenhouse conditions showed high infection rates by *Glomus intraradices*, which was quite rare in the field (Figure 10.6).

Approaches to Analyzing AMF Communities on the Mycorrhizal Roots Themselves

More recent studies have focused on direct detection of AMF in the roots of host plants. Cavagnaro et al. (2001) showed that the morphology of arbuscular mycorrhizae depends largely on the fungal partner rather than the plant partner. However, mycobiont identification based on features of AMF infection at best allows identification to the family level (Merryweather and Fitter 1998a). Specific staining techniques allow differentiation of some AMF species within certain groups (Vierheilig et al. 1998), but they are not adequate for general identification of AMF. For example, members of the Archaeosporaceae and Paraglomeraceae show weak or no staining with trypan blue, the most commonly used stain for mycorrhiza (Morton and Redecker 2001). Therefore, these techniques are of limited use under field conditions.

Within the whole spectrum of molecular methods, the high detection sensitivity of nested polymerase chain reaction (PCR) approaches is the most adaptable for amplifying target regions from the minute amounts of fungal DNA present in AM roots from the field (van Tuinen et al. 1998, Jacquot et al. 2000, Redecker 2000, Kjøller and Rosendahl 2000, 2001, Turnau et al. 2001). A problem with this method, however, is that DNA from all fungal

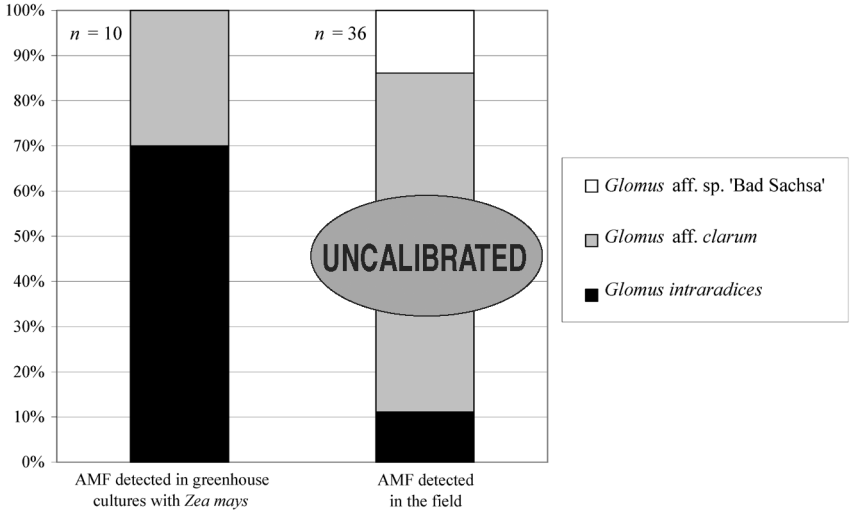

Figure 10.6 Survey of an AMF community from a phosphorus-contaminated grassland in the central Saale Valley 13 km north of Jena (Steudnitz, Thuringia, Germany; see Chapter 13 for details of the site) directly found in the field soil and in trap cultures with maize.

taxa of the roots, rhizoplane, and rhizosphere—the majority of which do not belong to AMF—are amplified when such universal primers for fungi as ITS1, ITS4 or ITS5 are used (White et al. 1990). To avoid this problem, most studies previously cited used primers with a narrow specificity for one to a few AMF taxa. This approach allows detection of specific mycobionts but fails to screen the entire diversity of AMF present in the field, which is necessary for ecological studies or for characterizing AMF populations adapted to specific stress conditions and to be used in restoration.

To resolve the conflict between amplification mainly of contaminants (non-AMF) and detection reduced to a narrow spectrum of AMF, a strategy was recently proposed based on a nested PCR in which one primer displays a specificity restricted to the glomalean fungi plus some groups in the basidiomycetes (Renker et al. 2003). Furthermore, the method tried to eliminate the most of the residual contaminants by performing a restriction between both reactions of the nested PCR with an enzyme that specifically does not cut the target region of AMF fungi. The first assessment of this method offered promising prospects for screening a wide range of AMF on field roots, even if it cannot reveal certain taxa of the most basal Glomeromycota.

