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Research review

Mycorrhizal fungi have a potential role in soil carbon storage under elevated CO_2 and nitrogen deposition

K. K. TRESEDER* AND M. F. ALLEN

Center for Conservation Biology, University of California at Riverside, Riverside, CA 92521, USA

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SUMMARY

In this review, we discuss the potential for mycorrhizal fungi to act as a source or sink for carbon (C) under elevated CO_2 and nitrogen deposition. Mycorrhizal tissue has been estimated to comprise a significant fraction of soil organic matter and below-ground biomass in a range of systems. The current body of literature indicates that in many systems exposed to elevated CO_2, mycorrhizal fungi might sequester increased amounts of C in living, dead and residual hyphal biomass in the soil. Through this process, the fungi might serve as a negative feedback on the rise in atmospheric CO_2 levels caused by fossil fuel burning and deforestation. By contrast, a few preliminary studies suggest that N deposition might increase turnover rates of fungal tissue and negate CO_2 effects on hyphal biomass. If these latter responses are consistent among ecosystems, C storage in hyphae might decline in habitats surrounding agricultural and urban areas. When N additions occur without CO_2 enrichment, effects on mycorrhizal growth are inconsistent. We note that analyses of hyphal decomposition under elevated CO_2 and N additions are extremely sparse but are critical in our understanding of the impact of global change on the cycling of mycorrhizal C. Finally, shifts in the community composition of arbuscular and ectomycorrhizal fungi with increasing CO_2 or N availability are frequently documented. Since mycorrhizal groups vary in growth rate and tissue quality, these changes in species assemblages could produce unforeseeable impacts on the productivity, survivorship, or decomposition of mycorrhizal biomass.

Key words: arbuscular mycorrhizal fungi, ectomycorrhizal fungi, elevated CO_2, external hyphae, interspecific variation, microbial communities, nitrogen deposition or fertilization, soil carbon sequestration.

INTRODUCTION

Land-based ecosystems in the northern hemisphere appear to remove, at least temporarily, a substantial portion of anthropogenic CO_2 from the atmosphere (Tans *et al.*, 1990; Ciais *et al.*, 1995; Schimel *et al.*, 1995; Keeling *et al.*, 1996; Fung *et al.*, 1997). The mechanisms behind this C sink are not well understood, even though knowledge of these processes is vital to predict and interpret the responses of ecosystems to global change (Field & Fung, 1999). Changes in plant productivity due to CO_2 enrichment (Friedlingstein *et al.*, 1995; Thompson *et al.*, 1996), nitrogen deposition (Nadelhoffer *et al.*, 1999), land use change (Houghton *et al.*, 1999), and climatic effects (Dai & Fung, 1993; Malmstrom *et al.*, 1997) have been investigated as potential components (Schimel *et al.*, 1995; Lloyd, 1999). However, the response of microbial communities to these perturbations, and their potential influence on C cycling, have received scarce attention.

Mycorrhizal fungi in particular might play an important role in the sequestration of C in soil under elevated CO_2 and N deposition. This group, which symbiotically colonizes plant roots, forms associations with 80% of plant species and is found in nearly every habitat in the world (Smith & Read, 1997). Plants allocate an estimated 10–20% of net photosynthate to mycorrhizal fungi, although this number can range from 5 to 85% among systems (reviewed by Allen, 1991).

A substantial amount of C allotted to mycorrhizal tissues could be long-lived in the soil. Chitin, which

*Author for correspondence (fax +1 909 787 4625; e-mail treseder@mail.ucr.edu).

is not readily decomposed (Gooday, 1994), can constitute up to 60% of fungal cell walls (Muzzarelli, 1977). Arbuscular mycorrhizal (AM) fungi are also the sole producers of glomalin, a potentially recalcitrant glycoprotein (Wright *et al.*, 1996; Wright & Upadhyaya, 1996, 1999). AM hyphae in the absorptive hyphal network (nonrunner hyphae) have lifespans of only 5–7 d (Friese & Allen, 1991a), and with each cycle residual hyphal C should remain in the soil. Furthermore, some micro-arthropods prefer to graze on nonmycorrhizal fungi rather than on a variety of AM fungi (Klironomos & Kendrick, 1996; Klironomos & Ursic, 1998; Klironomos *et al.*, 1999), and therefore might not necessarily speed up tissue turnover significantly. As a result, glomalin alone can account for 30–60% of C in undisturbed soils (calculated from data of Wright & Upadhyaya (1996), assuming that the protein is 30% C by weight M. C. Rillig, pers. comm.). Likewise, portions of ectomycorrhizal (ECM) biomass (sheaths, Hartig nets and fruit bodies) were responsible for approx. 15% of soil organic matter in two hardwood forests (Vogt *et al.*, 1982, cited by Vogt *et al.*, 1991). Carbon derived from mycorrhizal tissue can account for a significantly sized pool within ecosystems (Vogt *et al.*, 1982; Fogel & Hunt, 1983; Vogt *et al.*, 1991; O'Neill, 1994; Allen *et al.*, 1995; Rillig & Allen, 1999) and globally.

