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How Do Composition, Structure, and Function of Mycorrhizal Fungal Communities Respond to Nitrogen Deposition and Ozone Exposure?

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39.1 INTRODUCTION

This review will focus specifically on how mycorrhizal fungal (MF) communities are structured by, and function under, air pollution. In order to understand the impacts of air pollution on MF communities, and the functional significance of those impacts, we must consider both the empirical evidence for air pollution effects on those communities and the mechanisms by which fungal communities are likely to be affected. By understanding these mechanisms, we should also gain some insight into the potential functional consequences of community change.

Human effects on atmospheric chemistry have been dramatic, including increases in chemical oxidants (e.g., SO_2, NO_2, O_3), nutrient and acidifying compounds (e.g., SO_4^{2-}, NO_3^-, NH_y), and greenhouse gases (e.g., CO_2, CH_4, N_2O) driven largely by increased industrial and agricultural activity (Schlesinger, 1997). For example, anthropogenic N fixation has led to a doubling of terrestrial N fixation, and greatly elevated levels of N deposition (Galloway and Cowling, 2002); average tropospheric ozone (O_3) levels have increased from preindustrial levels of <20 ppb (Mickley et al., 2001) to current average levels of over 50 ppb, with high variability leading to regional levels of over 100 ppb (Ehhalt et al., 2001); and atmospheric CO_2 has increased over 30% compared with preindustrial levels (Houghton et al., 2001). The increases in these compounds have altered ecosystem structure and function in a variety of ways. Most relevant to the current chapter,

there are indications that diversity and community structure of mycorrhizal fungi have been affected by pollutants. Much less clear is what the exact causal mechanisms and functional consequences of these changes are.

This chapter will focus largely on the effects of atmospheric N and chemical oxidants, especially O_3, on mycorrhizal fungal communities. Along with CO_2, these are the pollutant classes with the most ubiquitous effects on ecosystems. The effect of changes in atmospheric CO_2 concentrations on mycorrhizal fungal communities is addressed elsewhere in this volume (Chapter 36), so we will address CO_2 primarily in terms of possible interaction with reactive N and O_3. We will attempt to answer the following questions: What are the potential pathways of pollutant effects on mycorrhizal fungi? Is there any evidence that mycorrhizal fungal communities are affected by air pollution? What are the potential functional consequences of mycorrhizal fungal community change? Where are our knowledge gaps and how might they be filled? It is hoped that this chapter will complement other excellent reviews of pollutant effects (e.g., Dighton and Jansen, 1991; Wallenda and Kottke, 1998; Erland and Taylor, 2002; Andersen, 2003).

39.2 POTENTIAL PATHWAYS OF POLLUTANT EFFECTS

In order to understand how air pollution might affect mycorrhizal fungi, the first point to consider is how the unique traits of mycorrhizal fungi define pathways of pollutant exposure. One of the properties of filamentous fungi that distinguishes them from most other sessile soil microorganisms is their ability to capture and use spatially discrete resources. The importance of this trait is obvious in the mycorrhizal fungi. By symbiotically bridging the root and soil environments, they are able to gain access to two valuable resource pools: root carbohydrates and soil resources. Although the advantages of this habit are made clear by mycorrhizal fungal diversity (Horton and Bruns, 2001) and dominance in many soils (e.g., Wallander et al., 2001), a disadvantage is that mycorrhizal fungi are vulnerable to environmental factors that alter resources and conditions in the host and soil environment.

Reactive nitrogen (NO_y, NH_x) and acidifying and oxidant air pollution are prime examples of such environmental factors, having the potential to affect mycorrhizal fungi via alteration of both host and soil environments (Figure 39.1). The likely pathway of these effects will differ depending on the mode of pollutant action.

39.2.1 Chemical Oxidants

We will focus on ozone (O_3) as the best-studied oxidant, although SO_2 is another environmentally important oxidant. At sufficiently high levels, O_3 damages leaves, reducing photosynthetic C gain, increasing leaf metabolic costs, and possibly reducing phloem loading (Andersen, 2003 and references therein). As a result, with sufficient doses O_3 often leads to reduced host C flux to roots and mycorrhizae (Andersen, 2003; Figure 39.1). Thus, initial effects on mycorrhizal fungi are likely to be via changes in C availability and perhaps host receptivity, although it is possible that longer-term effects could occur via indirect effects of oxidants on plant nutrition arising from reduced nutrient uptake, or via alteration of soils resulting from changes in quality and quantity of organic matter inputs (Figure 39.1).

39.2.2 N Deposition

In contrast to oxidants, N deposition has the potential to affect mycorrhizal fungi both via alterations in soils and consequent effects on soil resources and conditions experienced by

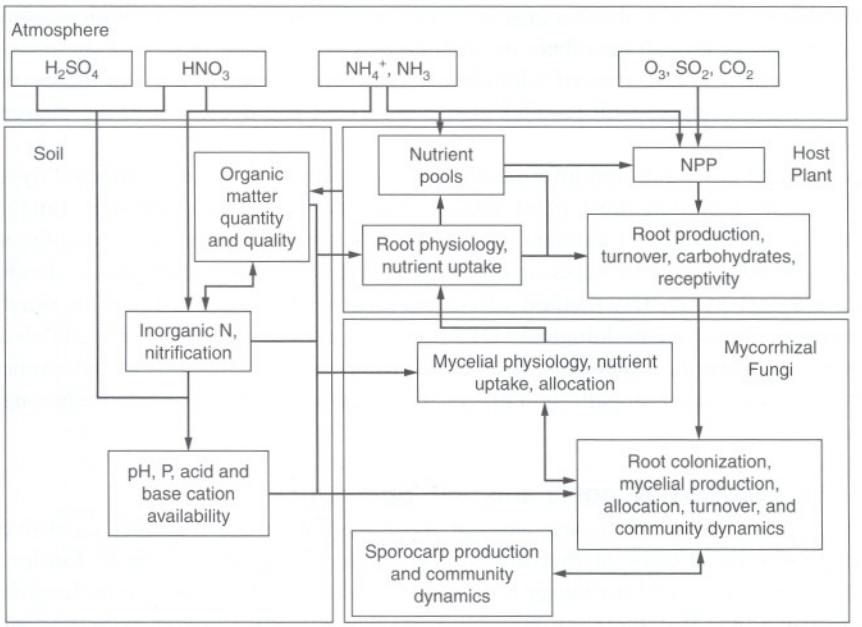

Figure 39.1 Conceptual diagram of the potential pathways of air pollution effects on mycorrhizal fungal communities.

MF (inorganic N availability, soil pH, base cation and P availability, acid cation availability, especially Al^{3+}) (Aber et al., 1998) and via alteration of host plant nutrition and the consequent effects on host C supply to roots and host receptivity to fungi (Figure 39.1).

It is important to know whether pollution-related changes in mycorrhizal fungal communities are driven largely by plant choice of ectomycorrhizal fungi (EMF), by fungal ability to colonize roots, or by a changing abiotic environment, because these mechanisms could lead to very different functional outcomes. Before we address these alternatives, we will consider the empirical evidence for mycorrhizal fungal community change.

39.3 EVIDENCE OF MYCORRHIZAL FUNGAL COMMUNITY CHANGE IN RESPONSE TO AIR POLLUTION

39.3.1 Variety of Study Types and Conditions

There are a range of approaches to investigating pollution effects on mycorrhizal fungi. The range of approaches varies with the type of pollutant. For all pollutants, experimental and gradient approaches have been used. The experimental approaches generally provide a higher degree of control but less realism. The gradient approach varies from large-scale regional gradients that usually are associated with variation not only in pollutants, but also in a range of other environmental variables, to more local gradients associated with point sources of air pollution, where extraneous environmental variables are held relatively constant.

For simulation of nutrient or acidity effects, the experimental approaches involve additions of nutrients in dry or liquid form. The timing of additions varies hugely, with some studies applying nutrients in one pulse in the first year only, one addition per year, or multiple additions per year, with the last most closely mimicking atmospheric deposition. Experimental settings include field plots, greenhouses, and growth chambers.

For oxidants there are also a range of exposure systems, with an evolution from studies carried out in growth chambers or greenhouses, to the field in closed chambers or open-topped chambers, to free-air addition of gases. The degree of realism increases with each of these changes, with parallel increases in cost and decreases in the degree of control.

Experimental outcomes and information derived from studies can be affected by a variety of factors, including host plant species and genotypes, host age/size, fungal inocula, variation in baseline or ambient pollution levels, amount and timing of additions or exposures, length of time of experimental exposures/treatments, soil physical, chemical, and biological properties, method of fungal identification, and a range of other factors. Given all of the methodological variability, it is not surprising that there is also variability in experimental outcomes. In the following, we will attempt to determine whether there is any consistent pattern in mycorrhizal fungal community response arising from these studies.

39.3.2 Field Studies: N Inputs and EMF Sporocarps

For ectomycorrhizal fungi, sporocarps are our most spatially and temporally extensive window into pollution effects on mycorrhizal fungi. This is especially true in Europe, where a long tradition of field mycology has resulted in some excellent long-term records of fungal distributions. It is therefore not surprising that some of the first evidence that ectomycorrhizal fungal communities might be negatively affected by air pollution came from longitudinal studies and spatial patterns of fungal sporocarps in Europe. These studies, summarized in Arnolds (1991), indicated that a broad range of ectomycorrhizal fungal species were decreasing in abundance or disappearing over large regions of Europe exposed to a broad range of pollutants. In contrast, no such declines were seen in saprophytic fungi. This suggested that some unique aspect of the mycorrhizal habit was leading to such declines. One interesting feature of these declines is that certain genera of ectomycorrhizal fungi appeared to be more affected than others (Figure 39.2).

Arnolds (1991) suggested multiple possible causal mechanisms for the decline. These included natural factors, sporocarp collection, forest management, and air pollution. Of these, he considered air pollution to be a likely causal agent. In particular, the patterns of sporocarp decline were most consistent with the effects of nitrogen fertilization. Fertilization experiments and deposition gradient studies demonstrated that additions of N can lead to changes in sporocarp production that paralleled those seen over time in Europe (Wallenda and Kottke, 1998 and references therein; Lilleskov et al., 2001; Peter et al., 2001). A larger-scale complex pollutant gradient study suggested that SO_2 and NH_3 were both negatively correlated with EMF species richness (Termorshuizen and Schaffers, 1987). A more localized N deposition gradient study also found very strong negative relationships between a broad range of N availability measures and sporocarp diversity (Figure 39.3; Lilleskov et al., 2001). Fertilization studies (Menge and Grand, 1978; Wästerlund, 1982; Termorshuizen, 1993; Wiklund et al., 1995; Brandrud and Timmerman, 1998; Peter et al., 2001) also found reduced species diversity and sporocarp production with elevated N inputs. These declines were not equal across taxa, with certain taxa declining in abundance and diversity more rapidly, including *Cortinarius* (Wästerlund, 1982; Termorshuizen, 1993; Brandrud, 1995; Brandrud and Timmermann, 1998; Lilleskov et al., 2001), *Russula* (Brandrud, 1995; Lilleskov et al., 2001), and *Suillus* (Menge and Grand, 1978; Wästerlund, 1982). Several other taxa appear to continue to fruit at higher deposition levels, including *Lactarius rufus* and *Paxillus involutus* (Hora, 1959; Laiho, 1970; Ohenoja, 1978; Salo, 1979; Wästerlund, 1982; Brandrud, 1995), *Lactarius theiogalus* (Brandrud,

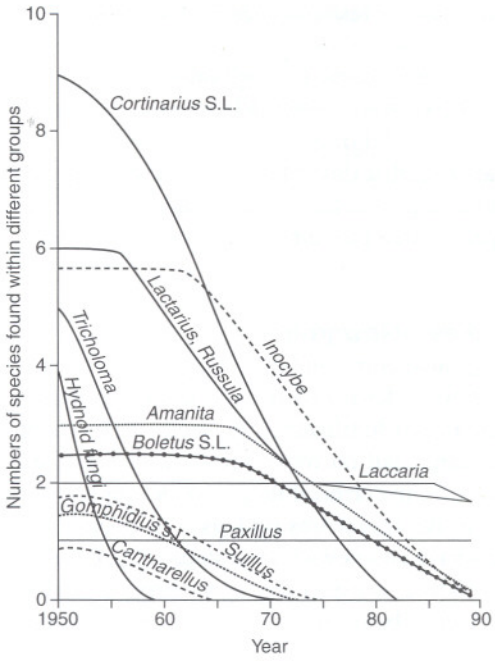

Figure 39.2 Temporal trends in species richness for sporocarp collections from different genera of ectomycorrhizal fungi over time in Europe. (From Arnolds, *Agriculture Ecosystems and Environment*, 35, 209–244, 1991. With permission.)

