

Ectomycorrhizas: their role in forest ecosystems under the impact of acidifying pollutants

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Abstract

The physiologically active lateral rootlets of all main trees in temperate forests are colonised by ectomycorrhizal fungi, forming so-called ectomycorrhizas. These symbiotic organs are the sites of exchange of nutrients, mainly P and N, provided from the fungal partner, and C from the host. Emerging from the ectomycorrhizas, fungal hyphae exploit the soil for the mobilisation and absorption of water and nutrient elements. By doing so, they connect the tree roots intimately with the soil and provide anchorage. The deposition of acidifying pollutants into forest ecosystems is a potential threat to the health and vitality of forest trees because it leads to the acidification and eutrophication of forest soils. Pollutants are also a threat to the functioning of ectomycorrhizas. Increased N concentrations in the soil lead to enhanced fungal N uptake and storage, and to enhanced N transfer to the host plants, and therefore to higher plant biomass of above ground parts. In consequence, there is a decrease of C allocation to the plant roots. This in turn leads to reduced ectomycorrhization, and to reduced production of external mycelia and fruiting bodies. Soil acidification leads to enhanced availability of Al, heavy metals, and radionuclides in the soil, all of which can be toxic to plants and fungi. Reduced growth of roots and hyphae are amongst the first symptoms. In ectomycorrhizas, the hyphae of the fungal tissues contain vacuolar polyphosphates which have the ability to bind Al, heavy metals, radionuclides and N. These electronegative polymers of phosphates represent an effective storage and detoxifying mechanism which otherwise is lacking in roots. Therefore, ectomycorrhizas have the potential to increase the tolerance of trees to acidifying pollutants and to the increased availability in the soil of toxic elements.

Key words: ectomycorrhizas, forests, heavy metals, nitrogen, radionuclides, soil acidification

Introduction

In the winters of 1990 and 1999, the hurricanes "Vivian" and "Lothar" swept across Europe and destroyed or damaged large areas of forest, and uprooted or snapped thousands of trees. In mountainous and subalpine regions of the Alps such destruction of forests has dramatic socio-economic and ecological

consequences for the valleys and their human populations. Damaged forests can no longer entirely fulfil their protective functions against avalanches, rockfalls, land slides, flooding, and erosion. Following these events, the question arose as to whether soil acidification and eutrophication from aerial

pollutants contributed to the problem because the trees had damaged roots and reduced anchorage in the soils. Many foresters remark that trees today appear to have smaller root systems than in earlier days.

In nature, all physiologically active lateral rootlets of the major trees of the forests in boreal or temperate regions are colonised by mycorrhizal fungi to form "mycorrhizas" (from the Greek meaning "fungus-root", Frank 1885). Because these mycorrhizas of trees have ectotrophic fungal tissues they are called "ectomycorrhizas" (Smith & Read 1997). Ectomycorrhizas are the sites for the exchange of nutrients between the plant and the fungus, and are therefore regarded as mutualistic symbioses (Smith & Read 1997). Ectomycorrhizas develop on the lateral rootlets of long roots and are composed of both fungal and plant tissues. An ectomycorrhiza consists of the following fungal and plant components: (i) the fungal mantle enveloping the rootlet, (ii) the intercellular fungal tissue (the so-called "Hartig net") occurring between the epidermal and cortical cells, (iii) plant epidermal, cortical and endodermal cells, (iv) the plant apical meristem, and (v) the plant stele. Root hairs are lacking because their formation is suppressed due to the fungal interactions with the rootlet. Instead of root hairs, starting from the fungal mantle, a vast external fungal mycelium invades the surrounding soil, penetrating into the finest soil pores. By doing that they connect the roots with the soil and provide stability to the trees. Due to their high absorption surfaces, fungal hyphae have higher capacities than root hairs for mobilising and absorbing water and nutrient elements. By exuding organic acids fungal hyphae are even able to enter into weatherable minerals and utilise mineral nutrients (Jongmans *et al.* 1997). Elements absorbed are transported in the fungal mycelium to the fungal mantle and Hartig net for metabolisation and storage. In the Hartig net, which represents the interfacial exchange zone of the two organisms, the fungus N and P are exchanged for plant C (Smith & Read 1997). As a result, ectomycorrhizal plants often have higher N and P contents than non-mycorrhizal plants (Colpaert *et al.* 1999; Brunner & Brodbeck 2001; Jentschke *et al.* 2001). They may also exhibit higher resistance against drought, frosts, and pathogens (Read 1999), and possibly higher anchoring capacities.

The ectomycorrhizal symbiosis

Ectomycorrhizal organs arise spontaneously when hyphae of ectomycorrhizal fungi come into contact with compatible and uncolonised young lateral rootlets. After the hyphae have contacted the root surface, which is associated with a switch of the hyphal growth pattern from an apical-dominated to a multibranched and multiple apices mode (Brunner & Scheidegger 1992), they penetrate the rootlets intercellularly. A densely interwoven, two-dimensional fungal tissue composed of so-called palmettes develops between the epidermal and cortical cells, and forms the Hartig net (Scheidegger & Brunner 1999; Fig. 1). The growth of the hyphae is probably restricted by increased levels of phenylpropanoids and cell wall thickenings (Weiss *et al.* 1999). As a result, apical meristem and stele remain uncolonised by fungal hyphae. The Hartig net tissue separates epidermal and cortical cells from each other, although they are still connected by plasmodesmata (Scheidegger & Brunner 1993). Root cap cells which accumulate polyphenolics are lysed by the fungal hyphae and incorporated into the fungal mantle (Weiss *et al.* 1997).