The Need to Consider the Soil Mycelium of AMF

Apart from these methodological issues, which should be resolvable by new molecular tools, another basic weakness of AMF community analyses based solely on morphological or molecular investigations on roots is that the ecological role of some species might be overestimated. Molecular approaches neglect the fact that the extraradical mycelium is at least as important as the inter- and intracellular mycelia for the nutrient supply of the host plant (Horton and Bruns 2001). Therefore, it might also be necessary to monitor the species distribution directly in the soil (Claassen et al. 1996).

These basic problems in monitoring AMF diversity may explain the great number of unresolved questions in field studies dealing with (1) species composition and community structure, (2) the influence of fungal community structure on plant biodiversity, and (3) the impact of fungal abundance on plant nutrition. Effective investigations of these topics under field conditions would require analyzing the AMF directly on roots and in the soil; to date, such analysis has been done only indirectly using spores, which makes no more sense than characterizing plant communities by analysis of seed banks in soils alone.

Problems in Handling Arbuscular Mycorrhizal Fungi: The Weakness of the Species Concept

Another source of complication, even for interpretation of the most recent molecular ecological studies, arises from biological traits of AMF themselves:

1. Hyphae of AMF have a siphonal structure, and there is evidence that genetically heterogeneous nuclei may coexist in mycelium, and even the multicopy regions of the ribosomal DNA (rDNA) of single nuclei are known to be heterogeneous (Sanders et al. 1996, Kuhn et al. 2001).
2. AMF do not display sexual reproduction (Sanders 1999); but in the genus *Glomus*, for example, somatic exchanges of nuclei were observed via anastomosis between hyphae, which originated not only from the same spore but also from different spores of the same isolate (Giovannetti et al. 1999, 2001). This indicates that beyond nutritional flow in AMF mycelial networks, a flow of genetic information also exists (Giovannetti et al. 2001).

Recent studies have also revealed that hybridization between different genera might be possible and that *Entrophospora infrequens* (I.R. Hall) R.N. Ames and R.W. Schneid. might be one of the outcomes (Rodriguez et al.

2001). Consequently, genetic variation within one population and among different isolates of one species is often quite high (Dodd et al. 1996, Lloyd-MacGilp et al. 1996, Antoniolli et al. 2000, Clapp et al. 2001). A prerequisite to any study of population structure and diversity is the clarification of the species concept for AMF (Dodd et al. 1996). However, based on the lack of sexual reproduction in AMF, it has been suggested that applying the species concept could be difficult and that it would be more appropriate to base the description of AMF biodiversity on genetic diversity (Sanders et al. 1996).

Toward a Molecular Ecological Approach to Understanding How AMF Rule the Assembly of Plants in Communities

So far, we have on the one hand reviewed facts and experiments that support the preeminent role of AMF in ruling the formation of plant communities. On the other hand, we have emphasized that the geobotany of AMF, which is urgently needed in this context, is hampered by biological traits of AMF (see the immediately preceding section, "Problems in Handling Arbuscular Mycorrhizal Fungi"). To resolve this conflict, we must develop molecular ecological tools that will allow us to monitor AMF directly in the field accurately enough to demonstrate their role in ruling the assembly of plants.