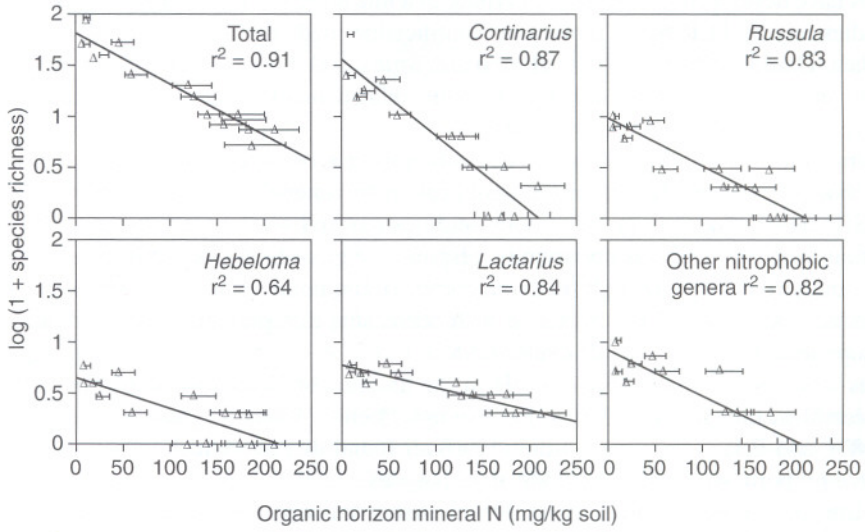

Figure 39.3 Soil inorganic N extractable pools as a predictor of sporocarp species richness for all species, and for the most species-rich genera over a nitrogen deposition gradient in Alaska. (From Lilleskov et al., *Ecological Applications* 11:397–410, 2001. With permission.)

1995; Lilleskov et al., 2001), and *Laccaria* species (Termorshuizen, 1993; Lilleskov et al., 2001).

The exact pattern of response to fertilization seems to vary among studies, with some studies showing declines in fruiting of all taxa, some showing increases in apparently nitrophilic taxa, and others showing initial increases followed by declines. Lilleskov et al. (2001) discuss this pattern in detail, hypothesizing that this variation might be a function of how quickly fertilization shifts forests into a eutrophic state, as a function of the natural N status of the site, land use history, tree species, tree age, amount of N deposited on the site, and size of the initial fertilization.

39.3.3 N Inputs and EMF Communities Belowground

Sporocarp production does not reflect belowground communities directly: first, fungi can reduce allocation to sporocarps in response to N fertilization, without any change in community structure; second, even in the absence of fertilization, ectomycorrhizal fungal species are not equally represented as sporocarps and on roots, presumably because of differences in allocation to sporocarps. Not all ectomycorrhizal fungi produce conspicuous epigeous sporocarps. Some produce thin crust-like sporocarps on logs or leaf litter (e.g., Thelephoraceae and Corticiaceae), and others have no known sexual stage (e.g., *Cenococcum geophilum*). Of those fungi that do produce conspicuous sporocarps, a species' sporocarp production does not necessarily reflect its belowground abundance (e.g., Gardes and Bruns, 1996).

The lack of direct correspondence between communities described via sporocarps and on root tips necessitates the use of belowground studies to characterize the EMF community response. This is more challenging, because identification of fungi on roots is harder, and communities exhibit high diversity and spatial variability. Until the advent of molecular methods, most studies were limited to morphological typing, or morphotyping. This morphotype information is not easily comparable among studies, and morphotyping can lead to false lumping and splitting of taxa (Mehmann et al., 1995). Although some labs have developed morphological typing to a fine art (e.g., Agerer, 1991), currently most studies rely on PCR-based molecular identification methods to obtain reliable community data (Gardes and Bruns, 1996; Horton and Bruns, 2001). These methods are mostly applied to individual root tips, although recently related methods are being applied to fungal hyphae in the soil (Dickie et al., 2002).

Many earlier pot studies of acidic deposition effects on mycorrhizal fungi usually included both HNO_3 and H_2SO_4 in the acidification treatments, although some studies used H_2SO_4 alone. The former were both acidification and fertilization studies. Some studies found no mycorrhizal community response (e.g., Adams and O'Neill, 1991), whereas others found apparent shifts in mycorrhizal community structure. Meier et al. (1989) found that *C. geophilum* increased with decreasing rain pH, an observation anecdotally supported by Stroo and Alexander (1985).

It is worth noting that change in pH in the absence of N addition can apparently alter mycorrhizal fungal communities. For example, Lehto (1994), Dighton and Skeffington (1987), and Erland and Soderstrom (1990) all found that alterations in pH affected the relative proportions of different EMF morphotypes.

Belowground field studies have found that even when sporocarp production responses are rapid and dramatic, short-term mycorrhizal fungal community responses at the root level are minimal. Most short-term studies have shown little or no change belowground (Saunders et al., 1996; Brandrud and Timmermann, 1998; Jonsson et al., 2000; M.F. Allen, personal communication). Two studies (Kårén and Nylund, 1997; Peter et al., 2001) found a belowground response, but it was much less pronounced than the sporocarp

Table 39.1 A Comparison of Response to Atmospheric N Deposition or N Fertilization, and Growth on Protein N in Pure Culture, for Ectomycorrhizal Fungal Taxa for Which Information Is Available

Taxon	Response to N Addition[a]	Reference[b]	Growth on Protein N	Reference[b]
Lactarius theiogalus	+ + +	1, 3	No	9
Paxillus involutus	+ + +	1, 2	Variable	10–13, 18, 20
Lactarius rufus	+ + +	2, 16	Variable	9–12, 18
Laccaria bicolor	+ +[d]	3–5	No–poor	9, 10, 19
Thelephora terrestris	+	1, 2	Variable	10
Tylospora fibrillosa	= / +	1, 2	Variable	13, 18
Cenococcum geophilum	– / =	1, 2	Variable	9, 11, 14, 18
Russula spp.	– / +	3, 6, 7, 15, 17	Yes (1)[c]	18
Cortinarius spp.	– –	1–3, 5	Yes (6)	9, 18
Piloderma croceum group	– –	1, 2	Yes	10, 20
Tricholoma inamoenum	– –	1, 3	Yes	9
Suillus variegatus	– –	2	Yes	10
Suillus bovinus	– –[d]	8	Yes	11, 20

Note: Taxa are ordered by their response to increased N.

[a] For response to fertilization: +, slightly positive; + +, positive; + + +, very positive; =, neutral; –, negative response to fertilization; – –, very negative.

[b] References: 1, Lilleskov et al. (2002a); 2, Kårén (1997); 3, Lilleskov et al. (2001); 4, Ohenoja (1989); 5, Sagara (1992); 6, Brandrud (1995); 7, Shubin et al. (1977); 8, Wästerlund (1982); 9, Lilleskov et al (2002b); 10, Finlay et al. (1992); 11, Abuzinadah and Read (1986); 12, Keller (1996); 13, Ryan and Alexander (1992); 14, El Badaoui and Botton (1989); 15 Avis et al. (2003); 16, Wallenda and Kottke (1998, and references therein); 17, Peter et al. (2001); 18, Taylor et al. (2000); 19, Yamanaka (1999); 20, Bending and Read (1996).

[c] Number in parentheses indicates number of species in genus tested.

[d] Information available from studies of sporocarps only.

response. Whereas for sporocarps there was a significant decline in species richness and diversity, belowground there was no loss of richness or diversity in response to the treatments. However, they did observe shifts in community structure. Kårén and Nylund (1997) found an increase in a brown morphotype that included *Tylospora fibrillosa*, *Lactarius theiogalus*, and other taxa. Peter et al. (2001) found that certain taxa declined in frequency in response to N inputs (e.g., *Russula* spp.), whereas others did not (e.g., *Tylospora asterophora*).

In contrast, studies of the effects of long-term N inputs indicate that long-term effects can be much larger. In both a fertilization study (Kårén, 1997) and an N deposition gradient study (Lilleskov et al., 2002a), high N inputs were associated with strikingly similar changes in belowground ectomycorrhizal fungal communities, resulting in the loss of many ectomycorrhizal fungal taxa and a shift in the dominants (Table 39.1, Figure 39.4). Similarly, along a north–south transect in Europe in which N deposition increased toward the south, Taylor et al. (2000) found a negative relationship between morphotype richness and soil inorganic N in spruce stands and decline in some of the same taxa as in the smaller-scale studies. Although the latter must be viewed with caution

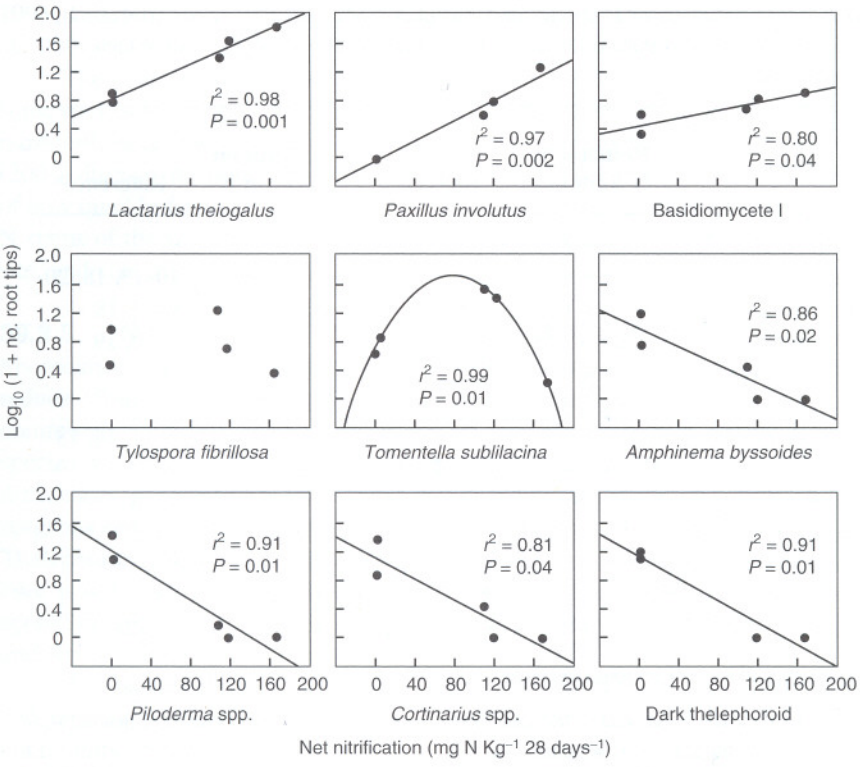

Figure 39.4 Regressions of percentage of white spruce root tips (log transformed) vs. net nitrification for individual ectomycorrhizal fungal taxa over an atmospheric nitrogen deposition gradient near Kenai, AK. (From Lilleskov et al., *Ecology,* 83:104–115, 2002a. With permission.)

given the other parameters varying along the gradient, its consistency with the fertilization and small-scale gradient results lends support to the claim that N deposition is driving belowground EMF community changes across Europe. Taxa that appeared to decline in these studies include *Cortinarius* (Kårén, 1997; Taylor et al., 2000; Lilleskov et al., 2002a), *Piloderma* (Kårén, 1997; Lilleskov et al., 2002a), *Suillus* (Kårén, 1997), many *Tomentella* species (Lilleskov et al., 2002a), *Tricholoma inamoenum*, and *Amphinema byssoides* (Lilleskov et al., 2002a). Taxa that appeared to increase or be relatively unaffected were *Tylospora fibrillosa* (Kårén, 1997; Taylor et al., 2000; Lilleskov et al., 2002a), *Lactarius rufus* (Kårén, 1997), *Lactarius theiogalus*, *Paxillus involutus*, *Tomentella sublilacina*, *Thelephora terrestris*, and an unidentified Corticioid (Lilleskov et al., 2002a). Thus, it appears that lags in the belowground response to N deposition are greater than for the sporocarp response, but long-term inputs are sufficient to cause significant declines in diversity and complete shifts in dominants, at least in boreal conifer forests.

Arnolds (1991) reported that diversity of sporocarps had declined more for conifer-associated than for deciduous-associated ectomycorrhizal fungal taxa. Is this also true belowground? Taylor et al. (2000) also examined beech stands and, in contrast with spruce stands, found a weak positive relationship between morphotype richness and soil inorganic N. In contrast, Avis et al. (2003), examining experimental plots in oak savannah in Minnesota that had been fertilized with complete fertilizer plus moderate or high N for

15 years, found clear declines in EMF sporocarp species richness and shifts in belowground community structure, with declines in *Cortinarius* and increases in *Russula*, but of a smaller magnitude than the shifts found in the long-term conifer studies. The smaller belowground community change compared with long-term conifer fertilization could be due to several factors: different host species, relatively low fertilizer inputs (maximum of 17 kg ha^{-1} $year^{-1}$ above ambient), a base level of complete fertilizer addition that could ameliorate some of the N effects, shorter period (15 vs. >25 years), or other factors such as soil characteristics and climate. These results clearly point to the need for a much more extensive study to determine the generality of mycorrhizal responses to long-term N inputs in different forest types.