In ectomycorrhizas, fungal hyphae take up nutrients and water from the pedosphere and transport them to the fungal mantle where they are metabolised and stored (Fig. 2). The Hartig net hyphae then transfer the nutrients to the host in exchange for plant C. The major nutrients which are taken up, metabolised, stored, and exchanged with the host are N and P (Read 1999). Absorbed inorganic N is metabolised in the fungal hyphae to the amino acids glutamate and glutamine (Fig. 3). There is evidence that glutamine from the fungus is exchanged for non-nitrogen containing organic molecules such as ketoacids from the plants (Botton & Chalot 1999; Hampp & Schaeffer 1999). However, the main plant carbohydrates which are taken up by the fungal Hartig net hyphae are glucose and fructose; these are derived from sucrose after hydrolysis by root cell wall acid invertase. After absorption by the fungal hyphae, these compounds are converted into the fungal carbohydrates trehalose, mannitol, and glycogen (Hampp & Schaeffer 1999). Absorbed inorganic phosphates are transferred into vacuolar pools of inorganic polyphosphates and stored as linear polymers in the

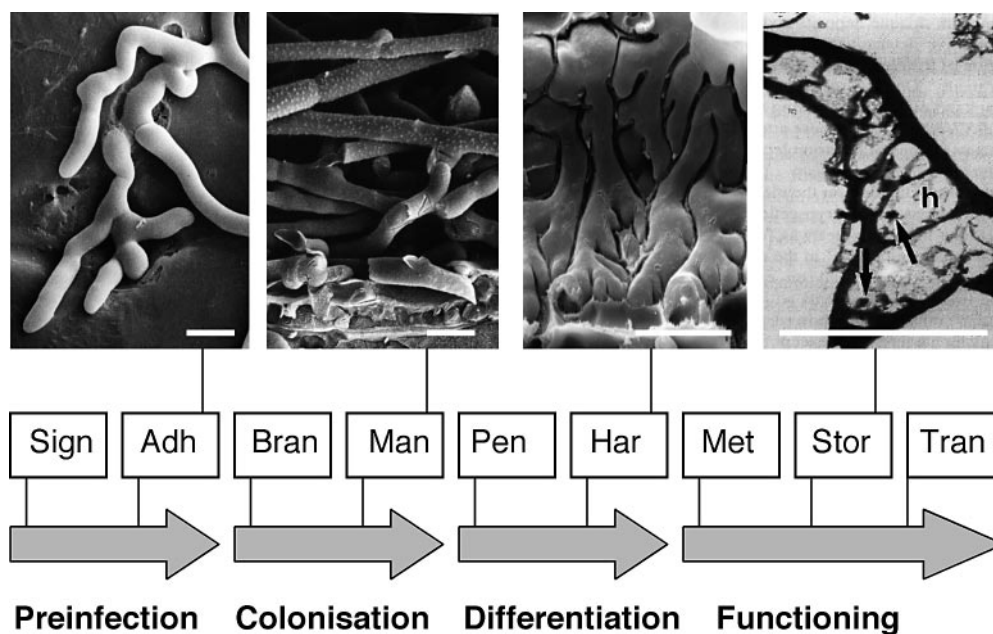


Fig. 1. Stages of ectomycorrhizal development (modified after Brunner & Scheidegger 1992; Martin & Tagu 1999; Scheidegger & Brunner 1999). Sign, signalling between fungal and root cells; Adh, adhesion of hyphae to root surface; Bran, branching of hyphae; Man, mantle formation; Pen, penetration of hyphae between root cells; Har, Hartig net formation; Met, alteration in metabolism; Stor, storage of elements in vacuoles and vacuolar P-rich particles (arrows) of Hartig net hyphae (h); Tran, transfer of nutrients between symbionts (bars, 5 μ m). Blackwell Science Ltd (Adh, Man; Brunner & Scheidegger 1992, *New Phytologist*, **120**, 359–369; Stor; Frey *et al.* 1997, *Plant, Cell and Environment*, **20**, 929–937) and Springer-Verlag (Har; Scheidegger & Brunner 1999, *Mycorrhiza: Structure, Function, Molecular Biology, and Biotechnology*, pp. 205–228, Springer, Berlin) are acknowledged as original sources of the photographs.

mantle and Hartig net hyphae. It is still a matter of debate to what extent these polyphosphates are present *in vivo* in a particulate form ("polyphosphate granules"; Bücking *et al.* 1998; Bücking & Heyser 1999) or dispersed in the vacuoles (Ashford *et al.* 1999). Polyphosphates give the ectomycorrhizal fungi the potential to accumulate phosphate and possibly re-mobilise it under low phosphate conditions in order to maintain a continuous P supply to the plant (Bücking & Heyser 1999, 2000). They are associated with accumulations of other nutritional elements such as Ca, K, Mg, N and S (Frey *et al.* 1997; Bücking *et al.* 1998; Bücking & Heyser 1999; Vesik *et al.* 2000). The exchange of nutrients between the two symbionts is such that the roots are a permanent sink for C, while the external mycelia are a sink for N and P. These symbiotic relationships mean that the fungal

partners play an active role in the acquisition of nutrients from localities and sources not available to roots, and to transport them over long-distances in hyphae or mycelial strands to the ectomycorrhizas (Brandes *et al.* 1998; Jentschke *et al.* 2001). As a result, ectomycorrhizal plants often have a higher uptake of P, N, K or Mg than non-mycorrhizal plants, resulting in higher plant tissues concentrations (Colpaert *et al.* 1999; Brunner & Brodbeck 2001; Jentschke *et al.* 2001).