Because mycorrhizal fungi acquire most or all their C directly from living plants, the nutrient status of foliage strongly affects mycorrhizal growth. As elevated CO_2 generally increases plant growth (Poorter, 1993) and root-to-shoot ratio (Rogers *et al.*, 1996), greater allocation of C to mycorrhizal structures might follow (Norby *et al.*, 1986; O'Neill *et al.*, 1987). Effects of elevated CO_2 on mycorrhizal growth have been reviewed by O'Neill (1994), Diaz (1996), Hodge (1996), and Staddon & Fitter (1998), with an emphasis on changes in percentage root length (or tips) colonized and total root length colonized per plant. These reviews indicate that the percentage of roots with mycorrhizal structures might not necessarily change under elevated CO_2. However, as root biomass tends to rise, total mycorrhizal biomass per plant might do so as well. This response varies among systems and does not necessarily occur universally. By contrast, increases in N availability through deposition or fertilization tend to reduce root colonization and fruit body production by ECM fungi (reviewed by Arnolds, 1988; Jansen & Dighton, 1990; Arnolds, 1991; Colpaert & van Tichelen, 1996; Wallenda & Kottke, 1998). Effects of CO_2 and N availability on the biomass or production of extraradical hyphae have been less intensively studied or summarized.

In this review, we address the current state of knowledge regarding the potential for mycorrhizal tissue (particularly hyphae) to form a sink or source of C in response to elevated CO_2 or N deposition. First, we present an overview of processes and pools involved in the cycling of mycorrhizal C and the relevance of various measures of mycorrhizal dynamics (e.g. percentage colonization, hyphal length, vital staining and ergosterol concentration). Inter- and intraspecific variations in traits that could affect C dynamics are considered. Second, we discuss known effects of CO_2 concentration on hyphal biomass, turnover, tissue quality and community composition. Next, we focus on the influence of N availability on these same factors, and finally we address potential interactions between elevated CO_2 and N availability.

MYCORRHIZAL CARBON CYCLING

Major fluxes and pools of carbon

Processes involved in the cycling of mycorrhizal C include production, survivorship and decomposition rates of tissue. As mycorrhizal tissue grows, C is transferred from the atmosphere via plants to the pool of live hyphae. Micro-arthropods might graze a fraction of live hyphae, but grazing on AM hyphae should be low, as in feeding trials mites and collembola appear to prefer nonmycorrhizal fungi (Klironomos & Kendrick, 1996; Klironomos & Ursic, 1998; Klironomos *et al.*, 1999). When grazing of AM fungal hyphae does occur, animals often only clip the hyphae, severing connections to the root but not ingesting mycelial mass (Klironomos & Ursic, 1998). Thus changes in micro-arthropod numbers might not have a major impact on C flux from AM hyphae to soil organic matter. Instead, death rates of live hyphae determine the flux of C from the live to the dead hyphal pool. At this point, dead tissue is distributed between active and slow soil organic matter pools as a function of tissue quality (following Parton *et al.*, 1988). Active soil organic matter includes sugars and other metabolites that are processed relatively quickly (in days to a few years) by decomposers; slow soil organic matter consists of recalcitrant components such as chitin and glomalin, and might last from years to decades in the soil. In plant tissues, higher N content generally speeds decomposition rates (Melillo *et al.*, 1982). Finally, as soil organic matter decomposes, a portion of C remains in decomposer tissues, and the rest returns to the atmosphere. Each of these fluxes and pools might be affected directly by elevated CO_2 and N deposition, or indirectly through changes in the composition of the mycorrhizal community.

The role of inter- and intraspecific variation

Groups of mycorrhizal fungi differ in several factors, including growth rate, that could influence C cycling. For example, isolates of ECM fungi vary markedly

in productivity, both within species (Wallander *et al*., 1999; reviewed by Cairney, 1999) and among species (Wallander *et al*., 1999). In AM fungi, Sanders *et al*. (1998) observed significant differences in hyphal biomass (after 18 wk growth on *Prunella vulgaris*) among three *Glomus* species. In addition, after 16 wk growth, total hyphal lengths of *Acaulospora denticulata* and *Scutellospora calospora* were significantly greater than those of two *Glomus* species in a glasshouse experiment with *Artemisia tridentata* (Klironomos *et al*., 1998). If mycorrhizal communities are altered by climate change, then variation in growth and biomass among groups could affect the amount of atmospheric C that is initially drawn into the pool of live hyphae.

Mycorrhizal groups also differ in tissue qualities that might affect the rate at which this mycorrhizal C is returned to the atmosphere. Wallander *et al*. (1997) found that five morphotypes of ECM fungi on field-grown *Pinus sylvestris* varied more than twofold in chitin concentration. Likewise, glomalin content in AM hyphae differed between *Gigaspora* and *Glomus* (approx. 20 and 60 μg protein mg^{-1} hyphae, respectively; Wright *et al*., 1996), and significantly between *Glomus caledonium* and *Glomus intraradices* (Wright & Upadhyaya, 1999). In addition, mean N concentrations in the hyphae of four isolates of *Paxillus involutus* (an ECM fungus) ranged from 5 to 9% when grown in culture (Wallander *et al*., 1999). Nitrogen content might be related to decomposability of fungal tissue (see 'Major fluxes and pools of carbon'). These results suggest that the identity, as well as the amount, of mycorrhizal fungi might be important in soil C dynamics.