The N-induced change in sporocarp production could feed back to affect belowground community composition if certain species require spore inputs to persist in the community. For example, one might expect that species colonizing mature forest stands would be effective at vegetative spread, but it appears that some *Russula* species from mature forests actually have numerous small genets, suggesting either that genets expand very slowly or that continued colonization by spores may be important (Redecker et al., 2001). Given that *Russula* sporocarp production declines with increasing N inputs (Mehmann et al., 1995; Wallenda and Kottke, 1998; Lilleskov et al., 2001; Peter et al., 2001; but see Avis et al., 2003 for an exception), this raises the possibility that reduced spore inoculum may be one mechanism leading to belowground decline of *Russula*. This effect might be weaker in a small-scale fertilization experiment than in a larger-scale regional decline in sporocarp production driven by N deposition because, in the former, local inoculum sources would persist in areas surrounding plots. Thus, small-scale studies might underestimate N deposition effects.

39.3.4 Arbuscular Mycorrhizal Fungi and N Deposition

In contrast with EMF, arbuscular mycorrhizal fungi (AMF) do not produce conspicuous epigeous sporocarps, so there is no long-term record of sporocarp production with which to compare experimental studies. In AMF, much of the information on community composition and structure is from asexual spores collected from the soil. This information has similar constraints to the EMF sporocarp information; i.e., spore abundance does not directly reflect fungal abundance in roots and soil. Molecular identifications, while attainable, are harder to derive from AM roots, because of lower quantities of DNA, and species mixtures in root sections.

There have been a few studies of AMF response to N deposition in coastal sage scrub ecosystems of California, and these suggest that there are significant community changes in AMF communities in response to N inputs. These trends have been indicated by studies of anthropogenic gradients and fertilization experiments (Egerton-Warburton and Allen, 2000) and longitudinal studies of archived soils (Egerton-Warburton et al., 2001). These studies provide multiple lines of evidence that with increasing N availability, there is a corresponding decline in diversity, driven largely by decline or disappearance of the large-spored genera (*Scutellospora*, *Acaulospora*, and *Gigaspora*) compared with smaller-spored *Glomus* species. As in EMF, AMF were more likely to be detected as hyphae in soil than as spores (Egerton-Warburton and Allen, 2000), suggesting that N effects are seen first in spore production for AMF as well.

It is unknown how general this response would be across AMF communities. One might expect a greater response to N inputs in N-limited ecosystems. The coastal sage scrub ecosystem appears to have low natural N availability (Egerton-Warburton and Allen, 2000), perhaps because of frequent fires. Thus, it is likely that these ecosystems are nitrogen

limited. Studies of effects of N fertilization alone on AMF community composition and structure have not been done in communities known to be P limited.

39.3.5 Ericoid Mycorrhizal Fungi and N Deposition

Plants in the Ericaceae typically live in N-limited, organic rich soils (Read, 1991). Some species in this family are declining in areas subject to high N deposition (Bobbink et al., 1998), and Ericaceae can be sensitive to N fertilization (e.g., Prescott et al., 1993). Ericoid mycorrhizal fungi (ErMF) are apparently more capable than other MF at accessing complex organic compounds, supplying otherwise unavailable nutrients to their hosts (Read, 1996; Cairney and Burke, 1998). As a result, one might expect ErMF to be the most sensitive to elevated N inputs. Short-term field fertilization studies of percent colonization have had varying results, with no negative effect in some (Caporn et al., 1995; Johansson, 2000) and ErMF declining in another (Yesmin et al., 1996). A gradient study suggested increasing colonization up to a threshold and then decline (Yesmin et al., 1996). There is no information on community response to elevated N, as ErMF species are difficult to distinguish morphologically, and only recently have the methods of community identification been developed and applied to them (e.g., Allen et al., 2003).

39.3.6 O_3 Effects on Mycorrhizal Community Structure

Although there have been a number of studies on O_3 or other oxidant effects on mycorrhizal fungi, for a variety of reasons they provide limited information on mycorrhizal fungal community responses. First, most studies were limited to examination of mycorrhizal colonization, without regard to the composition of the community. Second, many studies have been pot experiments. These experiments usually lack the full complement of mycorrhizal inoculum, resulting in low colonization percentages compared with natural settings. Third, most studies were carried out using morphotyping with the associated limitations described above. Therefore, interpretation of community response from these studies must be done with caution, and comparisons among studies are quite difficult. As a result, it is impossible to say whether there are any consistent taxonomic responses to increased O_3 exposure. Another difficulty lies with the spatial and temporal scales of exposure, and developmental stage of the plants. Most studies have been done in pots on seedlings, with experiments typically lasting from weeks to months, and only rarely for more than one growing season. The paucity of large-scale, long-term treatments is not surprising, because of the expense and difficulty of such studies. However, given the apparent temporal lags seen in community response to N fertilization (see above), long-term studies are essential to determine mycorrhizal fungal community response to O_3 or other oxidants.

When we add these limitations to the other variables affecting the outcome of experiments noted earlier, it is not surprising that we have limited community response information and find high variability among studies in the effect of O_3 on mycorrhizal fungal infection. Although many studies found decreases in mycorrhizal infection with increasing O_3 exposure, others found no change or increases (Table 39.2). Two factors that seem especially likely to affect mycorrhizal response to the treatments are O_3 concentration and exposure duration. The combination of these, or total dose, appears to be a good predictor of likely response (Väre et al., 1993). Most of the studies that found neutral or positive mycorrhizal responses to O_3 were found in short-term or relatively low-dose experiments. Consistent with this finding, when plants are exposed to a range of concentrations from subambient to ambient to superambient, quadratic response curves can occur (e.g., Stroo et al., 1988), with peak mycorrhizal colonization at or near ambient levels.

Andersen (2003) summarized the hypothesized causes of stimulation of mycorrhizal colonization with relatively short-term low doses. These include mobilization of stored

Table 39.2 Summary of Selected Studies of O_3 Effects on Mycorrhizal Fungal Colonization and Communities

Host/Fumigation System/Experiment Duration	O_3 Treatment: Concentration, Exposure, Timing	SO_2 Treatment	N Treatment	Acidity Treatment (pH)	CO_2	Mycorrhizal Treatment or ID Method	Mycorrhizal Response: Mycorrhizas (n), % Colonization (%), Mycorrhizas cm^{-1} (n/cm)	Community Structure: Mycorrhizas (n), % Colonization (%), Mycorrhizas cm^{-1} (n/cm)	Reference
Quercus rubra seedlings/growth chambers/52 days	20, 70, 120 ppb		SO_4^{-2}:NO_3^- 2:1 (mass ratio)	3, 4, 5		Total, no ID	Decreased (%) with lower pH (higher N); increased (%) with O_3; interaction with rain pH and soil	N.D.	Reich et al., 1985
Quercus rubra seedlings/open-topped chambers/56 days	Subambient, ambient, 1.5× ambient	0, 16 days @ 90 ppb, 29 days @ 100 ppb				Total, no ID	Decreased (%) with higher SO_2; increased (%) with higher O_3	N.D.	Reich et al., 1985
Betula papyrifera seedlings/closed chambers/84 days	0, 60–80 ppb		SO_4^{-2}:NO_3^- + NH4 10:7 equivalent ratio	3.5, 5.6		± *Pisolithus tinctorius*, ± sterile or natural inoculum, percent infection only	O_3 × pH interaction (%)	N.D.	Keane and Manning, 1988
Pinus strobus seedlings/growth chambers/3.5 months	20, 60, 100, 140 ppb, 3 days/ week		SO_4^{-2}:NO_3^- ratio 2:1	3.0, 3.5, 4.0, 5.6		No ID	Decreased (%, n) with decreasing pH; no O_3 effect	N.D.	Stroo et al., 1988
Pinus strobus seedlings/growth chambers/104 days	20, 60, 100, 140 ppb, 5 days/ week					No ID	Negative or quadratic response (%) to increasing O_3	N.D.	Stroo et al., 1988

Table 39.2 Summary of Selected Studies of O_3 Effects on Mycorrhizal Fungal Colonization and Communities (Continued)

Host/Fumigation System/Experiment Duration	O_3 Treatment: Concentration, Exposure, Timing	SO_2 Treatment	N Treatment	Acidity Treatment (pH)	CO_2	Mycorrhizal Treatment or ID Method	Mycorrhizal Response: Mycorrhizas (n), % Colonization (%), Mycorrhizas cm^{-1} (n/cm)	Community Structure: Mycorrhizas (n), % Colonization (%), Mycorrhizas cm^{-1} (n/cm)	Reference
Pinus taeda/open-topped chambers/165 days	20, 40, 50, 70, 90 ppb		SO_4^{-2}:NO_3^- 7:3 equivalent ratio	4.0, 5.3		Morphotyping (4 types)	No O_3 effect; increased (n) at lower pH	No O_3 effect; shift in morphotypes (n/cm, n, %) with changing pH; O_3*pH*soil mg interaction for one morphotype	Simmons and Kelly, 1989
Picea abies saplings/closed chambers/14 months	25, 50 ppb			3.0, 5.6, not factorial		Morphotyping	Increased nonmycorrhizal tips with + O_3, + acid mist	No significant treatment effect on morphotypes	Blaschke and Weiss, 1990
Pinus taeda seedlings/cstr[b] chambers/ 42–84 days	0, 50, 100, 150 ppb, 5 h/day, 5 days/week					Morphotyping	Decreased (%) with increasing O_3	Brown morphotype: decrease (n/cm), but no change (%) Coralloid: nonsignificant decrease (n/cm, %)	Meier et al., 1990
Pinus taeda/open-topped chambers/84 days	Ambient, ambient + 80, ambient + 160 ppb		Not reported	3.3, 4.5, 5.2		Inoculated with *Pisolithus tinctorius*	Decreased (%) with high O_3	N.D.	Adams and O'Neill, 1991

Pinus taeda/open-topped chambers/3 years	Subambient, 1×, 2× ambient		SO_4^{-2}:NO_3^- 7:3 equivalent ratio	3.8, 5.2		Morphotypes	Lower (%) in O_3 (p = 0.1)	Lower (%) for coralloid; rhizomorphic type in O_3 (p = 0.1)	Edwards and Kelly, 1992
Pinus rigida/growth chambers/91 days	0, 50, 100, 200 ppb					Inoculated with *Pisolithus tinctorius*	Decreased (%) with increasing O_3; change in ultrastructure	N.D.	Mcquattie and Schier, 1992
Pinus sylvestris and *Picea abies*/open-air fumigation/4 years	16–27 (100)[a], 17–32 (140) ppb	3–6 (60–112)[a], 9–14 (90–226), 13–22 (149–253) ppb				Sporocarp biomass and ID, morphotypes	No treatment effects	No treatment effects	Shaw et al., 1992
Pinus taeda seedlings/open-topped chambers/207 days	0.29×, 1×, 1.71×, 2.38× ambient		SO_4^{-2}:NO_3^- 7:3 equivalent ratio	3.3, 4.3, 5.3		4 morphotypes and *Cenococcum geophilum*	Increase (n/cm) with increasing O_3 for O_3-sensitive pine family	Increase (n/cm) with increasing O_3 for brown, tan morphotypes	Qiu et al., 1993
Picea abies/open-air fumigation/1–2 growing seasons	Ambient, 1.6× ambient					No identification	Increased (n) with higher O_3 (year 1), then decreased (n) (year 2, Ca stressed only)	N.D.	Rantanen et al., 1994
Pinus sylvestris/open-air fumigation/1–2 growing seasons	Ambient, 1.6× ambient					No identification	Increased (n) with higher O_3 (year 2)	N.D.	Ranatanen et al., 1994
Pinus sylvestris saplings/growth chambers/77 days	20, 55 ppb		0, 40 μg m^{-3}		Ambient, 700 ppm	Natural soil inoculum, % infection only	Decreased (%) with higher NH_3, O_3; $NH_3 \times O_3$ interaction	N.D.	Perez-Soba et al., 1995

Table 39.2 Summary of Selected Studies of O_3 Effects on Mycorrhizal Fungal Colonization and Communities (Continued)

Host/Fumigation System/Experiment Duration	O_3 Treatment: Concentration, Exposure, Timing	SO_2 Treatment	N Treatment	Acidity Treatment (pH)	CO_2	Mycorrhizal Treatment or ID Method	Mycorrhizal Response: Mycorrhizas (n), % Colonization (%), Mycorrhizas cm^{-1} (n/cm)	Community Structure: Mycorrhizas (n), % Colonization (%), Mycorrhizas cm^{-1} (n/cm)	Reference
Pinus halapensis/closed chambers/365 days	0, 50 ppb, factorial with SO_2	0, 40 ppb				Fungi isolated from root tips	Decreased (%) with $O_3 + SO_2$	Reduced coralloid tips (possibly *Suillus*); increased ectendomycorrhizae with elevated $SO_2 + O_3$	Diaz et al., 1996
Picea rubens saplings/open-topped chambers/4 years	0.5×, 1×, 1.5×, 2× ambient		~1.5, 3, 20 kg ha^{-1} $year^{-1}$	3.1, 4.1, 5.1		Morphotypes	No treatment effects	1 year: black type lowest in high pH (low N); $O_3 \times$ pH(N) × horizon interaction for 2 types 4 years: one type higher with increasing pH (lower N)	Roth and Fahey, 1998
Pinus sylvestris/open-topped chambers/2.5 years	0×, 1×, ~2× ambient				1×, ~1.7× ambient	4 morphotypes	1.5 years: increase (n) for elevated O_3; CO_2 eliminated O_3 effect 2.5 years: no effect	1.5 years: increase (n) in tuber-like; decrease in dichotomous type for elevated O_3; CO_2 eliminated O_3 effect 2.5 years: no O_3 effect	Kasurinen et al., 1999

Pinus halapensis and *Betula pendula*/growth chambers/68 days	Ambient, 200 ppb		Ambient, 700 ppm	Inoculated *Betula* with *Paxillus involutus*, *Pinus* uninoculated	Reduced fungal growth and higher colonization of *Pinus* with O_3	N.D.	Kytöviita et al., 1999
Pinus sylvestris/open-air fumigation/ 3 years	Ambient, 1.2×–1.7× ambient	32 kg ha^{-1}, 105 kg ha^{-1}		Morphotypes identified but not statistically analyzed	No O_3 effect; elevated N decreased mycorrhizal infection (n)	N.D.	Kainulainen et al., 2000
Pinus halapensis seedlings/growth chambers/94 days	Ambient, 200 ppb		Ambient, 700 ppm	Inoculated with *Paxillus involutus*	No difference (%); decreased soil volume explored with elevated O_3	N.D.	Kytöviita et al., 2001
Elymus glaucus/growth chambers/102 days	~15, ~120 ppm^{-h}			Inoculated with *Glomus intraradices*	Decreased arbuscules (%)	N.D.	Yoshida et al., 2001
Populus tremuloides and *Betula papyrifera*/open-air fumigation/7+ years	1×, 1.5× ambient		Ambient, 560 ppm	Sporocarp biomass and ID	Decreased sporocarp biomass with elevated O_3, O_3*CO_2 interaction	Decrease in *Leccinum* spp. sporocarp biomass with elevated O_3; increase with elevated CO_2; O_3*CO_2 interaction	Lilleskov, unpublished

Note: Exposures were either to O_3 alone or in combination with a variety of other pollution and soil treatments.