Ectomycorrhizal organs have a lifespan of one to two vegetation periods (Egli & Kälin 1991), but they can, after the regrowth of the apical meristems, become recolonised by the same or by another ectomycorrhizal fungus. In central Europe, about 1,500 fungus species or about one third of the known macromycete flora are thought to be ectomycorrhizal symbionts. They include many well known edible

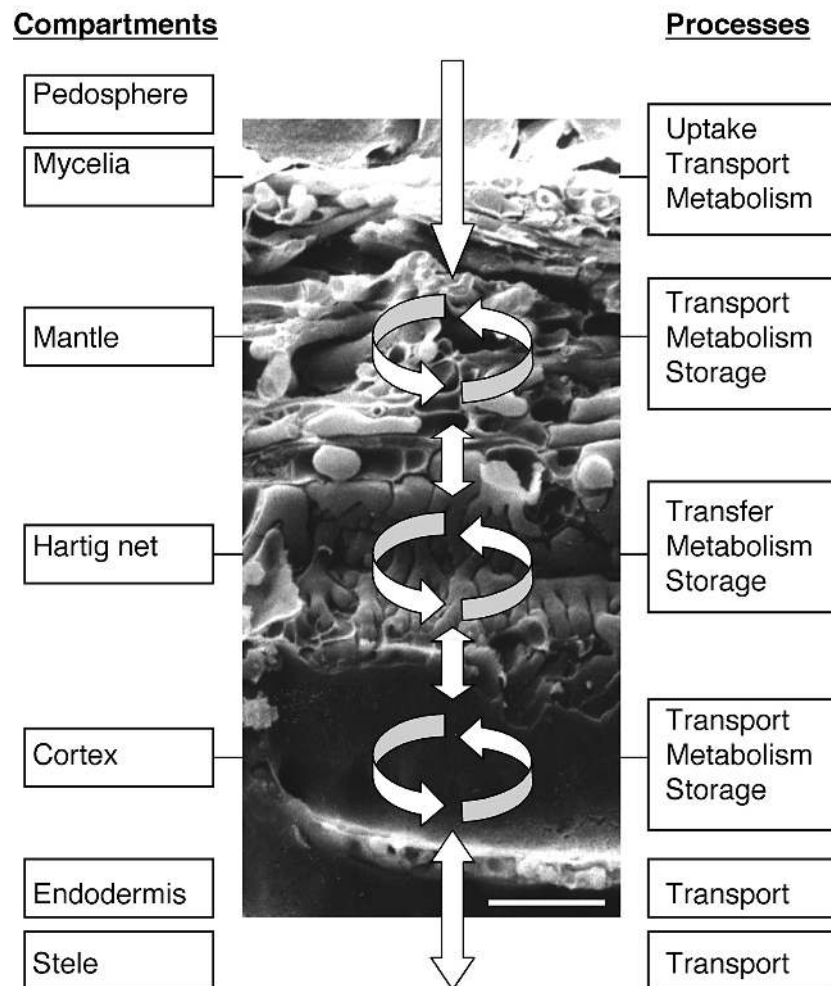


Fig. 2. Uptake, transport, metabolism, storage, and transfer of nutritional elements in ectomycorrhizas (modified from Brunner & Scheidegger 1995; Scheidegger & Brunner 1999; bar, 10 μm). Springer-Verlag is acknowledged as original source of the photograph (Scheidegger & Brunner 1999, *Mycorrhiza: Structure, Function, Molecular Biology, and Biotechnology*, pp. 205–228, Springer, Berlin).

basidiomycetes and ascomycetes such as boletus, truffles and chanterelles. Ectomycorrhizal fungi are polyphyletic, but they have in common, that they all depend upon the symbiotic stage to produce fruiting bodies in order to complete their life cycles. However, the successful production of fruiting bodies in association with their hosts has been possible in culture only for *Hebeloma cylindrosporum* and *Laccaria bicolor* (Debaud & Gay 1987; Godbout & Fortin 1992).