Measures of mycorrhizal response to environmental changes

As most mycorrhizal structures are relatively delicate and often below ground, measurements of mycorrhizal biomass, growth rate or turnover present some challenges. Most mycorrhizal studies under elevated CO_2 or N deposition have quantified changes in mycorrhizal colonization (percentage root length colonized by AM, or percentage root tips colonized by ECM). This measure might be an appropriate index for nutrient transfer to the host plant (in AM fungi the presence of internal structures such as arbuscules implies transfer of P). However, because extraradical hyphae account for a large portion of fungal biomass (30–87% of ECM fungi; Colpaert *et al*., 1992; Wallander & Nylund, 1992, cited by Ekblad *et al*., 1995), direct measures of hyphal length are a valuable indicator of the mycorrhizal C pool (Rillig & Allen, 1999). Furthermore, root colonization does not necessarily increase linearly with hyphal biomass, and environmental changes might alter relationships between the two variables. For instance, the ratio of AM hyphal length : total root length colonized by AM varied nearly twofold among CO_2 and N treatments in *Gutierrezia sarothrae* (Rillig & Allen, 1998), and was nearly three times greater under ambient versus elevated CO_2 in a serpentine grassland (Rillig *et al*., 1999a). In an additional study, Staddon *et al*. (1999) noted a decrease in this ratio with elevated CO_2 in *Plantago lanceolata* and *Trifolium repens*. For this reason we focus primarily on hyphal length or biomass in this review. We emphasize that hyphal length per unit soil area is a particularly meaningful variable in field studies, and might be used eventually to scale biomass to the ecosystem or regional level.

The life stage of hyphae is also an important consideration when measuring fungal productivity. Few CO_2 or N studies have made the distinction between live and dead hyphae when determining hyphal length. The size of this combined pool is a function of productivity, survival rate and initial decomposition rate, and therefore changes in any combination of these factors might influence the results. Although the magnitude of these two pools provides useful information regarding immobilization of C, the influence of underlying mechanisms must be interpreted with caution. To draw conclusions about productivity, the growth rate of live hyphae must be directly followed. Several techniques enable us to focus on this live pool. For example, ergosterol concentrations are thought to indicate relative amounts of living fungal cytoplasm (Salmanowicz & Nylund, 1988; Martin *et al*., 1990; Nylund & Wallander, 1992), and are an appropriate measure when no nonmycorrhizal fungi are present. In addition, stains such as fluorescein diacetate, differential fluorescent stain and immunofluorescent antibodies (Friese & Allen, 1991b; Morris *et al*., 1997) can distinguish between live and dead hyphae. Immunofluorescent antibodies are also specific to different genera and can be used to characterize the community composition of hyphae (Egerton-Warburton & Allen, 2000). This information, together with live and dead hyphal biomass, can be critical in analyses of mycorrhizal C dynamics.

EFFECTS OF ELEVATED CO_2

Hyphal biomass and productivity

Mycorrhizal fungi might provide a negative feedback on anthropogenic CO_2 emissions by responding to rising concentrations of this trace gas. Overall, elevated CO_2 tends to increase or produce no change in hyphal biomass or growth on both ECM and AM fungi (Table 1). These variables are determined independently of plant growth or biomass, and are therefore not an indication of relative allocation between plant and fungal tissue. Studies have reported variation among mycorrhizal species, plant