[a] Numbers in parentheses are annual hourly maxima.

[b] Continuously stirred tank reactor.

reserves in roots and increased membrane leakiness, both leading to short-term increases in C availability. Whatever the cause, if indeed low doses of O_3 provide increased C availability to mycorrhizal fungi, this would be likely to have much different effects on mycorrhizal fungal communities than decreased C availability expected as a result of damage to plant shoots. It is therefore critical to elucidate the temporal and dose response curves of O_3 effects on C availability to roots and mycorrhizae over the long term.

Compared with studies on total mycorrhizal colonization, there has been little work on the mycorrhizal fungal community response to O_3. Shifts in morphotype abundance have been seen (Meier et al., 1990; Edwards and Kelly, 1992; Diaz et al., 1996; Roth and Fahey, 1998; Kasurinen et al., 1999; Table 39.2). In several cases, decreases in dichotomous or coralloid morphotypes have been observed (Meier et al., 1990; Edwards and Kelly, 1992; Diaz et al., 1996; Kasurinen et al., 1999). It is unclear whether these always represent species shifts rather than morphological changes within species in response to inputs, although Diaz et al. (1996) found that *Suillus*-like mycorrhizas were being replaced by ectendomycorrhizae under high oxidant loads.

In contrast to N fertilization work, where the vast majority of early information on community response came from sporocarp response to N additions in the field, there are only two studies that have examined sporocarp communities of mycorrhizal fungi under O_3 fumigation. This is due to the much higher cost and effort involved in fumigation compared with fertilization. In the first, Shaw et al. (1992) examined *Pinus sylvestris* and *Picea abies* response to O_3 and SO_2 fumigation with relatively low ambient and additional doses. They found no effects of O_3 and only marginal effects of SO_2 fumigation. The second is an ongoing study of the effects of CO_2 and O_3, so it will be addressed in the following section.

39.3.7 O_3 Interactions with Other Pollutants

Pollutants rarely occur in isolation; therefore, understanding their interactions is critical to our ability to predict net pollutant effects in the field. Effects of some pollutants, e.g., oxidants, might be additive or synergistic. Diaz et al. (1996) found that O_3 or SO_2 alone had only nonsignificant negative effects on mycorrhizal infection or community structure, but in combination they had a larger significant negative effect.

Some pollutants may have antagonistic effects. Interactions between O_3 and acid deposition or soil characteristics (soil type, Ca or Mg concentration, soil horizon) have been observed in some studies for mycorrhizal colonization (Reich et al., 1985; Keane and Manning, 1988; Rantanen et al., 1994) or community structure (Simmons and Kelly, 1989; Roth and Fahey, 1998), but not in others (Qiu et al., 1993; Stroo et al., 1988), suggesting that under some conditions ozone effects on mycorrhizal communities are conditional on soil characteristics and plant nutrition. Similarly, Perez-Soba et al. (1995) found an interaction between the effect of NH_3 and O_3 on percent infection. Although individually they both suppressed mycorrhizal infection, in combination negative effects were eliminated. The cause of these interactions remains to be elucidated and might differ among host genotypes and species, depending on C allocation responses to nutrient addition, their nutrient requirements, and mechanisms of O_3 tolerance or avoidance.

The positive effect of CO_2 on carbon gain could be counteracted by O_3 damage to the foliage and consequent decreases in carbon gain. If CO_2 and O_3 effects on mycorrhizae are mediated solely by carbon gain, then one might expect CO_2 and O_3 combined to result in less change in mycorrhizal community composition than each pollutant individually.

One study examined the effect of exposure to elevated CO_2 and O_3 for 2 years on EMF communities of Scots pine (*Pinus sylvestris*) grown in open-topped chambers in Finland (Kasurinen et al., 1999). Using morphotyping, they found that O_3 exposure led

to an increase in the percentage of roots colonized by a tuber-like morphotype and a decrease in a dichotomous morphotype in the first year; in the next year, exposure to CO_2 led to a decrease in the percentage of roots colonized by a dichotomous, thin-mantled morphotype and an increase in a coralloid morphotype.

We are currently examining the effects of both CO_2 and O_3 on mycorrhizal fungal community structure at the AspenFACE site in Rhinelander, WI (Lilleskov, unpublished). This study, initiated in 1997, is designed to address the ecosystem consequences of CO_2 and O_3 enrichment on the growth of forest trees in a field setting, without the use of chambers (Dickson et al., 2000). Quaking aspen (*Populus tremuloides*) is planted in the 30-m-diameter rings, either alone or in combination with paper birch (*Betula papyrifera*) or sugar maple (*Acer saccharum*). Our preliminary results indicate a significant effect of both CO_2 and O_3 on sporocarp production by mycorrhizal fungi, with significantly lower sporocarp production under elevated O_3, a stimulatory effect of CO_2 on sporocarp production in some cases, but a much stronger CO_2 stimulation seen in combination with O_3. Furthermore, there has been a shift in the species composition of the dominant taxa fruiting, with a positive effect of CO_2 and a negative effect of O_3 seen for some taxa (e.g., *Leccinum* spp.) but not for others (e.g., *Hebeloma* spp.). The sporocarp effects have been quite strong, with virtually no sporocarp production by *Leccinum* in the O_3 treatments, whereas it is the dominant sporocarp biomass producer in the other treatments. We are presently in the process of analyzing the fungal communities on the root tips, to determine whether these changes in production reflect a shift in dominance on root tips or only allocation to sporocarp production.

39.4 MECHANISMS AND CONSEQUENCES OF MF COMMUNITY CHANGE

The consequences of MF community change for plant health and ecosystem function depend largely on whether, as mycorrhizal fungal communities change in response to alterations of resources and conditions, the consequent changes in community function buffer, are neutral with respect to, or exacerbate, the effect of these environmental changes. The answer to this question is fundamental to our understanding both of the mode of control of MF community structure and of the functional role of MF communities in ecosystems. In the following section we will review alternative models of community functional response and the evidence in support of these alternatives.

The core question we need to ask is: Are mycorrhizal communities in any sense optimized in terms of their supply of resources to their plant hosts? A related question relevant to the current discussion is: As resources and conditions change as a result of exposure to pollution or other stresses, do changes in fungal communities lead to new communities that are functionally optimized from the plant perspective? Or alternatively, do changes lead to less mutualistic communities? In order to answer these questions, we must first answer the following question: What do we mean by functional optimization?

39.4.1 What Do We Mean by Optimality?

If we think of optimality in terms of maximizing host fitness, for some herbaceous plants there is the potential to have direct measures of fitness. However, when working with long-lived woody perennials (including many hosts of AMF and almost all hosts of EMF), we must make certain assumptions, because host fitness of a long-lived perennial is very difficult to measure directly in the context of most experiments. Commonly used proxies for fitness are plant nutrition and growth, as both can be influenced by mycorrhizal fungi and are likely to be major determinants of fitness. Caveats for this definition are that high

growth rates may not always be optimal from the perspective of fitness, and defining the optimal nutrient status may be difficult.

39.4.2 How Would Mycorrhizal Fungal Optimal Function Change as N and C Availability Change?

In initially N-limited ecosystems, two aspects of mycorrhizal function are likely to have optima (from the host plant perspective) that shift in response to altered C and N availability: amounts and relative proportions of specific nutrients supplied to host plants, and the C cost of supplying those nutrients. As N availability increases, N limitation will become alleviated, initially leading to greater aboveground plant growth and decreased C flux belowground (Cannell and Dewar, 1994). Given sufficient inputs, other nutrients will become limiting, and these new nutrient limitations will also influence patterns of C flux belowground. Similarly, as C availability declines in response to oxidants, or increases in response to elevated CO_2, C available for nutrient uptake will theoretically be decreased or increased, respectively. Under these conditions, both changes in host requirements for specific nutrients and the availability of host C to the EMF could alter the suites of fungal traits that are functionally optimal.

39.4.2.1 Shifting Optima for Nutrient Supply

Under strongly N-limited conditions, a high affinity for inorganic N and enzymatic capabilities that permit access to complex organic N would both be beneficial, but one might expect that these traits would be less beneficial under high N conditions. Increased N supply can lower host plant demand for N, and high uptake rates of NH_4^+ impose a high C cost on EMF because of the need to incorporate excess NH_4^+ into amino acids, some of which are transferred back to hosts (Wallander, 1995; Wallander et al., 1999). These C costs in combination with nutrient effects on C allocation may contribute to reduced production of external mycelium in fertilized forests (Nilsson and Wallander, 2003). Consequently, traits that lead to reduced uptake of NH_4^+ but favor the uptake of other mineral nutrients should be beneficial to both host plant and EMF under conditions of excess N availability and low root C availability. Similarly, maintenance of extracellular proteolytic activity could also impose unnecessary costs under high N conditions. Limited evidence from pure culture experiments suggests that some dominant fungi under high N conditions grow more poorly with protein as a sole N source than mineral N, in contrast to many fungi from low N sites that grow equally well on protein and mineral N (Taylor et al., 2000; Lilleskov et al., 2002b; Table 39.1). These results require testing on a greater range of isolates and in symbiosis. Intraspecific variability in pure culture growth on protein has also been found (Table 39.1), but it is not known if this variation is related to N availability at the site of origin.

Under elevated N deposition, increased plant uptake, soil acidification, and leaching losses could all lead to reduced base cation and phosphorus (P) availability. If these nutrients become limiting to plant production, the optimality model would suggest that hosts would favor fungal symbionts with traits that maximize the gain of cations or P (e.g., Dighton et al., 1993). These traits could include high-affinity phosphate transporters that allow for uptake of P at low concentrations (Kothe et al., 2002) and increased production of acid phophatases (e.g., Antibus et al., 1997) and organic acids (e.g., Griffiths et al., 1994). The latter would be involved in mobilization of both P and cations.

39.4.2.2 Shifting pH and Mycorrhizal Function

Soil acidification under elevated N (or S) deposition could also lead to shifting optima for mycorrhizal function. For example, Lilleskov et al. (2002a) hypothesized that the decline

of a subset of EMF species over an N deposition gradient might have been driven by acidification rather than N availability per se. Because species function outside optimal pH ranges, nutrient uptake and other functions will be disrupted (Ek et al., 1994), reducing MF ability to be effective mutualists. These pH effects could be mediated by optima for membrane function or extracellular enzyme activity; shifts in forms and decreased availability of nutrients, especially cations and phosphorus; and increases in acid cations, especially Al^{3+}, that could affect root or fungal function. EMF species differ in their sensitivity to Al^{3+} in culture (Thompson and Medve, 1984). Dighton and Skeffington (1987) speculated that the decline in abundance of coralloid mycorrhizal morphotypes in response to high H_2SO_4 could be attributed to higher H^+ or Al^{3+} concentrations. *C. geophilum* appeared to be sensitive to pH in pot studies (Stroo and Alexander, 1985; Meier et al., 1989). Although it exhibited growth tolerance of a wide pH range in pure culture (Hung and Trappe, 1983), it was relatively sensitive to increased Al^{3+} concentrations (Thompson and Medve, 1984), suggesting that Al sensitivity rather than pH per se might be responsible for observed shifts. Ahonen et al. (2003) found differences between Al^{3+} tolerance and growth effects of two ectomycorrhizal fungal species. For a more in-depth treatment of acidification effects, see Dighton and Jansen (1991).