Inputs of acidifying pollutants into forest ecosystems

By-products of human activities since the onset of the industrial revolution have caused inadvertent changes to ecosystems. It is increasingly realised that pollution of soil, water, and air has economic, social and ecological consequences. Gaseous pollutants originate from the combustion of fossil fuels in power generation, industry and transportation. Pro-

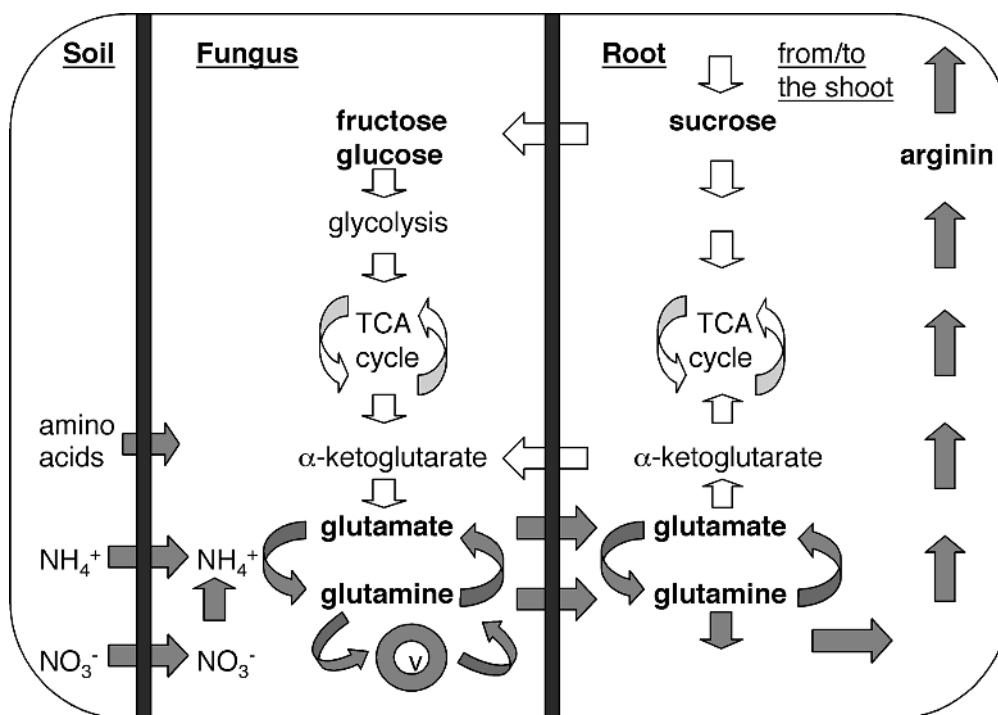


Fig. 3. Nitrogen (\Rightarrow) and C (\Leftrightarrow) metabolism in ectomycorrhizas under elevated N inputs (modified from Dähne *et al.* 1995; Botton & Chalot 1999; Hampp & Schaeffer 1999; v, vacuolar storage pool).

cesses related to agriculture and land use, such as decomposition of animal wastes in large-scale livestock production systems, paddy rice cultivation, and deforestation also release some of the same and other gases (Shafer & Schoeneberger 1991). Much of the interest in pollutant effects during the last decades has been focussed on forests, because of a widespread decline of tree health in both North America and Europe (Fowler *et al.* 1999). In central Europe, high atmospheric inputs of acidifying pollutants (SO_2 , SO_4^{2-} , NO , NO_2 , NO_3^- , NH_3 , NH_4^+ , H^+) in the last decades have led to an acceleration of soil acidification, the loss of base cations, and the release of Al ions into soil solution as a consequence of proton-buffer processes (Matzner & Murach 1995; Blaser *et al.* 1999). Acidification of soils also results in increased availability of trace elements including heavy metals and radionuclides. Excessive inputs of atmospheric N result in soil acidification and in nitrate leaching, and can lead to a relative shortage of other nutritional elements for

plants (Nihlgård 1985; Magill *et al.* 1997). In long-lived trees the impact of an altered soil chemistry can result in chronic stress (Shafer & Schoeneberger 1991).

Ectomycorrhizas in the challenge of altering forest soils

It is believed, that ectomycorrhizas have evolved to overcome the general deficiency of low nutrient availability in terrestrial ecosystems (Allen 1991; Colpaert & van Tichelen 1996; Cairney 2000). It has also been suggested that the ectomycorrhizal symbiosis made it possible for trees to colonise boreal zones where there is a low availability of N and P (Read 1991). In view of the association of ectomycorrhizas with low nutrient conditions it is not surprising that an altered soil chemistry due to air pollutants, with more available N or trace metals, can result in stress. In ectomycorrhizas, the site of action of a stress factor can either be the fungus or the plant, with