Table 1. *Effects of elevated CO_2 on growth or biomass of mycorrhizal hyphae*

Reference	Host plant or system	Mycorrhizal species	Growth environment*	Duration	CO_2 concentrations	Growth or biomass response (elevated: ambient CO_2)
Ectomycorrhizal fungi						
Ineichen *et al.* (1995)	*Pinus sylvestris* seedlings	*Pisolithus tinctoris*	Petri dishes, GC	25 d 56 d 91 d	350/600 ppm	NS NS ~2.0
Tingey *et al.* (1995)	*Pinus ponderosa* seedlings	Mixed (mostly *Thelephora terrestris*)	OTC	2.5 yr	Ambient/ambient +175 ppm/ambient +350 ppm	Increase across three N levels (Table 2)†
Godbold *et al.* (1997)	*Betula papyrifera* saplings *Pinus strobus* saplings	Mixed	GH	25 wk 35 wk	Ambient/700 ppm	1.6 NS
Rouhier & Read (1998a)	*Pinus sylvestris* seedlings	*Suillus bovinus* *Paxillus involutus*	Plexiglass observation chambers	87 d 55 d	350/700 ppm	2.3 4.4
Arbuscular mycorrhizal fungi						
Klironomos *et al.* (1996)	*Artemisia tridentata* seedlings	Mixed	Pots, GC	12 wk	350/700 ppm	~2.3‡
Klironomos *et al.* (1997)	*Populus tremuloides* saplings	Mixed	OTC	14 months	350/700 ppm	1.8 (low N; Table 2) NS (high N)
Klironomos *et al.* (1998)	*Artemisia tridentata* seedlings	*Glomus intraradices* *G. etunicatum* *Acaulospora denticulata* *Scutellospora calospora*	Pots, GC	16 wk	350/700 ppm	NS NS ~1.4 ~1.5
Lussenhop *et al.* (1998)	*Populus × euramericana* saplings (constructed ecosystem)	Mixed (inoculum from high fertility soil)	OTC	5 months	34.5/69.3 Pa	NS (low N; Table 2) NS (high N)
Rillig & Allen (1998)	*Gutierrezia sarothrae*	Mixed	Pots, GC	4 months	Ambient/750 ppm	~2.1 (low N; Table 2) NS (high N)
Rouhier & Read (1998b)	*Plantago lanceolata*	Mixed (inoculum from dune turf roots)	Pots, GH	41, 76, 104 d	350/540 ppm	NS at any harvest
Sanders *et al.* (1998)	*Prunella vulgaris*	*Glomus* isolates Bassle Pi and BEG 19	Pots, GC	20 wk	350/600 ppm	3.8 (1–2 cm from root) 3.9 (6.5–7.5 cm) 5.2 (13–14 cm)
Rillig *et al.* (1999a)	Serpentine grassland Sandstone grassland	Mixed	OTC	5.25 yr	Ambient/ambient+ 350 ppm	1.9 NS
Staddon *et al.* (1999)	*Plantago lanceolata* *Trifolium repens*	*Glomus mosseae*	Pots, OTC	37–71 d 42–75 d	400/650 ppm	Up to 1.7 Up to 2.3 (varied with harvest)

*GC, growth chamber; GH, glasshouse; OTC, open-top chamber. †Could include some nonmycorrhizal species. ‡Used DFS staining to measure hyphal biomass.

communities, N treatments or harvest dates; however none has detected a significant decrease in hyphal length or growth. This trend suggests a possible increase in global mycorrhizal biomass as atmospheric CO_2 levels rise, although the magnitude of this response might vary regionally and among species.

Long-term field-based studies of mycorrhizal biomass under elevated CO_2 are rare but critical in predicting responses of natural systems. Rillig *et al*. (1999a) found increases in AM hyphal biomass in a serpentine grassland (but no significant change in a sandstone grassland) after >5 yr CO_2 treatment. This change could be due to indirect effects of shifts in plant or fungal communities, or direct effects on plant C status. A similar increase occurred in AM fungi associated with trembling aspen after 14 months (Klironomos *et al*., 1997). Likewise, Tingey *et al*. (1995) noted a rise in the presence of ECM root tips and visible hyphae after 2.5 yr enrichment. With the exception of the sandstone grassland, these results are not consistent with the hypothesis that hyphal lengths in soils are already at a maximum under ambient CO_2 and will not increase as CO_2 levels rise (O'Neill, 1994; Allen *et al*., 1995). Each of these studies focused on changes in the incidence of live and dead hyphae combined.

Additional field studies have indicated that the quantity of soil organic matter derived from mycorrhizal tissue might rise under CO_2 enrichment. Rillig *et al*. (1999b) reported an increase in glomalin concentrations in soil from a chaparral system exposed to elevated CO_2 for 3 yr. In large macroaggregates (1–2 mm diameter) from the same ecosystem, the length of live AM hyphae increased 10-fold as CO_2 treatments varied from 250 to 650 ppm CO_2 (K. Treseder, unpublished). This increase in mycorrhizal biomass was accompanied by a 30-fold rise in C allocation to these macroaggregates. These field-based studies suggest that the combined influences of elevated CO_2 on mycorrhizal C dynamics (including community changes, productivity and decomposition) could ultimately produce an increase in the amount of C sequestered in intact hyphae and their residual components. However, many more studies of this nature are required before we can make general statements with any certainty. In addition, it is not clear whether these increases in hyphal biomass or residues will be maintained at equilibrium levels after the system has adjusted to the sudden rise in CO_2 that occurred at the onset of the experiment.

Controlled, smaller-scale experiments provide insights into the mechanisms underlying the increase in hyphal biomass in field systems. For example, Rouhier & Read (1998a) directly followed hyphal growth of two ECM fungi and noted a positive response in both under CO_2 enrichment. These measurements of actual growth rates are rare.