39.4.2.3 Availability of Host C to MF

Oxidative stress reduces C available for allocation to roots (Andersen, 2003). Similarly, allocation theory suggests that plants will respond to alleviation of N limitation by shifting the allocation of C away from the capture of belowground resources to the capture of aboveground resources (Ingestad and Agren, 1991). Source–sink models (Cannell and Dewar, 1994) predict that total belowground C flux will be reduced in response to the alleviation of nutrient limitations to growth because the C sink strength of shoots increases relative to the sink strength of roots. For example, the total quantity of C allocated belowground by stands of *Pinus radiata* and *Eucalyptus saligna* was reduced under conditions of high N availability (Ryan et al., 1996; Giardina and Ryan, 2002). In combination with a reduction in belowground C flux, Giardina and Ryan (2002) observed a dramatic reduction in EMF colonization rates of fine roots. Similarly, oxidant stress reduces the source pool of C, reducing available C for allocation to roots (Andersen, 2003).

However, sufficiently high inputs of N to N-limited ecosystems can lead to the development of limitations by other nutrients, such as P or cations. Of importance to EMF function, P vs. Mg or K limitation has opposite effects on belowground flux of C (Ericsson, 1995). P limitation, like N limitation, results in increased belowground C flux. This C flux can lead to substantial increases in EMF biomass under P limitation (Wallander and Nylund, 1992). In contrast, studies suggest that Mg, and possibly K, limitation results in reduced C flux belowground (Ericsson, 1995). Consistent with this, EM development is restricted under Mg limitation (Ericsson, 1995). Although limitation by these cations is relatively rare, when it occurs there would be limited ways that MF could alleviate limitation because lower C supply to roots might reduce MF ability to produce organic acids to mobilize cations.

39.4.2.4 Integrating Nutrient and C Availability

Thus, altered N availability affects both patterns of nutrient limitation and other aspects of soil chemistry, and the availability of C to obtain those nutrients. Therefore, one might hypothesize that optimal symbionts would shift under N deposition or oxidative stress. As N availability increases, the optimal EMF partner for the host plant would shift, tracking limiting nutrients, soil chemistry, and C availability:

1. Under low N conditions: Aboveground growth is N limited, belowground C flux is high, and fungi effective at supplying inorganic or organic N to hosts, with low to high C efficiency (because C is readily available), are optimal.
2. Under elevated N conditions where nutrition is relatively balanced: Aboveground growth is not nutrient limited, belowground C flux is reduced, and fungi with high C efficiency for uptake of a broad range of nutrients are optimal.
3. Under elevated N conditions where cations such as Mg and K are limiting and soils might be acidified: Belowground C flux is also low (because Mg limitation reduces soluble carbohydrate pools), so fungi with high C efficiency of supplying the limiting cation, possibly in acidified soils, are optimal.
4. Under elevated N conditions where P limitations are encountered, and soils may be acidified: Belowground C flux is again high, and fungi effective at supplying inorganic or organic P, possibly in acidic soils, are optimal.

In the case of elevated oxidants or CO_2, we can expect that the major changes in optimal fungal function would be in terms of the C efficiency of nutrient supply. Under elevated oxidants, more C-efficient fungi would be optimal. Thus, the optimal fungi under oxidant stress might be expected to be most similar to 2 or 3 above in the N deposition scenario, depending on nutrient limitations. The major difference would be that C allocation belowground would be reduced even under N or P limitation, so one would expect the optimal fungi to be those that acquired the most limiting resources, including N, for the lowest C cost.

Under elevated CO_2, fungi with higher rates of nutrient supply with relatively less regard for C efficiency would be optimal, although there would be clear interactions with soil nutrient availability and host limitations described above. The tendency would be for optimal fungi to be more like those under 1 or 4 above, depending on patterns of limitation.

The above discussion assumes that nutrients and C supply are the major controls on root receptivity to fungi. However, it is conceivable that other mechanisms could affect host receptivity to fungi. For example, oxidative stress to foliage could affect mycorrhizas by triggering a systemic induced defensive response against fungi (e.g., Eckey-Kaltenbach et al., 1994) that could inhibit mycorrhiza formation.

39.4.3 Are Mycorrhizal Fungal Communities Optimized as Resources and Conditions Change?

The above discussion of optimality assumes that mycorrhizal fungal communities are selected to optimize plant fitness, nutrition, and growth, and that mycorrhizal associations are predominantly mutualistic across a wide range of environmental conditions. The optimality model suggests that regulatory mechanisms within the host plant result in selection of the most effective (i.e., in terms of supply of limiting nutrients, C costs, or other attributes) fungal mutualists. For example, trees may reduce the supply of plant C to one MF species if there is no nutritional benefit to the host and allocate more to other MF or nonmycorrhizal roots as appropriate. Higher rates of mortality of root tips colonized by poor mutualists vs. good mutualists would lead to increased proportional colonization by more beneficial mutualists (Hoeksema and Kummel, 2003).

However, plant control over which mutualists colonize roots has not been clearly established, and evolutionary models of mutualism suggest that the stability of mutualistic associations depends on the ability of partners to detect parasitism and retaliate (Axelrod and Dion, 1988; Bull and Rice, 1991). The temporal or spatial scales at which plants can recognize and respond to parasitism will affect the fungal fitness consequences of parasitic vs. mutualistic interactions (Hoeksema and Kummel, 2003). Negative host

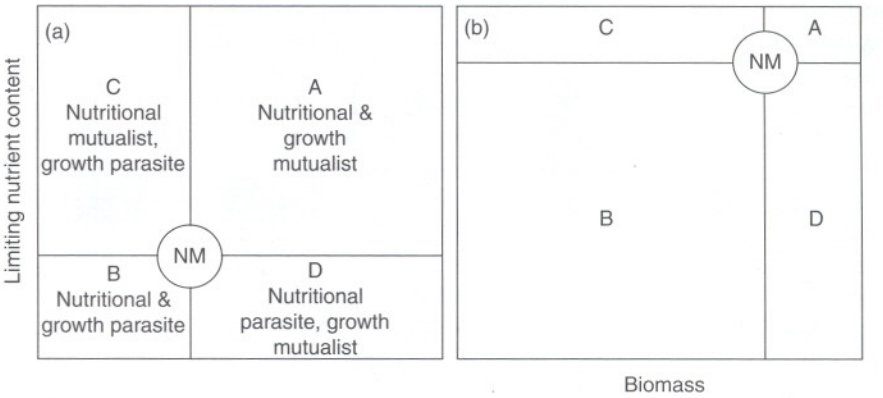

Figure 39.5 Mycorrhizal nutritional and growth mutualism and parasitism under low nutrient availability (a) and high nutrient availability (b). NM = nonmycorrhizal plant. Note that there is less room for mutualism as the fertilized plants approach maximal growth and optimal nutrient concentrations.

fitness consequences of colonization by MF could occur if plant mechanisms controlling choice of fungal partner are weak compared with other factors, such as highly effective root-colonizing ability of relatively parasitic fungi (Eissenstat et al., 1993; Johnson et al., 1997; Bever et al., 2001). In the present context we define parasitic MF as those fungi that provide low or no returns of a limiting resource in exchange for host C supplied, so that plant fitness or its proxies are lower than in the nonmycorrhizal state. Empirical evidence suggests that MF community composition could change in response to fertilization in ways that have suboptimal (but still positive), neutral, or even negative consequences for plant growth (Johnson et al., 1997). Although MF parasitism could theoretically occur under any conditions, it is thought that it should be more likely under fertile conditions. This is because lower belowground C availability and higher nutrient availability could permit nonmycorrhizal plants to achieve uptake of limiting nutrients at a lower cost than mycorrhizal plants (Figure 39.5). Fungal community optimization can only occur if the optimal fungal partner exists. Under extremely nutrient-rich, acidic, or C-limited conditions, the best fungal partner present in the community might not be able to provide resources as efficiently as nonmycorrhizal roots. In this case, the optimal state for the plant would be nonmycorrhizal, whereas the optimal state for the fungus is always in symbiosis. Fungi that are better able to gain access to host C under these conditions (more aggressive strains, *sensu* Johnson, 1993) would by definition be parasitic (Johnson, 1993).

Empirical investigations in AMF plant communities have suggested that fertilization might shift EMF communities toward the parasitic end of the mutualism–parasitism spectrum (Johnson, 1993). Johnson (1993) found that arbuscular mycorrhizal (AM) inoculum from completely fertilized plots, when compared with inoculum from unfertilized plots, had less positive effects on host growth and number of inflorescences. Similarly, Corkidi et al. (2002) compared the relative benefit of inoculum from unfertilized and N-fertilized soils from two sites in the western U.S., when used to inoculate two grass species in either low or high N soils. They found that in both cases, most host parameters measured were improved more by the inoculum from low N soils than those from high N soils, especially when grown in high N soils, and inoculum from high N soils had no significant benefit compared with uninoculated controls (Figure 39.6). Some caution must be used in interpreting this as solely a mycorrhizal effect, because inoculum of pathogenic micro-

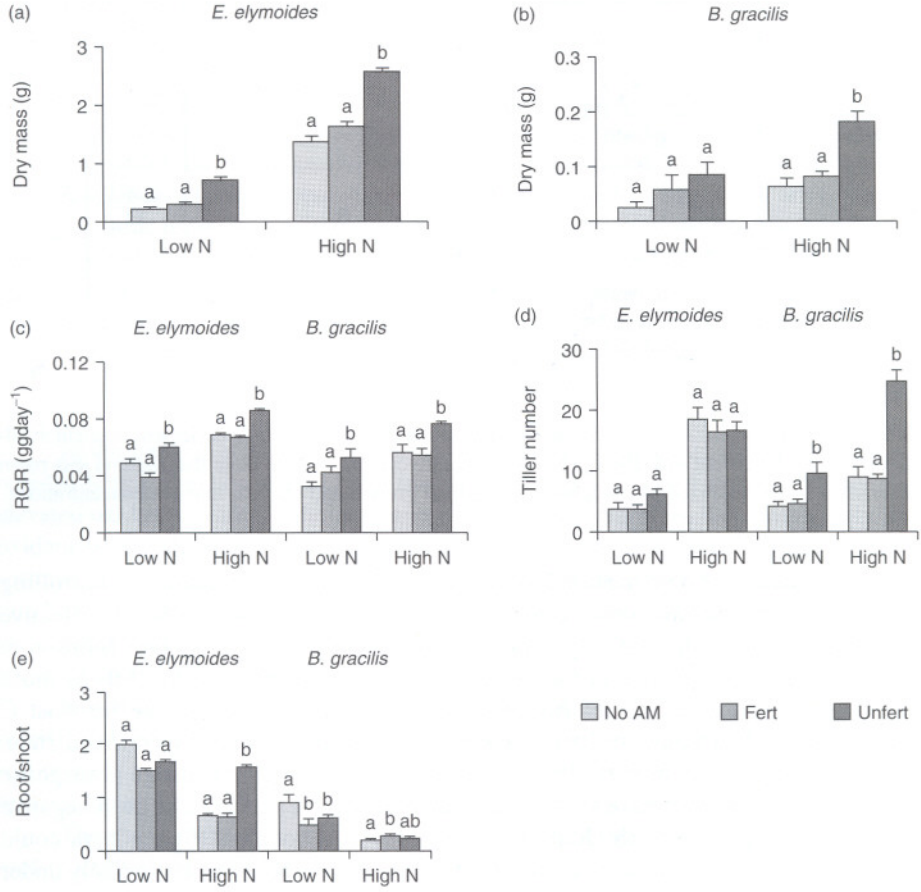

Figure 39.6 Growth response of *Elymus elymoides* and *Bouteloua gracilis* inoculated with fertilized (Fert), unfertilized (Unfert), and nonmycorrhizal (No AM) soil from Shortgrass Steppe, Colorado. Plants were grown in high N and low N conditions for 12 weeks. (a, b) Dry mass. (c) Relative growth rate (RGR). (d) Tiller number. (e) Root/shoot. Bars represent the standard error of the mean of 10 replicates. Within each nutrient treatment, different letters indicate significant differences between soil inoculum treatments at p 0.05. Letters above bars of different N treatments cannot be compared. (From Corkidi et al., *Plant and Soil*, 240, 299–310, 2002. With permission.)

organisms could also have differed among soil types. However, the authors report seeing little evidence of pathogens.

It is unclear how general these results are, as no study of this sort has been repeated in EMF or other mycorrhizal fungal communities. Lilleskov et al. (2002a) hypothesized that the increase in abundance of *Paxillus involutus* with increasing N inputs might be the result of a functional shift to fungi adapted to conditions of low pH and P limitation. This species appears to be specialized for high N/low P environments. It is more efficient at inorganic P uptake than N uptake (Ekblad et al., 1995; Högberg et al., 1999), has higher acid phosphatase activity (Pacheo et al., 1991), and supplies relatively more P than N to seedlings than other species, including *Piloderma croceum*, a species sensitive to high N inputs (Wallander et al., 1997; Högberg et al., 1999). Similarly, *Laccaria bicolor* — another species that appears to be relatively tolerant of high N conditions — grows poorly on organic N (e.g., Lilleskov et al., 2002b), provides relatively low amounts of N and high amounts of

P to hosts, and is tolerant of high aluminum availability (Ahonen et al., 2003). These traits could be beneficial under high N conditions and are consistent with an optimization model of community change. However, the generality of these traits, among both different strains and species from high N sites, and the relative benefits of EMF communities from high vs. low N sites under N-enriched conditions remain to be tested. I am aware of no tests of the functional consequences of community change driven by oxidants. This must in part be a consequence of the paucity of data available characterizing community response to oxidants.