This last section will focus on a critical review of some molecular biological techniques that have been used to study the genetic diversity of AMF, based on analyses of glycoproteins (Wright et al. 1987, Hahn 1993, Thingstrup et al. 1995), isozymes (Rosendahl 1989), fatty acid patterns (Jabaji-Hare 1988, Bentivenga and Morton 1994), PCR coupled with restriction fragment length polymorphisms (RFLP) of target regions within the rDNA (Sanders et al. 1996), random amplified polymorphic DNA-PCR (RAPD, Abbas et al. 1996, Lanfranco et al. 1995), or microsatellite PCR (Longato and Bonfante 1997, Zézé et al. 1997) (for a detailed description of most methods, see Weising et al. 1995). Recent studies indicate that the most adequate techniques are based on sequence analyses of target regions within the nuclear rDNA, which combine the advantages of high copy numbers, highly conserved sequence tracks for the annealing of the primers, and variable regions between the priming sites (Simon et al. 1992, Abbas et al. 1996, Lloyd-MacGillp et al. 1996, Helgason et al. 1999, Antoniolli et al. 2000). Works focus either on the 18S gene encoding for the ribosomal small subunit (SSU) or on the internal transcribed spacer (ITS) region, which encompasses the two nonencoding spacers ITS1 and ITS2 separated by the 5.8S gene of the nuclear rDNA. Some studies analyze the 28S gene of the rDNA, which encodes for the ribosomal large subunit (LSU) (van Tuinen et al. 1998; Kjøller and Rosendahl 2000, 2001). So far, the number of available

sequence data for this last region is quite low, so it seems preferable to consider the SSU or the ITS in ecological studies. The ITS displays the greatest polymorphism. In Ascomycota and Basidiomycota, it is adequate to differentiate species and in some groups to resolve intraspecific variations, whereas the SSU polymorphism is more adequate at higher taxonomic levels (Bruns et al. 1992, Horton and Bruns 2001). Therefore, the ITS is generally considered to be the most convenient region for investigating the structure of natural fungi communities in the field (Buscot et al. 2000). Within the Glomeromycota, analysis of ITS is also well established and is used mainly to study inter- or even intraspecific variations (for example, Lloyd-MacGilp et al. 1996, Antoniolli et al. 2000). In contrast, studies of the SSU deal mainly with higher taxonomic units, although they can be used even at the species level (Helgason et al. 1998, 1999; Daniell et al. 2001; Schwarzott et al. 2001; Schüßler et al. 2001). Once the chosen target region is amplified by simple or nested PCR, various approaches, reviewed below, can be used to study its polymorphism.

Restriction Fragment Length Polymorphism of the Internal Transcribed Spacer (ITS-RFLP)

RFLP of the ITS region is a powerful tool for characterizing the fungal partners of ectomycorrhizal symbiosis (Horton and Bruns 2001). In the Glomeromycota, the intraspecific genetic variability of the rDNA and the multinucleate state complicate its use, because even one single spore may display several restriction patterns (Figure 10.7). Therefore, it is necessary to test a large number of enzymes on several PCR products of one "species" from pure cultures to understand the variability of its ITS before being able to monitor this species in the field. This limitation makes this method less interesting, apart from the fact that the more common types of AMF in the field might not be detectable in trap cultures.

Terminal Restriction Fragment Length Polymorphism (T-RFLP)

T-RFLP is based on a PCR in which one of the primers is labeled fluorescently. The PCR products obtained are digested with one single restriction enzyme or a set of restriction enzymes. The restriction bands obtained at the labeled end of the amplification product are separated on a polyacrylamide gel and detected by laser-induced fluorescence (LIF). Initial results using this technique in AMF have been published by Tonin et al. (2001).