Most investigations have been conducted in growth chambers or glasshouses for periods from several weeks to months (Table 1). Many of these have reported augmentation of hyphal length under CO_2 enrichment. However, the duration and scale of these experiments impose some limitations in scaling up to ecosystem-level dynamics or in assessing underlying mechanisms (Allen, 1996). As the plants and fungi were not grown in an intact community, they were not necessarily subject to competition or interactions from higher trophic levels. In addition, the bacterial community might not have been representative of those found in natural systems. Decomposition might also be a factor in experiments lasting more than a few weeks, and could introduce some unknown degree of error into interpretation of growth rates. Klironomos *et al*. (1996) used differential fluorescent staining to restrict biomass measurements to live hyphae, and found a more than twofold increase with elevated CO_2. This response might also have been affected somewhat by changes in lifespan of the fungi. Nevertheless, the general trend toward increases in hyphal biomass (usually associated with increasing plant biomass) under elevated CO_2 in pot experiments indicates that the abundance of mycorrhizal hyphae could rise in a number of AM and ECM fungal species and, potentially, ecosystems. Notably, the hyphal lengths of ECM fungi do not appear to demonstrate a greater frequency or magnitude of response to CO_2 than do hyphae of AM fungi, as suggested by O'Neill (1994).

Shifts in the mycorrhizal community

Mycorrhizal groups vary in the magnitude of their responses to elevated CO_2 (Table 1), resulting in shifts in the mycorrhizal community structure (O'Neill, 1994; Cairney & Meharg, 1999). In AM fungi, hyphal lengths of *Acaulospora denticulata* and *Scutellospora calospora* increased in response to CO_2 enrichment, while those of two *Glomus* species did not (Klironomos *et al*., 1998). These genera were each grown separately in pots (with *Artemisia tridentata*) and did not compete for resources. In a complementary chaparral-based field experiment, the abundance of hyphae from four AM genera (*Acaulospora*, *Gigaspora*, *Glomus* and *Scutellospora*) was evenly distributed in chambers exposed to CO_2 concentrations of 250–350 ppm, but *Acaulospora* and *Scutellospora* dominated at 450–650 ppm (K. Treseder, unpublished). The results of these two studies indicate that *Acaulospora* and *Scutellospora* might become more prevalent as CO_2 levels rise.

Interspecific variation and shifts in community composition have also been documented in ECM fungi. For example, Rouhier & Read (1998a) reported that the biomass of *P. involutus* responded more strongly to a doubling of CO_2 concentrations than did that of *Suillus bovinus*. Likewise, in

mycorrhizal root tips of *Betula pendula* seedlings *Leccinum* dominated at elevated CO_2, but at ambient CO_2 species were more evenly distributed (Rey & Jarvis, 1997). In addition, in *Betula papyrifera* the relative abundance of ECM morphotypes changed significantly under elevated CO_2 (Godbold *et al*., 1997), with a shift toward morphotypes with higher numbers of associated hyphae and rhizomorphs (Godbold & Berntson, 1997). Finally, in young *Pinus sylvestris* trees, CO_2 enrichment reduced by half the presence of the dominant (dichotomous) morphotype (Kasurinen *et al*., 1999), although this response could have been strictly morphological. As mycorrhizal groups vary in tissue quality and growth rate, these shifts in the mycorrhizal community might feed back to affect several processes involved in the cycling of mycorrhizal C.

Life span and decomposition of mycorrhizal tissue

The effects of elevated CO_2 on the life span or turnover of individual mycorrhizal hyphae (ECM or AM) are not known. Rygiewicz *et al*. (1997) used minirhizotrons to track the length of time between formation and disappearance of mycorrhizal root tips on seedlings of *Pinus ponderosa* and found that elevated CO_2 had no significant effect. Moreover, as high CO_2 increased production of new root tips, a greater C flux through mycorrhizas was implied. Single hyphae might respond in a similar manner, and studies of their dynamics are required. Additionally, nutrient concentrations might be related to decomposition rates in mycorrhizal tissue (as in plant tissue), and P concentrations in ECM tips of *P. involutus* and *S. bovinus* declined significantly with CO_2 enrichment (Rouhier & Read, 1998a). Nitrogen content also decreased in *P. involutus*. Finally, we note that elevated CO_2 can increase fine-root mortality (Pregitzer *et al*., 1995), which might be followed by a decrease in the life span of relatively long-lived ECM root tips and rhizomorphs.

Summary of CO_2 effects

Although approaches and scopes have varied widely among studies of elevated CO_2 on mycorrhizal dynamics, several lines of evidence indicate that pools of mycorrhizal C might increase as CO_2 levels rise. With exceptions, growth rate and biomass of total live and dead hyphae tend to increase for both AM and ECM fungi both at plant and ecosystem level. This response is probably related to concurrent increases in plant biomass (Staddon & Fitter, 1998) or to changes in plant or fungal community composition. Hyphal length rarely, if ever, decreases. In addition, preliminary evidence indicates that soil organic matter associated with mycorrhizal hyphae might become more prevalent. However, few data exist regarding changes in life span or decomposition rate under CO_2 enrichment, and these processes have strong controls over the long-term accumulation of soil organic matter pools over time. In addition, shifts in community composition of mycorrhizal fungi with CO_2 concentration might affect all aspects of mycorrhizal C cycling in an unforeseeable manner. Additional research on ecosystem-level responses and underlying mechanisms is critical.