In summary, our understanding of the mechanisms and consequences of mycorrhizal fungal community change in response to air pollution is at present quite crude. Future experiments should explore the match between MF communities and the soils from which they are derived, specifically addressing the alternate hypotheses of MF community optimization vs. parasitism under changing resource conditions.

39.5 RECOVERY AND RESTORATION

As pollution abatement measures are implemented, ecosystem perturbation will be reduced, and the potential for community recovery exists. In Europe, economic and technological trends and specific pollution regulation are leading to reduced inputs of N over broad regions (Erisman et al., 2003). What is the potential for recovery of EMF diversity? This question is especially difficult to answer, given that we do not know baseline diversity, the extent of diversity losses, or the mechanisms controlling diversity and composition. If, for example, plant nutrition, soil N availability, and soil pH all affect mycorrhizal communities, then considerable lags are possible in community response, and species-specific patterns of recovery are to be expected. Strengbom et al. (2001) examined plots fertilized with nitrogen 9 and 47 years previously and found patterns indicative of lags in community recovery. Although sampling was limited, there were significant residual effects of N fertilization on sporocarp diversity at both sites, with reduced sporocarp production by *Cortinarius* in fertilized plots at both sites.

One approach to deal with eutrophication effects is active restoration, at least at a small scale. Efforts in the Netherlands involving removal of humus layers from stands with dramatically reduced sporocarp production have resulted in partial recovery of EMF communities that has persisted for 5 years (Smit et al., 2003).

In the case of oxidants, assuming that oxidant effects led to significant community change, one might expect a much more rapid response in the fungal community if, as hypothesized, the primary pathway of pollutant effect is via C allocation. The major factor limiting rates of recovery would likely be the lags involved with host recovery of photosynthetic capacity and species turnover on roots. Consistent with this hypothesis, dramatic reductions in SO_2 and NO_x emissions (88 and 79%, respectively) in the Czech Republic from the late 1980s to the late 1990s have been associated with a parallel dramatic increase in EMF sporocarp production and diversity in the Giant Mountains (Fellner and Landa, 2003). The potential nutrient, acidification, and oxidant effects of these pollutants or their reaction products make it difficult to ascribe the changes strictly to removal of oxidants; however, it is indicative of the potential for relatively rapid recovery from some pollution stresses.

39.6 KNOWLEDGE GAPS

The above should make it clear that air pollution has the potential to alter mycorrhizal fungal communities, with potential biodiversity and functional consequences. However, it

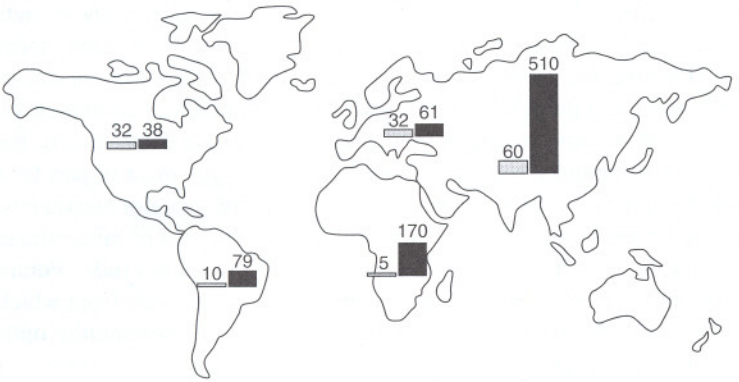

Figure 39.7 Comparison of contemporary and possible future reactive nitrogen creation rates in various regions of the world (1995 vs. max population) (Tg N year^{-1}). (From Galloway and Cowling, *Ambio*, 31, 64–71, 2002. With permission.)

should also be clear that in order to assess the full magnitude and significance of community change, we must fill a number of critical gaps in our understanding of the patterns, mechanisms, and consequences of community change. Besides the obvious gaps in our knowledge pointed out above, I wish to highlight several other knowledge gaps that are worth considering as we develop future research programs.

39.6.1 Generality of Mycorrhizal Fungal Community Responses to Pollutants

As should be clear from the earlier discussion of empirical results, there are data from multiple studies suggesting responses of MF communities to N deposition. However, most of this work has been carried out in relatively few ecosystem types: for ectomycorrhizal fungi, most of the work has been done in conifer forests; for AM fungi, most of the work has been done in coastal sage scrub ecosystems (community characterization) or grasslands (community function). Virtually no information is available on the effects of ozone or fertilization on ericoid, arbutoid, and orchidoid mycorrhizal communities. Relatively little work has been done with deciduous trees, including both temperate and tropical ectomycorrhizal and AM tree species. Given the projections for dramatic increases in N deposition in the developing world, especially Asia (Galloway and Cowling, 2002; Figure 39.7), the lack of tropical data is of particular concern. It is possible that the ectomycorrhizal dipterocarps of tropical Asia could be especially sensitive to N deposition.

39.6.2 What Is the Baseline Community?

In order to accurately characterize biodiversity consequences of air pollution, we must have a baseline against which to measure these changes. Unfortunately, for globally mixed pollutants such as CO_2 there is no possibility of establishing a baseline unless archived or otherwise preserved samples can be accessed (e.g., Egerton-Warburton et al., 2001). As noted earlier, current global CO_2 concentrations are ~30% above preindustrial levels. As an added complication, the idea of a baseline for CO_2 may be unrealistic, as even before industrialization the baseline for CO_2 was not stable, but rather varied repeatedly between 180 and 280 ppm during the Pleistocene (Houghton et al., 2001).

In contrast, preanthropogenic levels of atmospheric N deposition and oxidants were low and believed to be relatively stable (Holland et al., 1999). It is still possible to find

regions of the globe at or near preindustrial levels (e.g., Lilleskov et al., 2001, 2002a). However, many fertilization experiments have been carried out in regions already experiencing elevated nitrogen deposition or oxidant concentrations. In these cases, the control communities will not be equal to the baseline, unpolluted communities. Similarly, many studies of oxidant effects use ambient levels as the baseline. However, these studies are often carried out in areas where the baseline O_3 levels are substantially elevated above preindustrial levels. It is essential that studies be carried out in regions with preindustrial levels of oxidants and N deposition in order to establish reliable baselines.

As a result, the amount of good community baseline data is relatively limited, making determination of large-scale pollutant effects more difficult. The best baseline data are from sporocarp records in Europe, made possible by the long tradition of fungal taxonomy there. Monitoring of long-term trends of sporocarp production in Europe was one of the first clues that air pollution might be affecting mycorrhizal fungal communities. Most other regions of the world do not have similar records. If we do not act soon in those areas (especially North America, Asia, Africa, and South America), it may be difficult to find baseline communities for some forest types.

39.6.3 Biodiversity and Biogeography

This lack of extensive baseline data, combined with the challenges involved in species identification for fungi, has limited our knowledge of biogeographic patterns of mycorrhizal fungi. While site-level (alpha) diversity of fungi can be calculated from individual studies, understanding the scales at which both fungal species and air pollutants vary is required to determine the likely regional and global biodiversity consequences of air pollution. Would elevated air pollution likely eliminate genotypes within populations, entire populations, or entire species? Pollutants differ in the spatial scales at which they are elevated. Nitrogen and ozone pollutants have a shorter atmospheric residence time, so they are characterized by local to regional hot spots, with remote areas having relatively low concentrations. As noted above, CO_2 has a longer atmospheric residence time and tends to be relatively well mixed globally, so even remote regions are exposed to elevated CO_2.

In contrast to the patterns of air pollution, we have relatively little information on the biogeographic patterns of species and populations of mycorrhizal fungi (Halling, 2001). In particular, we have very little idea of how much endemism exists in mycorrhizal fungi. Studies suggest high rates of endemism in Australia (Castellano and Bougher, 1994) and New Zealand (McKenzie et al., 2000), and for boletes in Costa Rica and Colombia (Halling, 1996). What about North America, Europe, Africa, and Southeast Asia? Many morphological species appear to be shared across north-temperate regions, but biogeographic differentiation within these species groups is routinely discovered (e.g., Martin et al., 2002). Even within species there is the potential for regional differentiation among isolated populations that needs to be taken into consideration in estimates of loss of biodiversity.

In order to fill these gaps in baseline data, it is critical that large-scale sampling and mapping programs be undertaken to determine mycorrhizal fungal community composition and structure in a broad range of ecosystem types, as is occurring at present in parts of Europe and North America (Arnolds, 2001).

39.6.4 Critical Loads

Critical loads are "a quantitative estimate of an exposure to one or more pollutants below which significant harmful effects on specified sensitive elements of the environment do not occur according to present knowledge" (Nilsson and Grenfelt, 1988). Critical loads for N effects on mycorrhizal fungi have never been estimated. Bouwman et al. (2002) have

calculated critical loads of nitrogen as a nutrient, although these are not based on mycorrhizal fungal responses. They indicate that critical loads for maintenance of biodiversity in response to N eutrophication were exceeded in significant areas of Eastern Europe (47%), Western Europe (38%), the U.S. (24%), and Southeast Asia (23%), based on a medium estimate of critical loads. By 2015, the areas exceeding these critical loads are projected to decline for Europe, stay stable for the U.S., and increase to 30% for Southeast Asia.

Can we say what a likely critical load for nitrogen deposition would be for mycorrhizal communities? Wallander and Kottke (1998) suggested that a critical load of 20 to 30 kg ha^{-1} $year^{-1}$ could be too high for sensitive EMF communities. If we can link changes in mycorrhizal communities to specific processes already addressed by critical load models, such as changes in nitrification, then we might be able to use them to refine current models to predict critical loads for mycorrhizal fungal diversity. Increase in net nitrification is believed to be an important indicator of significant N eutrophication (Aber et al., 1998). Most studies of mycorrhizal fungi have not linked community response to specific N cycling parameters such as nitrification. Taylor et al. (2000) and Lilleskov et al. (2001, 2002a) (Figure 39.3 and Figure 39.4) found negative correlations between diversity of EMF and various metrics of soil inorganic N pools. More studies must establish links between N cycling parameters and mycorrhizal community diversity, composition, and structure before we can determine appropriate critical loads for EMF diversity. Metrics of this sort are likely to be more useful predictors than nitrogen inputs because the latter do not take into account the other factors that could influence N cycling, such as soils, site history, climate, productivity, and vegetation.

39.7 CONCLUSIONS

There is good evidence that nitrogen deposition is one of the major factors contributing to decline in diversity of EMF sporocarps over broad regions of Europe. Although there is less available evidence, experiments and gradient studies suggest that sites with long-term N inputs are also losing diversity belowground. Much less good MF community information is available for oxidant effects. Although lab experiments suggest that oxidants can have negative effects on mycorrhizal fungi, there is a need for field experiments that address long-term oxidant effects at realistic concentrations. More extensive biogeographic data are necessary to assess the full biodiversity impacts of mycorrhizal fungal community change. Similarly, functional consequences of community change are largely unknown. Limited research with AMF communities indicates the potential for fertilization-mediated transitions to communities of less beneficial mycorrhizal fungi, but the generality of this phenomenon across mycorrhizal classes and plant communities must be determined. The development of DNA-based tools has provided us with an unprecedented opportunity to characterize community composition and structure. This fundamental community information will allow us to make meaningful inroads into the complex questions related to mycorrhizal community function. Clearly, there are many urgent and exciting challenges ahead for the current generation of mycorrhizologists.

REFERENCES

Aber J, McDowell W, Nadelhoffer K, Magill A, Berntson G, Kamakea M, McNulty S, Currie W, Rustad L, Fernandez I. (1998). Nitrogen saturation in temperate forest ecosystems. *Bioscience* 48:921–934.

Abuzinadah RA, Read DJ. (1986). The role of proteins in the nitrogen nutrition of ectomycorrhizal plants. 1. Utilization of peptides and proteins by ectomycorrhizal fungi. *New Phytologist* 103:481–493.

Adams MB, O'Neill EG. (1991). Effects of ozone and acidic deposition on carbon allocation and mycorrhizal colonization of *Pinus-taeda* L. seedlings. *Forest Science* 37:5–16.

Agerer R. (1991). Characterization of ectomycorrhiza. *Methods in Microbiology* 23:25–73.

Ahonen JU, Goransson A, Finlay RD. (2003). Growth and nutrient uptake of ectomycorrhizal *Pinus sylvestris* seedlings in a natural substrate treated with elevated Al concentrations. *Tree Physiology* 23:157–167.

Allen TR, Millar T, Berch SM, Berbee ML. (2003). Culturing and direct DNA extraction find different fungi from the same ericoid mycorrhizal roots. *New Phytologist* 160:255–272.

Andersen CP. (2003). Source-sink balance and carbon allocation below ground in plants exposed to ozone. *New Phytologist* 157:213–228.