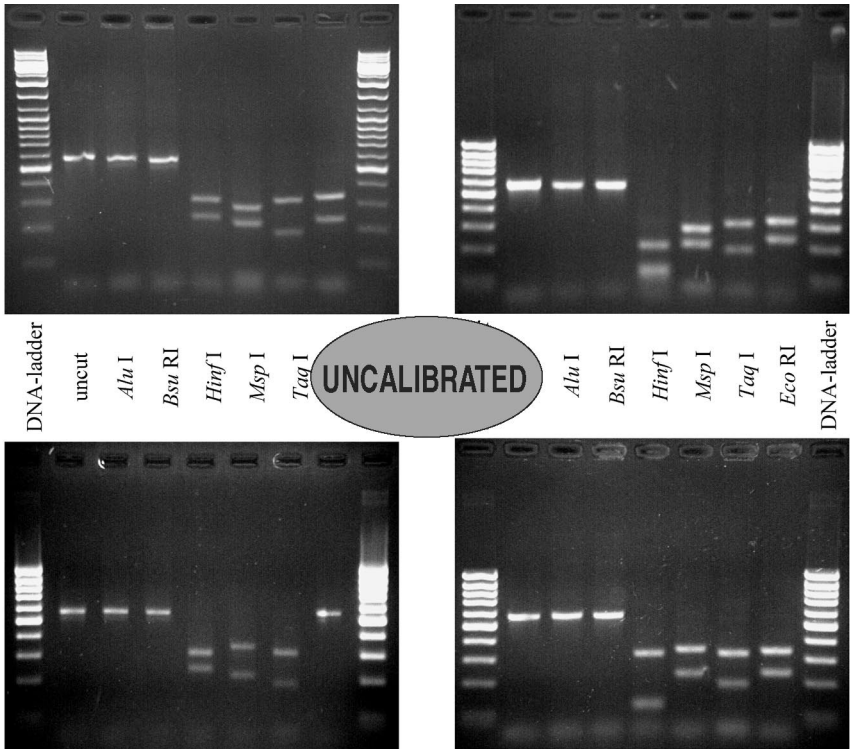

Figure 10.7 Four different PCR-RFLP patterns of maize roots inoculated with *Glomus intraradices* BEG 140 from one pot culture, illustrating the intraspecific variability of AMF. PCR products of the ITS were amplified with the primer pair ITS4/ITS5. Restriction fragments were separated on 2 percent agarose gels. Lanes 1 and 9 Gene Ruler DNA Ladder Mix.

Polymerase Chain Reaction–Single-Strand Conformation Polymorphism (PCR-SSCP)

PCR-SSCP is a reliable method for rapid and efficient detection of mutations and polymorphisms in genomic sequences. Target sequences are separated by polyacrylamide gel electrophoresis into a single-stranded state. Mutations are detected as mobility shifts resulting from changes in the conformation of single-stranded amplification products (Makino et al. 1992). This method has proved to be helpful in studies of genetic diversity for broad screening of different sequence types (Kjøller and Rosendahl 2000, 2001). However, as mentioned above, small changes in the genome are common in Glomeromycota, which might result in different patterns within the same

"species." Therefore, this time-consuming method is not adequate for a broad screening of AMF in the field.

Denaturing Gradient Gel Electrophoresis (DGGE)

DGGE is a further development of the SSCP technique and is based on mobility differences of DNA fragments in a polyacrylamide gel, which result from both the base composition and the size of the product. DGGE polyacrylamide gels contain a gradient of a denaturant (usually formamide and urea). Double-stranded DNA molecules that migrate through the gradient gel become single-stranded at a position (that is, a denaturant concentration) that corresponds to their melting points, depending on the GC content of the molecules. When this point is reached, denaturation results in a sharp reduction of mobility. The fragment virtually "stops."

One disadvantage of these analyzing methods is that the different banding patterns they produce do not allow us to identify the detected species nor to assess the part of polymorphism resulting from intraspecific variations. Therefore, a prerequisite to investigations with these methods is a thoroughly determination of the species and their intraspecific variation by sequencing the used DNA target region. Another residual problem, which is omnipresent in soil microbiology, is the inability to exclude PCR products of nontarget species, which may greatly increase the number of different banding patterns.