EFFECTS OF NITROGEN DEPOSITION

Biomass and productivity

Elevated CO_2 often occurs concurrently with several other aspects of global change, including widespread N deposition. Increasing N availability might reduce investment by plants in mycorrhizal fungi. This response is especially likely for ECM fungi, as this group acts as an important mechanism of N acquisition for plants (Read, 1991). The decline in abundance of fruiting bodies of ECM fungi in European forests has been well documented (Arnolds, 1988, 1991; Jansen & Dighton, 1990; Colpaert & van Tichelen, 1996; Wallenda & Kottke, 1998; Cairney & Meharg, 1999), and N deposition is considered a major contributing factor. Nitrogen fertilization has often been used to simulate effects of N deposition, and usually produces decreases in mushroom production (Menge & Grand, 1978; Ruhling & Tyler, 1991; Termorshuizen, 1993). This decline in fruiting bodies might in itself represent a noteworthy decrease in ECM fungal biomass, but might not necessarily be accompanied by a decrease in the presence of below-ground tissue (Termorshuizen, 1993).

Extraradical hyphae can also account for a substantial portion of mycorrhizal biomass, and ecosystem-level responses of hyphae to N fertilization vary (Table 2). For example, the incidence of ECM biomass (including hyphae) in root tips was not affected by long-term fertilization in *P. ponderosa* seedlings (Tingey *et al*., 1995) or in a *Picea abies* forest (Karen & Nylund, 1997). In AM fungi, Klironomos *et al*. (1997) reported a significant reduction in hyphal length associated with *Populus tremuloides* saplings after 14 months of N additions, but Eom *et al*. (1999) observed an increase in biomass in a tallgrass prairie fertilized for 10 yr. Of these four studies, all but Karen & Nylund (1997) included both live and dead hyphae in their measurements of biomass. These inconsistencies in N response could be attributable to the initial N status of the systems, and to varying influences of alterations in growth rate, decomposition, life span or community structure.

Nitrogen treatments also have inconsistent effects on hyphal growth rate (Table 2). Arnebrant (1994) documented significant decreases in growth with increasing N availability in five ECM isolates.

Table 2. *Effects of nitrogen availability on growth or biomass of mycorrhizal hyphae*

Reference	Host plant or system	Mycorrhizal species	Growth environment*	Duration	Nitrogen additions	Growth or biomass response (N addition: control)
Ectomycorrhizal fungi						
Wallander & Nylund (1992)	*Pinus sylvestris* seedlings	*Laccaria bicolor* *Suillus bovinus*	Semi-hydroponic	6–9 wk	NH_4Cl in 1–10 or 100–200 mg N l^{-1}	~0.3† ~0.1
Arnebrant (1994)	*Pinus contorta* seedlings *P. contorta* seedlings *P. sylvestris* seedlings *P. sylvestris* seedlings *P. sylvestris* seedlings	*Paxillus involutus* isolate 1 *P. involutus* isolate 2 *S. bovinus* Unidentified Unidentified	Microcosms	2–4 months	$(NH_4)_2SO_4$, $NaNO_3$, $NH_4^+ + NO_3^-$ in 1, 2, or 4 mg N g^{-1} peat	0.8 0.4 0.3 0.5 0.3
Wallander *et al.* (1994)	*P. sylvestris* seedlings	*Hebeloma crustuliniforme*	Semi-hydroponic	5 wk	75 mg l^{-1} type of N not given	Decrease†
Ekblad *et al.* (1995)	*P. sylvestris* seedlings *Alnus incanta* seedlings	*P. involutus*	Pots, GC	13 wk 10 wk	NH_4NO_3 in 6 or 54 mg kg^{-1}	~1.5
Tingey *et al.* (1995)	*P. ponderosa* seedlings	Mixed (mostly *Thelephora terrestoris*)	OTC	2.5 yr	$(NH_4)_2SO_4$ in 0, 100 or 200 kg ha^{-1} yr^{-1}	NS in three CO_2 levels (Table 1)
Karen & Nylund (1997)	*Picea abies* adults	Mixed	Forest	4 yr	$(NH_4)_2SO_4$ in 0 or 100 kg ha^{-1} yr^{-1}	NS (biomass in root tips)†
Wallander *et al.* (1999)	*Pinus sylvestris* seedlings	*P. involutus* 1 (low-N habitat) *P. involutus* 2 (moderate N) *P. involutus* 3 (high N) *P. involutus* 4 (moderate N)	Microcosms	3–8 wk	$(NH_4)_2SO_4$ in 1, 2, or 4 mg N per g peat	NS
Arbuscular mycorrhizal fungi						
Klironomos *et al.* (1997)	*Populus tremuloides* saplings	Mixed (soil inoculum)	OTC	14 months	Soil with high (348 μg g^{-1} d^{-1}) or low (45 μg g^{-1} d^{-1}) N mineralization	0.3 across CO_2 levels (Table 1)
Lussenhop *et al.* (1998)	*Populus* × *euramericana* saplings (constructed ecosystem)	Mixed (inoculum from high-fertility soil)	OTC	5 months	High-(15.1 g N kg^{-1}) or low-(4.6 g N kg^{-1}) N soil	2.3 across CO_2 levels (Table 1)
Rillig & Allen (1998)	*Gutierrezia sarothrae*	Mixed	Pots, GC	4 months	$CaNO_3 + NH_4NO_3$ in 100 kg N ha^{-1} yr^{-1}	NS (ambient CO_2; Table 1) ~0.5 (elevated CO_2)
Bethenfalvay *et al.* (1999)	Soybean	Mixed (soil)	GH	9 wk	1 mM NH_4NO_3, 3 mM $Ca(NO_3)_2$ or 4 mM urea-$[CO(NH_2)_2]$	NS
Eom *et al.* (1999)	Tallgrass prairie	Mixed	Prairie	10 yr	NH_4NO_3 in 10 g N m^{-2} yr^{-1}	1.2