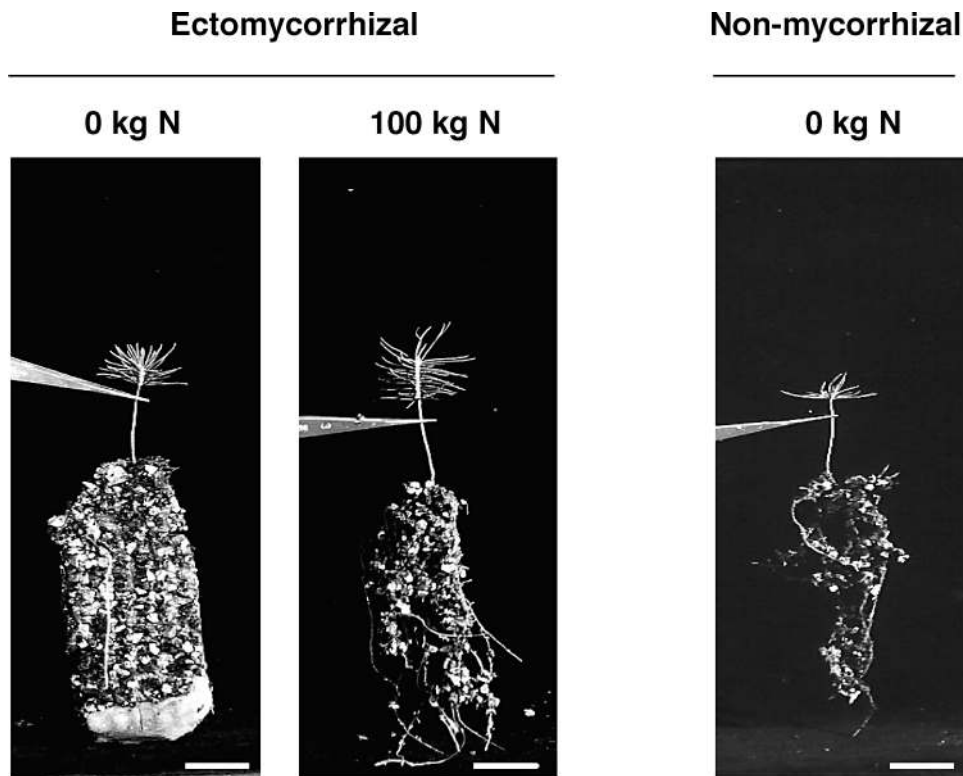


Fig. 4. Effects of varying N loads (0 or 100 kg N ha⁻¹ year⁻¹ as NH₄NO₃) on the substrate-attachment (vermiculite) of the root systems of ectomycorrhizal Norway spruce seedlings associated with *Hebeloma crustuliniforme* or of non-mycorrhizal control plants (bars, 2 cm).

primary responses being either positive or negative (Anderson & Rygielwicz 1991; Colpaert & van Tichelen 1996). Furthermore, primary (direct) responses may be followed by secondary (indirect) responses. Consequences of stress can either be altered plant C supply to the roots, altered fungal absorption of nutrients from the soil, or altered exchange capacities between the Hartig net and the host cells (compare also Dighton & Jansen 1991). Through a series of metabolic feedbacks, ectomycorrhizas eventually reach a new steady state enabling the symbiosis to be stress tolerant (Anderson & Rygielwicz 1991). Indicators of stress in ectomycorrhizas include alterations in the accumulation and metabolism of elements, and ultrastructural changes. Furthermore, the abundance and diversity of external mycelia, of ectomycorrhizas, and of fruiting bodies are also thought to be sensitive indicators of anthropogenic pollutants.

Nitrogen

Agriculture, combustion of fossil fuels, and other human activities have altered the global cycle of N substantially, and increased both the availability and the mobility of N over large regions. In terrestrial ecosystems the consequences of these changes are (i) higher input rates of N into N cycles, (ii) increased concentrations of the potent greenhouse gas N₂O, (iii) increased transfer of N, mainly nitrate, into ground water, (iv) soil acidification, (v) losses of soil nutrients such as Ca and K, (vi) increased storage of organic C in terrestrial ecosystems, and (vii) accelerated losses of biological diversity of plants, animals and microorganisms (Vitousek *et al.* 1997).

Tree growth in boreal and temperate regions is typically N-limited (Vitousek & Howarth 1991). There is considerable evidence that microbial processes in forest soils,

metabolic processes in forest trees and forest ecosystem functioning tend to be adapted to N limitations rather than N excess (Rennenberg & Gessler 1999). However, atmospheric inputs of reactive N compounds have increased in the last decades from less than 10 kg N ha⁻¹ year⁻¹ to values of 60 kg N ha⁻¹ year⁻¹ or more in polluted regions (Rennenberg & Gessler 1999). If ectomycorrhizas have evolved to overcome the general stress of low nutrient availability in terrestrial ecosystems (Allen 1991; Colpaert & van Tichelen 1996), the increased N availability is likely to have considerable impact upon the function of ectomycorrhizas as organs of nutrient uptake, transport, metabolism, storage and transfer.

It is widely accepted that the provision of plant photoassimilates to the fungal partners is the key factor for the formation and maintenance of ectomycorrhizas (see also Wallenda & Kottke 1998). Increased N inputs into trees leads to an increase of above ground biomass (van Dijk *et al.* 1990; Flückiger & Braun 1998) but to a reduction of C allocation to the roots (Wallenda *et al.* 1996). This leads in turn to a C deficiency for ectomycorrhizal fungi, which is reflected in a decrease of ectomycorrhization (Haug & Feger 1990/1991), reduced amounts of external mycelia (Wallander & Nylund 1992; Arnebrant 1994), and lower production of fruiting bodies (Godbout & Fortin 1992). An obvious visible indication of the inhibited growth of the external mycelia is the small amounts of substrate which are attached to root systems developed at elevated N loads (Fig. 4). Further investigation under experimental conditions reveals a decrease of the fungal tissues within rootlets and a decrease of fungus-specific compounds such as ergosterol, trehalose and mannitol (Wallander & Nylund 1991; Wallenda *et al.* 1996). High N concentrations also cause enlarged cortical cells (Brunner & Scheidegger 1995), and contacts with fungal hyphae induce cell wall thickenings (Haug *et al.* 1992; Brunner & Scheidegger 1995) which contain elevated Ca concentrations (Frey *et al.* 1997) and callose (Brunner & Schneider 1996). These responses are similar to the defense responses of hosts upon attack by pathogenic fungi. Intracellular penetrations of hyphae into cortical cells (Jentschke 1990; Holopainen & Heinonen-Tanski 1993; Brunner & Scheidegger 1995) support the suggestion that high N concentrations affect the mutualistic symbiosis of ectomycorrhizas and alter it to a more

antagonistic relationship. This alteration is possibly caused by a disturbed recognition between the two partners (Anderson 1988). Elevated N conditions at polluted sites or after fertilisation treatments also induce changes in the species composition of the fungal partners in the ectomycorrhizal root tips and in the fruiting bodies (Arnolds 1991; Arnolds & Jansen 1992; Brandrud 1995; Karen & Nylund 1997; Peter *et al.* 2001).