Sequence Analysis

An important step for reaching the species level in molecular biological studies is the sequence analysis. In PCR products of AMF, each band may correspond to products with divergent sequences; resulting, for example, from the intraspecific polymorphism of DNA target regions. This makes it necessary to clone PCR products and to sequence a minimum number of clones systematically in preliminary work. SSCP was used in several recent studies to detect different patterns in PCR products. This allows one to reduce the number of samples to be sequenced (Kjøller and Rosendahl 2000, 2001, Clapp et al. 2001).

In recent years, a powerful tool has been established to analyze sequences: big sequence databases, such as GenBank and EMBL, with rapidly increasing amounts of sequence data sets for different fungal groups. Sequences of ITS and SSU rDNA of about one-third of the described Glomeromycota are already available. Using the databases in combination with programs such as BLAST (http://www.ncbi.nlm.nih.gov/; Altschul et al.

1997), the approximate taxonomic position of AMF can be determined on the basis of newly obtained sequences.

A large problem in using these databases is the uncertainty of the given species names, because the original determination of a taxon for which a sequence was deposed could be based on weak morphological observations. Another problem is misidentified sequence data belonging to contaminating fungi. An evaluation of the databases is urgently needed to remove such erroneous data, but this seems unlikely in view of the amount of new sequence data deposed daily. However, phylogenetic analysis including sequences of possible contaminants should be performed to identify an organism based on its sequence (Schüßler 1999).

In addition to these problems, the taxon sampling and sequencing must be completed to increase the chance of proper identification. To reach this goal, the available strains in the various culture collections (BEG—La Banque Européene des Glomeromycota/European Bank of Glomeromycota, with 142 registered isolates; INVAM—International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi, with reference cultures of 77 species from all glomalean genera; GINCO—Glomeromycota In Vitro Collection, with isolates of 8 species available) should be screened completely. Alignments of sequence data should also be made available in Treebase (http://www.herbaria.harvard.edu/treebase/) or EMBL-Align (Multiple Sequence Alignment Database of European Molecular Biology Laboratory, http://www.ebi.ac.uk/embl/Submission/alignment.html) to allow fast access to such data sets for further studies.

Future Perspectives for the Use of Molecular Tools

A combination of molecular biology methods may be the most promising way to monitor the community structure and biodiversity of AMF in the field. Sequencing PCR products derived from nested PCR with mycorrhizal roots and also from products on spores will help bring new insights into "species" diversity. T-RFLP or DGGE will help to increase the number of analyzed samples, once a large number of roots and spores have been checked. Many of the gathered banding patterns should be addressable by the sequence data; then only samples of unknown origin detected at this level would have to be sequenced to determine their taxonomic status. This procedure will lead to an overview of biodiversity (sequencing) and community structure (T-RFLP/DGGE). For ecological studies in the future, development of microarrays or microchips might be very helpful to reduce the time needed to monitor AMF in field. By hybridization of given DNA

fragments on the arrays or chips with amplified PCR products from the field, a fast and proper identification should be possible for large numbers of samples. Before reaching this stage in ecological studies, however, much work remains to be done. For example, all the unknown molecular strains should be sequenced to assess whether the commonly estimated diversity of only 150 to 200 AMF species worldwide is a reality, since it is based on the original classification system that used weak morphological characteristics. This work is an important prerequisite to systematic analysis of relationships between AMF diversity and plant communities.

Back to the Roots

Molecular AMF community studies of seminatural forests and arable fields in the United Kingdom that were based on sequencing of the 18S rRNA gene of Glomeromycota have revealed at least 18 AMF sequence groups detected from five natural and four crop plant species (Clapp et al. 1995; Helgason et al. 1998, 1999; Daniell et al. 2001) (Table 10.1). Only six of these taxa have shown close sequence similarity with reference species (*Glomus mosseae*, *G. geosporum*, *G. intraradices*, *Acaulospora scrobiculata* Trappe, *A. rugosa* J.B. Morton, *Scutellospora dipurpurescens* J.B. Morton and Koske). The taxonomic identity of the remaining fungi has not been established so far but awaits more extensive spore surveillance and trap-culturing studies in natural systems. Interestingly, Öpik et al. (unpublished data) were able to detect six of these sequence types in trap seedlings and established native plants of two *Pulsatilla* spp. in boreal forest and grassland ecosystems in Estonia (see Table 10.1). Four other sequence types detected by these researchers, including the dominating sequence in natural plant roots at four sites, apparently represent new AMF sequence types (taxa).