Antibus RK, Bower D, Dighton J. (1997). Root surface phosphatase activities and uptake of P-32-labelled inositol phosphate in field-collected gray birch and red maple roots. *Mycorrhiza* 7:39–46.

Arnolds E. (1991). Decline of ectomycorrhizal fungi in Europe. *Agriculture Ecosystems and Environment* 35:209–244.

Arnolds E. (2001). The future of fungi in Europe: threats, conservation and management. In *Fungal Conservation: Issues and Solutions*, Moore D, Nauta MM, Evans SE, Rotheroe M. (Eds.). Cambridge, U.K., Cambridge University Press.

Avis PG, McLaughlin DJ, Dentinger BC, Reich PB. (2003). Long-term increase in nitrogen supply alters above- and below-ground ectomycorrhizal communities and increases the dominance of *Russula* spp. in a temperate oak savanna. *New Phytologist* 160:239–253.

Axelrod R, Dion D. (1988). The further evolution of cooperation. *Science* 242:1385–1390.

Bending GD, Read DJ. (1996). Nitrogen mobilization from protein-polyphenol complex by ericoid and ectomycorrhizal fungi. *Soil Biology and Biochemistry* 28:1603–1612.

Bever JD, Schultz PA, Pringle A, Morton JB. (2001). Arbuscular mycorrhizal fungi: more diverse than meets the eye, and the ecological tale of why. *Bioscience* 51:923–931.

Blaschke H, Weiss M. (1990). Impact of ozone, acid mist and soil characteristics on growth and development of fine roots and ectomycorrhiza of young clonal Norway spruce. *Environmental Pollution* 64:255–263.

Bobbink R, Hornung M, Roelofs JGM. (1998). The effects of air-borne nitrogen pollutants on species diversity in natural and semi-natural European vegetation. *Journal of Ecology* 86:717–738.

Bouwman AF, Van VDP, Derwent RG, Posch M. (2002). A global analysis of acidification and eutrophication of terrestrial ecosystems. *Water Air and Soil Pollution* 141:349–382.

Brandrud TE. (1995). The effects of experimental nitrogen addition on the ectomycorrhizal fungus flora in an oligotrophic spruce forest at Gardsjon, Sweden. *Forest Ecology and Management* 71:111–122.

Brandrud TE, Timmermann V. (1998). Ectomycorrhizal fungi in the NITREX site at Gardsjon, Sweden; below and above-ground responses to experimentally-changed nitrogen inputs 1990–1995. *Forest Ecology and Management* 101:207–214.

Bull JJ, Rice WR. (1991). Distinguishing mechanisms for the evolution of cooperation. *Journal of Theoretical Biology* 149:63–74.

Cairney JWG, Burke RM. (1998). Extracellular enzyme activities of the ericoid mycorrhizal endophyte *Hymenoscyphus ericae* (Read) Korf & Kernan: their likely roles in decomposition of dead plant tissue in soil. *Plant and Soil* 205:181–192.

Cannell MGR, Dewar RC. (1994). Carbon allocation in trees: a review of concepts for modelling. In *Advances in Ecological Research*, Vol. 25, Begon M, Fitter AHE. (Eds.). London, Academic Press, pp. 59–104.

Caporn SJM, Song W, Read DJ, Lee JA. (1995). The effect of repeated nitrogen fertilization on mycorrhizal infection in heather (*Calluna vulgaris* (L.) Hull). *New Phytologist* 129:605–609.

Castellano MA, Bougher NL. (1994). Consideration of the taxonomy and biodiversity of Australian ectomycorrhizal fungi. *Plant and Soil* 159:37–46.

Corkidi L, Rowland DL, Johnson NC, Allen EB. (2002). Nitrogen fertilization alters the functioning of arbuscular mycorrhizas at two semiarid grasslands. *Plant and Soil* 240:299–310.

Diaz G, Barrantes O, Honrubia M, Gracia C. (1996). Effect of ozone and sulphur dioxide on mycorrhizae of *Pinus halepensis* Miller. *Annales des Sciences Forestieres* 53:849–856.

Dickie IA, Xu B, Koide RT. (2002). Vertical niche differentiation of ectomycorrhizal hyphae in soil as shown by T-RFLP analysis. *New Phytologist* 156:527–535.

Dickson RE, Lewin KF, Isebrands JG, Coleman MD, Heilman WE, Riemenschneider DE, Sober J, Host GE, Zak DF, Hendrey GR, Pregitzer KS, Karnosky DF. (2000). Forest atmosphere carbon transfer and storage (FACTS II). The aspen free-air CO_2 and O_3 enrichment (FACE) project: an overview. In *General Technical Report NC-214*. USDA Forest Service North Central Research Station.

Dighton J, Jansen AE. (1991). Atmospheric pollutants and ectomycorrhizae: more questions than answers. *Environmental Pollution* 73:179–204.

Dighton J, Poskitt JM, Brown TK. (1993). Phosphate influx into ectomycorrhizal and saprotrophic fungal hyphae in relation to phosphate supply: a potential method for selection of efficient mycorrhizal species. *Mycological Research* 97:355–358.

Dighton J, Skeffington RA. (1987). Effects of artificial acid precipitation on the mycorrhizas of Scots pine seedlings. *New Phytologist* 107:191–202.

Eckey-Kaltenbach H, Ernst D, Heller W, Sandermann H. (1994). Biochemical plant responses to ozone. IV. Cross-induction of defensive pathways in parsley (*Petroselinum crispum* L.) plants. *Plant Physiology* 104:67–74.

Edwards GS, Kelly JM. (1992). Ectomycorrhizal colonization of loblolly-pine seedlings during 3 growing seasons in response to ozone, acidic precipitation, and soil Mg status. *Environmental Pollution* 76:71–77.

Egerton-Warburton LM, Allen EB. (2000). Shifts in arbuscular mycorrhizal communities along an anthropogenic nitrogen deposition gradient. *Ecological Applications* 10:484–496.

Egerton-Warburton LM, Graham RC, Allen EB, Allen MF. (2001). Reconstruction of the historical changes in mycorrhizal fungal communities under anthropogenic nitrogen deposition. *Proceedings of the Royal Society of London Series B: Biological Sciences* 268:2479–2484.

Ehhalt D, Prather M, Dentener F, Derwent R, Dlugokencky E, Holland E, Isaksen I, Katima J, Kirchhoff V, Matson P, Midgley P, Wang M. (2001). Atmospheric chemistry and greenhouse gases. In *Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change*, Houghton JT, et al. (Eds.). Cambridge, U.K., Cambridge University Press, pp. 239–287.

Eissenstat DM, Graham JH, Syvertsen JP, Drouillard DL. (1993). Carbon economy of sour orange in relation to mycorrhizal colonization and phosphorus status. *Annals of Botany* 71:1–10.

Ek H, Andersson S, Arnebrant K, Soderstrom B. (1994). Growth and assimilation of NH_4^+ and NO_3^- by *Paxillus-involutus* in association with *Betula-pendula* and *Picea-abies* as affected by substrate pH. *New Phytologist* 128:629–637.

Ekblad A, Wallander H, Carlsson R, Huss Danell K. (1995). Fungal biomass in roots and extramatrical mycelium in relation to macronutrients and plant biomass of ectomycorrhizal *Pinus sylvestris* and *Alnus incana*. *New Phytologist* 131:443–451.

El Badaoui K, Botton B. (1989). Production and characterization of exocellular proteases in ectomycorrhizal fungi. *Annales des Sciences Forestieres Paris* 46:728S–730S.

Ericsson T. (1995). Growth and shoot:root ratio of seedlings in relation to nutrient availability. *Plant and Soil* 168/169:205–214.

Erisman JW, Grennfelt P, Sutton M. (2003). The European perspective on nitrogen emission and deposition. *Environment International* 29:311–325.

Erland S, Soderstrom B. (1990). Effects of liming on ectomycorrhizal fungi infecting *Pinus-sylvestris* L. 1. Mycorrhizal infection in limed humus in the laboratory and isolation of fungi from mycorrhizal roots. *New Phytologist* 115:675–682.

Erland S, Taylor AFS. (2002). Diversity of ecto-mycorrhizal fungal communities in relation to the abiotic environment. In *Ecological Studies*, Vol. 157, *Mycorrhizal Ecology*, van der Heijden Marcel GA, Sanders-Ian RE. (Eds.). Berlin, Springer-Verlag, pp. 163–200.

Fellner R, Landa J. (2003). Mycorrhizal revival: case study from the Giant Mts., Czech Republic. *Czech Mycology* 54:193–203.

Finlay RD, Frostegard A, Sonnerfeldt AM. (1992). Utilization of organic and inorganic nitrogen-sources by ectomycorrhizal fungi in pure culture and in symbiosis with *Pinus-contorta* Dougl Ex Loud. *New Phytologist* 120:105–115.

Galloway JN, Cowling EB. (2002). Reactive nitrogen and the world: 200 years of change. *Ambio* 31:64–71.

Gardes M, Bruns TD. (1996). Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: above- and below-ground views. *Canadian Journal of Botany-Revue* 74:1572–1583.

Giardina CP, Ryan MG. (2002). Total belowground carbon allocation in a fast-growing *Eucalyptus* plantation estimated using a carbon balance approach. *Ecosystems* 5:487–499.

Griffiths RP, Baham JE, Caldwell BA. (1994). Soil solution chemistry of ectomycorrhizal mats in forest soil. *Soil Biology and Biochemistry* 26:331–337.

Halling RE. (1996). Boletaceae (Agaricales): latitudinal biodiversity and biological interactions in Costa Rica and Colombia. *Revista de Biologia Tropical* 44:111–114.

Halling RE. (2001). Ectomycorrhizae: co-evolution, significance, and biogeography. *Annals of the Missouri Botanical Garden* 88:5–13.

Hoeksema JD, Kummel M. (2003). Ecological persistence of the plant-mycorrhizal mutualism: a hypothesis from species coexistence theory. *American Naturalist* 162:S40–S50.

Högberg P, Högberg MN, Quist ME, Ekblad A, Nasholm T. (1999). Nitrogen isotope fractionation during nitrogen uptake by ectomycorrhizal and non-mycorrhizal *Pinus sylvestris*. *New Phytologist* 142:569–576.

Holland EA, Dentener FJ, Braswell BH, Sulzman JM. (1999). Contemporary and pre-industrial global reactive nitrogen budgets. *Biogeochemistry Dordrecht* 46:7–43.

Hora FB. (1959). Quantitative experiments on toadstool production in woods. *Transactions of the British Mycological Society* 42:1–14.

Horton TR, Bruns TD. (2001). The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. *Molecular Ecology* 10:1855–1871.

Houghton JT, Ding Y, Griggs DJ, Noguer M, van der Linden PJ, Dai X, Maskell K, Johnson CA. (2001). *Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge, U.K., Cambridge University Press.

Hung LL, Trappe JM. (1983). Growth variation between and within species of ectomycorrhizal fungi in response to pH *in-vitro*. *Mycologia* 75:234–241.

Ingestad T, Agren GI. (1991). The influence of plant nutrition on biomass allocation. *Ecological Applications* 1:168–174.

Johansson M. (2000). The influence of ammonium nitrate on the root growth and ericoid mycorrhizal colonization of *Calluna vulgaris* (L.) Hull from a Danish heathland. *Oecologia* 123:418–424.

Johnson NC. (1993). Can fertilization of soil select less mutualistic mycorrhizae? *Ecological Applications* 3:749–757.

Johnson NC, Graham JH, Smith FA. (1997). Functioning of mycorrhizal associations along the mutualism-parasitism continuum. *New Phytologist* 135:575–586.

Jonsson L, Anders D, Tor-Erik B. (2000). Spatiotemporal distribution of an ectomycorrhizal community in an oligotrophic Swedish *Picea abies* forest subjected to experimental nitrogen addition: above- and below-ground views. *Forest Ecology and Management* 132:143–156.

Kainulainen P, Utriainen J, Holopainen JK, Oksanen J, Holopainen T. (2000). Influence of elevated ozone and limited nitrogen availability on conifer seedlings in an open-air fumigation system: effects on growth, nutrient content, mycorrhiza, needle ultrastructure, starch and secondary compounds. *Global Change Biology* 6:345–355.

Kårén O. (1997). Effects of Air Pollution and Forest Regeneration Methods on the Community Structure of Ectomycorrhizal Fungi. Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, Uppsala. Ph.D. dissertation.

Kårén O, Nylund JE. (1997). Effects of ammonium sulphate on the community structure and biomass of ectomycorrhizal fungi in a Norway spruce stand in southwestern Sweden. *Canadian Journal of Botany* 75:1628–1642.

Kasurinen A, Helmisaari HS, Holopainen T. (1999). The influence of elevated CO_2 and O_3 on fine roots and mycorrhizas of naturally growing young Scots pine trees during three exposure years. *Global Change Biology* 5:771–780.

Keane KD, Manning WJ. (1988). Effects of ozone and simulated acid-rain on birch seedling growth and formation of ectomycorrhizae. *Environmental Pollution* 52:55–65.