Ectomycorrhizas and their extramatrical mycelia influence N mobilisation, uptake and metabolism, and, as a consequence, the N status of the whole plant. The uptake of N is enhanced due to the extramatrical mycelia exploiting the substrate efficiently and enlarging the absorbing surface, and due to the exudation of organic acids and enzymes responsible for mobilising and taking up inorganic and organic N resources (Chalot & Brun 1998; Read 1999). Ectomycorrhizal hyphae take up inorganic N (NH₄⁺, NO₃⁻) as well as organic N compounds such as amino acids, and metabolise and store N as amino acids and proteins in the hyphae of the fungal mantles (Chalot & Brun 1998; Wallenda *et al.* 2000). In fungal mantles, the concentrations of N ranges from 2.9–4.4% whereas in the inner part of ectomycorrhizas it is only 0.9–2.1% (Högberg *et al.* 1996). Recent investigations after applying fertilisers have shown that storage of N in mantle hyphae mainly occurs in vacuolar deposition bodies (Kottke *et al.* 1995). Bücking *et al.* (1998) proposed that these N storage bodies are identical to the polyphosphate granules in vacuoles, and that, as has been shown for *Neurospora*, these negatively charged granules can bind basic amino acids such as arginin. In ectomycorrhizas of *Xerocomus badius*, N was concentrated chiefly in large, rather diffuse-lined vacuolar bodies but not in small, well-defined bodies (Kottke *et al.* 1998). Kottke *et al.* (1995), Beckmann *et al.* (1998) and Turnau *et al.* (2001) observed that the numbers and N contents of these granules increased in hyphae of ectomycorrhizal mantles after N fertilisation.

A significant increase is also evident in the N concentration in root tips colonised by *Paxillus involutus* following N additions (Wallander *et al.* 1999).

In general N fertilisation leads to increased plant biomass, but reduces ectomycorrhiza formation and decreases the root/shoot ratio. Further, while N concentrations in the plants usually increase, P and K concentrations tend

to decrease (Termorshuizen & Ket 1991; Seith *et al.* 1996; Wallenda *et al.* 1996). In a greenhouse study, in which varying N loads were applied to ectomycorrhizal Norway spruce seedlings associated with *Hebeloma crustuliniforme* or with *Laccaria bicolor* or to non-mycorrhizal controls, elevated N loads led to enhanced nitrate reductase activities in fine roots and ectomycorrhizas (Brunner *et al.* 2000), and to enhanced N concentrations in the plants (Brunner & Brodbeck 2001). Phosphorus and Zn concentrations decreased under high N loads. In the same experiment, ectomycorrhization led to enhanced N and P but decreased Mn concentrations (Table 1; Brunner & Brodbeck 2001). Thus, to a certain extent, the ectomycorrhization compensated for the decrease in plant P concentration caused by enhanced N loads.

Aber *et al.* (1998) posed the hypothesis that ectomycorrhizal assimilation and exudation is the dominant process involved in immobilisation of added N due to incorporation of N into soil organic matter. During N saturation, the composition of microbial communities shifts from a high abundance of fungi, probably ectomycorrhizal fungi, to dominance by bacteria (Tietema 1998). This loss of ecto-

mycorrhizal function results not only in increased nitrification and nitrate mobility (Aber *et al.* 1998), but also to changes in the element supply to plants.

Metals and radionuclides

Atmospheric pollution leading to soil acidification and elevated concentrations of trace metals is a significant threat to many forest ecosystems (Innes 1993; Godbold 1994). Increased inputs of acidifying substances accelerate weathering processes and increase the availability of Al and heavy metals in soils. Additionally, heavy metals enter into both agricultural and non-agricultural lands via many routes including disposal of industrial effluents, sewage sludge, deposition of airborne industrial wastes, mining, industrial solid waste disposal, and use of agricultural chemicals (Saxena *et al.* 1999). The metal species commonly found include Cd, Co, Cu, Hg, Ni, Pb and Zn. Although some of these metals are required in small amounts by plants for their normal physiological activities, excessive accumulation is toxic. The problem of metal toxicity is further aggravated by the

Table 1. Mean element concentrations (mg g⁻¹) in ectomycorrhizal *Picea abies* seedlings associated with *Hebeloma crustuliniforme* or *Laccaria bicolor* or non-mycorrhizal controls after treatment with various N loads of nitrate (kg N ha⁻¹ year⁻¹) (modified from Brunner & Brodbeck 2001). Probability level for 2-factorial ANOVA: ns, not significant; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; ****, $P \leq 0.0001$.