Some ecological specificity of AMF is apparent from these data, since only 3 of 18 sequence types from natural plant roots have been detected in both arable and woodland systems; namely, *G. intraradices*, *A. scrobiculata*, and *S. dipurpurescens*, 5 from arable fields and 10 from woodland only. Five sequences by Öpik et al. (unpublished data) represent woodland sequences and one, *G. intraradices*, is an arable land sequence detected from 2-month-old bait seedlings.

In addition to differences in AMF colonization patterns among plant species, temporal and spatial changes obviously occur in AMF community composition and species frequencies within an ecosystem type (Helgason et al. 1999, Daniell et al. 2001).

TABLE 10.1

AMF Taxa Detected by Two Research Groups in Different Ecosystems in the United Kingdom (UK) and Estonia (EST)[1]

AMF sequence group	Closest relative	Seminatural woodlands (UK)[2]	Arable fields (UK)[3]	Boreal forests and meadows (EST)[4]
Acau1	*A. scrobiculata*	+	+	
Acau2[5]/MO-A1[6]	*A. rugosa*	+		+
Acau3		+		
Acau4		+		
Acau5		+		
Glo1A	*G. geosporum*		+	
Glo1B	*G. mosseae*		+	
Glo2/MO-G5		+		+
Glo3/MO-G2		+		+
Glo4			+	
Glo5		+		
Glo6				
Glo7/MO-G6		+		+
Glo8/MO-G3	*G. intraradices*	+	+	+
Glo9/MO-G7		+		+
Glo10			+	
Glo11		+		
MO-G1				+
MO-G4				+
MO-G8				+
MO-G9				+
Scut1	*S. dipurpurescens*	+	+	

[1]*A. Acaulospora, G. Glomus, S. Scutellospora.*
[2]Data from Helgason et al. (1998, 1999).
[3]Data from Helgason et al. (1998), Daniell et al. (2001).
[4]Data from Öpik et al. (unpublished).
[5]Sequence group designations after Helgason et al. (1998, 1999), Daniell et al. (2001).
[6]Sequence group designations after Öpik et al. (unpublished data).

Another approach, using taxon-specific nested PCR of the 25S rRNA gene combined with root staining, has been used recently to detect and quantify AMF species *in planta* found as spores in the vicinity of roots of *Fragaria vesca* L. in a zinc waste site in Poland (Turnau et al. 2001). Five AMF taxa were identified—*Archaeospora gerdemannii* (S.L. Rose, B.A. Daniels and Trappe) J.B. Morton and D. Redecker, *Glomus mosseae*, *G. intraradices*, *G. claroideum*, and *Paraglomus occultum* (C. Walker) J.B. Morton and D. Redecker—the first of them being the most efficient colonizer. However, 12 percent of colonized roots contained fungi other than the morphologically recognized species. It is worth noting that the *Archaeospora* and *Paraglomus* sequences are probably not detectable with the primers used by these researchers.

Conclusions

Many questions still remain regarding the impact of arbuscular mycorrhizal fungi (AMF) on assembly in ecosystems and on the structure, dynamics, and restoration of plant communities. Nevertheless, promising results have been obtained recently by combining classical ecology, morphology, and molecular biology in field studies. Especially in the field of restoration ecology, successful applications have been conducted, promoting acceptance of the positive influence of AMF on plant establishment and growth on degraded soils. In addition, the influence of AMF on plant community structure, one of the most important questions, has been addressed by several field studies. Further investigation is needed to determine the importance of AMF more precisely.

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