*GC, growth chamber; GH, glasshouse; OTC, open-top chamber. †Used ergosterol as index of hyphal biomass.

However, in another ECM experiment Wallander *et al*. (1999) found no significant N effect on growth rates of four isolates of *P. involutus* collected from regions exposed to varying levels of N deposition. Aside from these two studies, few direct assessments of N effects on ECM or AM growth rates are reported in the literature. In experiments that have measured ECM biomass after weeks or months of growth, decreases (Wallander & Nylund, 1992; Wallander *et al*., 1994); lack of response (Wallander & Nylund, 1992); or increases (Ekblad *et al*., 1995) under higher N availability have each been observed. Likewise, the responses of AM biomass to N concentration have been positive (Lussenhop *et al*., 1998), negative (Rillig & Allen, 1998), or not significant (Rillig & Allen, 1998; Bethlenfalvay *et al*., 1999).

Much of the inconsistency in N effects among and within studies might be due to variation in responses among mycorrhizal groups or among plant/fungal combinations. Wallander and Nylund (1992) reported that *S. bovinus* was more sensitive to N additions than was *Laccaria bicolor* when both were grown on *Pinus sylvestris* in a semi-hydroponic medium. In a microcosm experiment conducted by Arnebrant (1994), two isolates of *P. involutus*, one isolate of *S. bovinus*, and two additional unidentified species of ECM fungi were exposed to increasing N availability. The growth rate of *S. bovinus* and one unknown species declined markedly, while one *P. involutus* isolate was only slightly affected. In a separate study by Wallander *et al*. (1999), four isolates of *P. involutus* and two of *S. bovinus* grown in culture (as a complement to the seedling experiment summarized in Table 2) also displayed different sensitivities to N additions. One *P. involutus* isolate from a low-N deposition site, another from a moderate-N site, and an isolate of *S. bovinus* from a low-N site each grew significantly more slowly when supplied with excess N. Additional isolates from moderate- to high-N (or unknown) sites had no significant response. These N effects were not consistent with those of the seedling experiment, in which hyphal growth rates were not affected in any group. Nevertheless, these three studies suggest that ECM fungi might differ in productivity under N deposition, and that *S. bovinus* appears particularly susceptible. It remains to be seen whether AM groups might vary as well.

Shifts in community composition

Several studies have detailed changes caused by N deposition or fertilization in the species assemblage of mushrooms (Menge & Grand, 1978; Arnolds, 1988, 1991; Termorshuizen, 1993). However, as the community composition of ECM fruiting bodies can differ from that of below-ground ECM structures (Gardes & Bruns, 1996), and below-ground biomass could be a substantial C pool, this review focuses on the species composition of fungal structures in the soil. In both a Swedish *Picea abies* forest (Karen & Nylund, 1997) and a Scottish Sitka spruce plantation (Taylor & Alexander, 1989), frequencies of ECM morphotypes on root tips shifted upon long-term N fertilization. Likewise, in *Pinus sylvestris* forests in Sweden, colonization of 'bait' seedlings by one particular morphotype was less frequent in N-fertilized than in control plots (Arnebrant & Soderstrom, 1992).

Composition of AM communities can also shift with N availability. In a natural N deposition gradient in southern California coastal sage scrub, spores of *Scutellospora* and *Gigaspora* species became less prevalent with increasing deposition. Conversely, spores of certain *Glomus* species (e.g. *G. aggregatum*, *G. leptotichum* and *G. geosporum*) proliferated under the same conditions (Egerton-Warburton & Allen, 2000). In addition, in tallgrass prairie the abundance of spores from *Gigaspora gigantea* and *Glomus mosseae* increased with N fertilization, while that of *Entrophospora infrequens* declined significantly (Eom *et al*., 1999). Johnson (1993) noted an increase in the presence of *Gigaspora gigantea*, *Gigaspora margarita*, *Scutellospora calospora* and *Glomus occultum*, and a decrease in *Glomus intraradix* after 8 yr fertilization with a suite of nutrients including N and P. Alterations in the species assemblage of mycorrhizal fungi, either directly through N availability or indirectly through shifts in plant communities, appear to be a likely outcome of widespread N deposition.