Elements	N-loads (N) (kg N ha ⁻¹ yr ⁻¹)	Fungus inoculations (F)			ANOVA		
		Control	<i>H. crustuliniforme</i>	<i>L. bicolor</i>	N-load	Fungus	NxF
N	0	10.9	13.1	16.7	****	****	***
	50	21.9	22.9	23.1			
	100	20.6	23.5	23.3			
	800	27.7	27.4	27.7			
P	0	2.27	4.97	5.85	****	****	*
	50	2.91	3.83	4.38			
	100	2.57	3.95	4.11			
	800	1.75	2.98	2.99			
Mn	0	0.26	0.22	0.27	ns	****	ns
	50	0.32	0.18	0.22			
	100	0.37	0.19	0.20			
	800	0.42	0.24	0.24			
Zn	0	0.19	0.17	0.12	***	ns	ns
	50	0.19	0.20	0.16			
	100	0.22	0.17	0.20			
	800	0.06	0.08	0.13			

persistence of the metals in the environment. Radionuclides, on the other hand, have been deposited within the past decades as fallout from nuclear weapon testing and nuclear accidents. The predominant isotopes are ^{137}Cs and ^{90}Sr . The very long half-life times of these radionuclides, 30.2 and 28.5 years respectively, renders them problematic. It is probable that they are fixed in bacteria and fungal hyphae of forest soils (Guillitte *et al.* 1994), and very high activities have been measured in the edible fruiting bodies of ectomycorrhizal fungi (Haselwandter & Berreck 1994).

An excess of heavy metals causes cell death in plants and fungi. These metals inactivate enzymes and structural proteins by acting on metal-sensitive groups such as sulfhydryl or histidyl groups (van Assche & Clijsters 1990; Gadd 1993); as a result they diminish the integrity of biomembranes and reduce the activity of key enzymes such as nitrate reductase (Ernst 1996). Metals also bind to DNA and affect cell division and cell elongation by disruption of DNA synthesis (Godbold 1994). Toxic concentrations of metals in plants cause a wide range of morphological and structural effects such as decreased root elongation, root tip damage, collapse of root hairs, enhancement of suberisation and lignification, and structural alterations of hypo- and endodermis (Barcelo & Poschenrieder 1999). They also influence the uptake of other mineral elements (Kabata-Pendias & Pendias 1992; Turner 1994; Ernst 1996). Furthermore, metals stimulate the formation of free radicals and

reactive oxygen species, resulting in an increase of antioxidative enzymes as a detoxification mechanism (Dietz *et al.* 1999). The degree of cell damage depends on the formation of these free radicals or reactive oxygen species, and on the efficiency and capacity of detoxification and repair mechanisms.

Heavy metals induce both plant and fungal cells to produce a wide range of low molecular weight polypeptides and proteins with high cysteine contents; these are the so-called metallothioneins and metallothionein-like proteins (Gadd 1993; Prasad 1999). One of the most important compounds in plants is a metallothioneine of class III ("phytochelatin"), an oligomer of glutathione, which is induced predominantly by Cd, Cu and Pb stress (Zenk 1996). Phytochelatins form complexes with metals and thus decrease the concentrations of free metal cations in the cytoplasm. The metal-phytochelatin-complex can subsequently be detoxified after transportation into vacuoles (Dietz *et al.* 1999). Recently a gene coding for the enzyme phytochelatin synthase was discovered and sequenced from *Arabidopsis* (Clemens *et al.* 1999; Ha *et al.* 1999; Vatamaniuk *et al.* 1999). Homologs of this gene family have also been found in yeasts. Less is known about such proteins in ectomycorrhizal fungi. The only report is from Howe *et al.* (1997) who isolated metallothionein-like proteins from Cu-tolerant strains of *Laccaria laccata* and *Paxillus involutus*. The proteins had similar weights (2.2–2.8 kDa) to those of known Cu-metallothioneins in *Neurospora* or *Agaricus*.

Table 2. Mean net counts of metals in compartments of freeze-fractures of ectomycorrhizas of *Picea abies* seedlings associated with *Hebeloma crustuliniforme* after treatments with Al, Cd, Ni, or Zn, and after measurements using a SEM-EDX (modified from Brunner & Frey 2000; highest values are in bold print; nd, not detected; detection limit 80 counts).

Metals	Treatments	Mantle	Hartig net	Cortex		Stele
		Hyphae	Hyphae	Cell walls	Cell lumen	Cell walls
Al	Control	nd	nd	nd	nd	nd
	1 mM Al ³⁺	83	90	148	nd	102
	10 mM Al ³⁺	778	2437	2617	208	197
Cd	Control	nd	nd	nd	nd	nd
	1 mM Cd ²⁺	131	713	137	nd	nd
Ni	Control	nd	nd	nd	nd	nd
	5 mM Ni ²⁺	772	1373	1198	768	406
Zn	Control	nd	nd	nd	nd	nd
	2 mM Zn ²⁺	172	134	116	81	nd

Table 3. Mean element concentrations (mmol kg⁻¹ dry weight) of cell compartments in cryosections within ectomycorrhizas of *Picea abies* seedlings associated with *Hebeloma crustuliniforme* after Cs and Sr exposure, and after measurements using a STEM-EDX (modified from Frey *et al.* 1997; highest values are in bold; nm, not measured).