Kothe E, Mueller D, Krause K. (2002). Different high affinity phosphate uptake systems of ectomycorrhizal *Tricholoma* species in relation to substrate specificity. *Journal of Applied Botany* 76:127–131.

Kytöviita MM, Le Thiec D, Dizengremel P. (2001). Elevated CO_2 and ozone reduce nitrogen acquisition by *Pinus halepensis* from its mycorrhizal symbiont. *Physiologia Plantarum* 111:305–312.

Kytöviita MM, Pelloux J, Fontaine V, Botton B, Dizengremel P. (1999). Elevated CO_2 does not ameliorate effects of ozone on carbon allocation in *Pinus halepensis* and *Betula pendula* in symbiosis with *Paxillus involutus*. *Physiologia Plantarum* 106:370–377.

Laiho O. (1970). *Paxillus-involutus* as a mycorrhizal symbiont of forest trees. *Acta Forestalia Fennica* 106:5–72.

Lehto T. (1994). Effects of pH soil and calcium on mycorrhizas of *Picea-abies*. *Plant and Soil* 163:69–75.

Lilleskov EA, Fahey TJ, Horton TR, Lovett GM. (2002a). Belowground ectomycorrhizal fungal community change over a nitrogen deposition gradient in Alaska. *Ecology* 83:104–115

Lilleskov EA, Fahey TJ, Lovett GM. (2001). Ectomycorrhizal fungal aboveground community change over an atmospheric nitrogen deposition gradient. *Ecological Applications* 11:397–410.

Lilleskov EA, Hobbie EA, Fahey TJ. (2002b). Ectomycorrhizal fungal taxa differing in response to nitrogen deposition also differ in pure culture organic nitrogen use and natural abundance of nitrogen isotopes. *New Phytologist* 154:219–231.

Martin F, Diez J, Dell B, Delaruelle C. (2002). Phylogeography of the ectomycorrhizal *Pisolithus* species as inferred from nuclear ribosomal DNA ITS sequences. *New Phytologist* 153:345–357.

McKenzie EHC, Buchanan PK, Johnston PR. (2000). Checklist of fungi on *Nothofagus* species in New Zealand. *New Zealand Journal of Botany* 38:635–720.

Mcquattie CJ, Schier GA. (1992). Effect of ozone and aluminum on pitch pine (*Pinus-rigida*) seedlings: anatomy of mycorrhizae. *Canadian Journal of Forest Research* 22:1901–1916.

Mehmann B, Egli S, Braus GH, Brunner I. (1995). Coincidence between molecularly or morphologically classified ectomycorrhizal morphotypes and fruitbodies in a spruce forest. In *Biotechnology of Ectomycorrhizae: Molecular Approaches*, Stocchi V, Bonfante P, Nuti ME. (Eds.). New York, Plenum Press, pp. 41–52.

Meier S, Grand LF, Schoeneberger MM, Reinert RA, Bruck RI. (1990). Growth, ectomycorrhizae and nonstructural carbohydrates of loblolly-pine seedlings exposed to ozone and soil-water deficit. *Environmental Pollution* 64:11–27.

Meier S, Robarge WP, Bruck RI, Grand LF. (1989). Effects of simulated rain acidity on ectomycorrhizae of red spruce seedlings potted in natural soil. *Environmental Pollution* 59:315–324.

Menge JA, Grand LF. (1978). Effect of fertilization on production of epigeous basidiocarps by mycorrhizal fungi in loblolly pine plantations. *Canadian Journal of Botany* 56:2357–2362.

Mickley LJ, Jacob DL, Rind D. (2001). Uncertainty in preindustrial abundance of tropospheric ozone: implications for radiative forcing calculations. *Journal of Geophysical Research* 106:3389–3399.

Nilsson J, Grenfelt P. (Eds.). (1988). Critical loads for sulphur and nitrogen. Copenhagen, Denmark, NORD 1988:15, Nordic Council of Ministers.

Nilsson LO, Wallander H. (2003). Production of external mycelium by ectomycorrhizal fungi in a Norway spruce forest was reduced in response to nitrogen fertilization. *New Phytologist* 158:409–416.

Ohenoja E. (1978). Mushrooms and mushroom yields in fertilised forests. *Annales Botanici Fennici* 15:38–46.

Ohenoja E. (1989). Forest fertilization and fruiting body production in fungi. In *Fourth Convegno Internazionale Micologia*, Vol. 7, *Attivitata del Centro Studi per la Flora Mediterannea*, Borgo Taro, Italy, pp. 233–252.

Pacheo S, Cambraia J, Kasuya MCM. (1991). Effect of different levels of phosphorus on acid phosphatase activity and mineral composition of some ectomycorrhizal fungi. *Revista de Microbiologia* 22:345–348.

Perez-Soba M, Dueck TA, Puppi G, Kuiper PJC. (1995). Interactions of elevated CO_2, NH_3 and O_3 on mycorrhizal infection, gas-exchange and N-metabolism in saplings of Scots pine. *Plant and Soil* 176:107–116.

Peter M, Ayer F, Egli S. (2001). Nitrogen addition in a Norway spruce stand altered macromycete sporocarp production and below-ground ectomycorrhizal species composition. *New Phytologist* 149:311–325.

Prescott CE, Coward LP, Weetman GF, Gessel SP. (1993). Effects of repeated nitrogen fertilization on the ericaceous shrub, salal (*Gaultheria shallon*), in two coastal Douglas-fir forests. *Forest Ecology and Management* 61:45–60.

Qiu Z, Chappelka AH, Somers GL, Lockaby BG, Meldahl RS. (1993). Effects of ozone and simulated acidic precipitation on ectomycorrhizal formation on loblolly-pine seedlings. *Environmental and Experimental Botany* 33:423–431.

Rantanen L, Palomaki V, Holopainen T. (1994). Interactions between exposure to O_3 and nutrient status of trees: effects on nutrient content and uptake, growth, mycorrhiza and needle ultrastructure. *New Phytologist* 128:679–687.

Read DJ. (1991). Mycorrhizas in ecosystems. *Experientia* 47:376–391.

Read DJ. (1996). The structure and function of the ericoid mycorrhizal root. *Annals of Botany* 77:365–374.

Redecker D, Szaro TM, Bowman RJ, Bruns TD. (2001). Small genets of *Lactarius xanthogalactus*, *Russula cremoricolor* and *Amanita francheti* in late-stage ectomycorrhizal successions. *Molecular Ecology* 10:1025–1034.

Reich PB, Schoettle AW, Stroo HF, Troiano J, Amundson PG. (1985). Effects of ozone, sulfur dioxide and acidic rain on mycorrhizal infection in northern red oak *Quercus-Rubra* seedlings. *Canadian Journal of Botany* 63:2049–2055.

Roth DR, Fahey TJ. (1998). The effects of acid precipitation and ozone on the ectomycorrhizae of red spruce saplings. *Water Air and Soil Pollution* 103:263–276.

Ryan EA, Alexander IJ. (1992). Mycorrhizal aspects of improved growth of spruce when grown in mixed stands on heathlands. In *Mycorrhizas in Ecosystems*, Read DJ, Lewis DH, Fitter AH, Alexander IJE. (Eds.). Wallingford, England, CAB International, pp. 237–245.

Ryan MG, Hubbard RM, Pongracic S, Raison RJ, McMurtrie RE. (1996). Foliage, fine-root, woody-tissue and stand respiration in *Pinus radiata* in relation to nitrogen status. *Tree Physiology* 16:333–343.

Sagara N. (1992). Experimental disturbances and epigeous fungi. In *Mycology Series*, Vol. 9, *The Fungal Community: Its Organization and Role in the Ecosystem*, 2nd ed., Carroll GC, Wicklow DT. (Eds.). New York, Marcel Dekker, pp. 427–454.

Salo K. (1979). Mushrooms and mushroom yield on transitional peatlands in central Finland. *Annales Botanici Fennici* 16:181–192.

Saunders EM, Taylor AFS, Read DJ. (1996). Ectomycorrhizal community response to simulated pollutant nitrogen deposition in a Sitka spruce stand, North Wales. In *First International Conference on Mycorrhizae*, Szaro TM, Bruns TD, Eds., Berkeley, CA, p. 106.

Schlesinger WH. (1997). *Biogeochemistry: An Analysis of Global Change*, 2nd ed. San Diego, Academic Press.

Shaw PJA, Dighton J, Poskitt J, McLeod AR. (1992). The effects of sulphur dioxide and ozone on the mycorrhizas of Scots pine and Norway spruce in a field fumigation system. *Mycological Research* 96:785–791.

Shubin VI, Ronkonen NI, Saukkonen AV. (1977). Effect of fertilizer on fructification of macromycetes on young birch trees. *Mikologiya i Fitopatologiya* 11:294–303.

Simmons GL, Kelly JM. (1989). Influence of O_3, rainfall acidity, and soil Mg status on growth and ectomycorrhizal colonization of loblolly-pine roots. *Water Air and Soil Pollution* 44:159–171.

Smit E, Veenman C, Baar J. (2003). Molecular analysis of ectomycorrhizal basidiomycete communities in a *Pinus sylvestris* L. stand reveals long-term increased diversity after removal of litter and humus layers. *FEMS Microbiology Ecology* 45:49–57.

Strengbom J, Nordin A, Nasholm T, Ericson L. (2001). Slow recovery of boreal forest ecosystem following decreased nitrogen input. *Functional Ecology* 15:451–457.

Stroo HF, Alexander M. (1985). Effect of simulated acid rain on mycorrhizal infection of *Pinus-Strobus*. *Water Air and Soil Pollution* 25:107–114.

Stroo HF, Reich PB, Schoettle AW, Amundson RG. (1988). Effects of ozone and acid rain on white pine *Pinus-strobus* seedlings grown in five soils. II. Mycorrhizal infection. *Canadian Journal of Botany* 66:1510–1516.

Taylor AFS, Martin F, Read DJ. (2000). Fungal diversity in ectomycorrhizal communities of Norway spruce (*Picea abies* (L.) Karst.) and Beech (*Fagus sylvatica* L.) in forests along north-south transects in Europe. In *Carbon and Nitrogen Cycling in European Forest Ecosystems*, Vol. 142, Schulze ED. (Ed.). Heidelberg, Springer-Verlag, pp. 343–365.

Termorshuizen AJ. (1993). The Influence of nitrogen fertilizers on ectomycorrhizas and their fungal carpophores in young stands of *Pinus-sylvestris*. *Forest Ecology and Management* 57:179–189.

Termorshuizen AJ, Schaffers AP. (1987). Occurrence of carpophores of ectomycorrhizal fungi in selected stands of *Pinus-sylvestris* in the Netherlands in relation to stand vitality and air-pollution. *Plant and Soil* 104:209–217.

Thompson GW, Medve RJ. (1984). Effects of aluminum and manganese on the growth of ectomycorrhizal fungi. *Applied and Environmental Microbiology* 48:556–560.

Väre H, Bäck J, Hyvärinen M. (1993). Experimental design in fumigation experiments in relation to results. *Aquilo Ser Botanica* 32:55–67.

Wallander H. (1995). A new hypothesis to explain allocation of dry-matter between mycorrhizal fungi and pine-seedlings in relation to nutrient supply. *Plant and Soil* 169:243–248.

Wallander H, Nylund JE. (1992). Effects of excess nitrogen and phosphorus starvation on the extramatrical mycelium of ectomycorrhizas of *Pinus-sylvestris* L. *New Phytologist* 120:495–503.

Wallander H, Arnebrant K, Dahlberg A. (1999). Relationships between fungal uptake of ammonium, fungal growth and nitrogen availability in ectomycorrhizal *Pinus sylvestris* seedlings. *Mycorrhiza* 8:215–223.

Wallander H, Nilsson LO, Hagerberg D, Baath E. (2001). Estimation of the biomass and seasonal growth of external mycelium of ectomycorrhizal fungi in the field. *New Phytologist* 151:753–760.

Wallander H, Wickman T, Jacks G. (1997). Apatite as a P source in mycorrhizal and non-mycorrhizal *Pinus sylvestris* seedlings. *Plant and Soil* 196:123–131.

Wallenda T, Kottke I. (1998). Nitrogen deposition and ectomycorrhizas. *New Phytologist* 139:169–187.

Wästerlund I. (1982). Do pine mycorrhizae disappear following fertilization? *Svensk Botanisk Tidskrift* 76:411–417.

Wiklund K, Nilsson LO, Jacobsson S. (1995). Effect of irrigation, fertilization, and artificial drought on basidioma production in a Norway spruce stand. *Canadian Journal of Botany* 73:200–208.

Yamanaka T. (1999). Utilization of inorganic and organic nitrogen in pure cultures by saprotrophic and ectomycorrhizal fungi producing sporophores on urea-treated forest floor. *Mycological Research* 103:811–816.

Yesmin L, Gammack SM, Cresser MS. (1996). Effects of atmospheric nitrogen deposition on ericoid mycorrhizal infection of *Calluna vulgaris* growing in peat. *Applied Soil Ecology* 4:49–60.

Yoshida LC, Gamon JA, Andersen CP. (2001). Differences in above- and below-ground responses to ozone between two populations of a perennial grass. *Plant and Soil* 233:203–211.