Life span and decomposition of mycorrhizal tissue

Nitrogen effects on the survivorship and decomposition of mycorrhizal hyphae have received little attention. Using minirhizotrons, Majdi & Nylund (1996) found that N fertilization significantly reduced the life span of ectomycorrhizal short roots from 240 to 210 d in a 30-yr-old Norway spruce stand in southwest Sweden. The authors did not speculate on mechanisms underpinning this change, but we suggest that alterations of the ECM community could have been one factor. In another minirhizotron experiment, Rygiewicz *et al*. (1997) recorded no effect of N fertilization on the length of time between appearance and disappearance of ECM tips. This latter result includes both life span and decomposition rate of the fungal tissue. These studies present valuable information on the dynamics of mycorrhizal root tips. However, alterations in turnover of extraradical ECM and AM hyphae by N additions have yet to be reported in the literature. Nitrogen concentrations of hyphae from one *P. involutus* isolate were reported to increase with higher N availability in a culture experiment

(Wallander *et al*., 1999), with possible shifts in decomposition rate. Additionally, root turnover can increase with N fertilization (Pregitzer *et al*., 1995; Majdi & Nylund, 1996), and this response could produce a corresponding decrease in the life span of ECM structures such as mycorrhizal root tips and rhizomorphs.

Summary of N effects

Increases in N availability inconsistently affect hyphal dynamics (especially growth and biomass). In field studies, this variation in response could be partially attributable to the initial N status of the ecosystem; N-limited systems might respond differently from systems in which another factor (e.g. P or water) limits primary productivity. Nutrient limitation of plants is an important consideration because mycorrhizal fungi derive the majority of their C from the host. However, this possibility has not been explicitly tested. At this point we can only suggest that C storage in living and recently dead mycorrhizal tissue might only be affected (e.g. augmented or reduced) in certain systems exposed to N deposition. Furthermore, in glasshouse or culture studies the use of different fungal species or isolates might contribute to conflicting responses of hyphal growth or biomass. Even isolates of the same species can vary in response to N availability (Arnebrant, 1994; Wallander *et al*., 1999).

Overall, alteration in the community composition of mycorrhizal fungi (probably due to differences among groups in sensitivity to N) appears to be the most general response to N addition. Because mycorrhizal groups can vary in chitin content and growth rate, these shifts could have important consequences for C immobilization in live hyphal tissue or its residual soil organic matter. However, a more complete understanding of differences in tissue quality and physiology among groups is necessary in order to predict their influence on C dynamics.

Interactions between elevated CO_2 and N deposition

Effects of elevated CO_2 and N availability can interact to influence hyphal biomass. In a 14-month experiment on *P. tremuloides* seedlings, AM hyphal lengths increased with CO_2 in the low-N but not the high-N treatment (Klironomos *et al*., 1997). Arbuscular mycorrhizal hyphae associated with *Gutierrezia sarothrae* responded in a similar manner after 4 months' exposure to high and low treatments of CO_2 and N (Rillig & Allen, 1998). Apparently, N additions can negate CO_2 effects on mycorrhizal biomass in some systems. This interaction is not universal, however. Lussenhop *et al*. (1998) noted no significant CO_2 by N effects in AM biomass of a constructed ecosystem of *Populus* × *euramericana* seedlings. Tingey *et al*. (1995) also found no CO_2 and N interaction on the presence of ECM root tips and hyphae on *P. ponderosa* seedlings. Further investigations of this interaction and its effects on processes including turnover will be of interest. Unlike elevated CO_2, which is a global phenomenon, N deposition is greatest near regions with dense human populations. Therefore interactions between these two elements of global change will be factors primarily in ecosystems surrounding urban and agricultural areas, and investigations should focus on these habitats.

CONCLUSION

The potential for mycorrhizal fungi to influence the sequestration of soil C under various aspects of global change has been frequently suggested in the literature, although rarely directly addressed. The current body of work on mycorrhizal dynamics suggests that elevated CO_2 might augment global pools of C in living, dead and residual mycorrhizal tissue by increasing productivity in numerous habitats. By contrast, the (smaller-scale) influence of N deposition on ecosystem-level responses is less general than that of CO_2, and might rely on several factors, including the initial N status of the vegetation. Preliminary evidence that N fertilization increases turnover rates of hyphae indicates that C sequestration in hyphal biomass might decrease in areas exposed to N deposition. Elevated CO_2 and N deposition could therefore have conflicting influences on mycorrhizal C.

Shifts in the community composition of AM and ECM fungi with CO_2 and N enrichment could have important influences on mycorrhizal C dynamics. For instance, since *Scutellospora* and *Acaulospora* species appear to proliferate under elevated CO_2, and *Glomus* species can be more abundant with N deposition, predictions of the community structure under both disturbances are challenging. Nevertheless, our understanding of these changes is important; AM genera and species can vary in tissue quality and therefore affect C transformations. ECM fungi present similar issues. Compared with *Suillus bovinus*, *P. involutus* can respond more strongly to elevated CO_2 and can be less sensitive to N addition, with potential consequences for species composition and C storage. However, these patterns are derived from very few studies. Our knowledge indicates that the influence of mycorrhizal fungi on C dynamics under global change remains largely unknown, but could be a significant factor in soil C sequestration.

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