Elements	Cortical cells			Interface	Hartig net hyphae	
	Cell walls	Cytoplasm	Vacuoles	Cell walls	Vacuoles	Particles
Ca	65	9	2	118	12	64
Cl	23	59	161	11	216	25
K	48	221	54	37	309	31
Mg	14	32	37	17	14	12
N	nm	nm	nm	nm	nm	nm
Na	11	23	12	19	32	7
P	59	295	128	29	161	406
S	34	57	99	29	118	91
Cs	34	78	22	43	134	26
Sr	21	61	2	71	19	84

Recent studies have indicated that colonisation of tree roots by ectomycorrhizal fungi can increase tolerance of their hosts to metals present in toxic concentrations in the soil (Godbold 1994; Turner 1994; Wilkinson & Dickinson 1995; Leyval *et al.* 1997; Godbold *et al.* 1998). Studies under experimental conditions have shown, that a lower phytochelatin content in roots of Cd-treated Norway spruce can be observed when the plants were ectomycorrhizal with *Laccaria laccata* compared to the non-mycorrhizal controls (Galli *et al.* 1993). Ectomycorrhizal fungi confer metal tolerance by binding metals to electronegative sites on the cell walls of the hyphae, or binding to phosphates and sulfhydryl compounds within the cells (Galli *et al.* 1994; Godbold *et al.* 1998). High amounts of metals in ectomycorrhizas of Norway spruces seedlings associated with *Hebeloma crustuliniforme* can be found predominantly in the fungal mantle, Hartig net and cortical cells (Table 2; Brunner & Frey 2000). In *Rhizopogon roseolus* ectomycorrhizas from calamine dumps, Turnau *et al.* (1996) found that Cd was concentrated in the cytoplasm, whereas Al was bound to P in vacuoles, and also extracellularly on the surface of the fungal cell walls. In contrast, in *Hebeloma crustuliniforme* ectomycorrhizas treated with heavy metals, there was cytosolic sequestration of Zn but extracellular complexation of Cd in the Hartig net (Frey *et al.* 2000). In *Xerocomus badius* ectomycorrhizas from acidic soils, Kottke *et al.* (1998) demonstrated the occurrence of small distinct vacuolar bodies containing P and Al.

Such electron-beam dense particles consisting of polyphosphates have been shown to bind not only Al (Väre 1990; Kottke & Martin 1994; Martin *et al.* 1994), but also heavy metals such as Cd and Zn (Turnau *et al.* 1993, 1996; Bücking & Heyser 1999) and the radionuclide Sr (Table 3; Frey *et al.* 1997).

It is a matter of debate to what extent ectomycorrhizas can ameliorate metal stress in plants (Godbold *et al.* 1998). Whether non-mycorrhizal or ectomycorrhizal plants contain more or less of the metals appears to depend on the ectomycorrhizal fungal species used and on the treatments applied. In investigations under experimental conditions, Colpaert & van Tichelen (1996) found similar Zn contents in the shoots of Scots pines inoculated with *Laccaria laccata* and in non-inoculated plants. In contrast, in *Suillus bovinus* inoculated plants, most of the Zn was bound in the external mycelium, and there were lower Zn concentrations in shoots compared to the uninfected controls. Jentschke *et al.* (1999) found that Norway spruces inoculated with *Laccaria bicolor* or *Paxillus involutus* and treated with Cd did not have significantly altered Cd contents in the needles compared with non-mycorrhizal plants. In the needles of Scots pines associated with *Suillus luteus* or *Pisolithus tinctorius*, van Tichelen *et al.* (1999) found significantly lower concentrations of Cu than in plants which were non-mycorrhizal. Hartley-Whitaker *et al.* (2000) observed that *Paxillus involutus* inoculated Scots pines had reduced Cd and Zn concen-

trations in the shoots, whereas *Suillus variegatus* inoculated plants did not. Riesen & Brunner (1996) found in Norway spruces exposed to the radionuclides ^{134}Cs and ^{85}Sr that plants associated with *Hebeloma crustuliniforme* had significantly lower activities than non-mycorrhizal controls, but only when a high N treatment was applied. However, in a similar study using ^{134}Cs , activities were only reduced significantly when plants were treated with a Cs/K ratio above 1 (Brunner *et al.* 1996).

In view of the increasing metal deposition and metal solubility in the soils due to human activities, the metal binding capacities of ectomycorrhizal fungal mycelia and of ectomycorrhizas potentially gain an important role in making trees tolerant of the new conditions. Some frequently found ectomycorrhizas, e.g. *Xerocomus badius*, have a high capacity for element storage, including Zn and Fe, in particular in acidic soils (Kottke *et al.* 1998). This is, among others, one important reason why ectomycorrhizal fungi are often considered in restoration programs for contaminated soils (Haselwandter & Bowen 1996; Haselwandter 1997; Leyval *et al.* 1997).

Concluding remarks

Whether ectomycorrhizas can contribute significantly to the resistance of forest trees against acidifying pollutants or whether they suffer in a similar manner to tree roots cannot be finally concluded. In ectomycorrhizas, the binding of N, metals and radionuclides to polyphosphates in the vacuoles of fungal hyphae represents a detoxifying mechanism which plant tissues alone do not have. Thus, one of the advantages of the ectomycorrhizal symbiosis could be in improving the tolerance of the trees to acidifying pollutants (compare also Leyval *et al.* 1997). On the one hand, ectomycorrhizal systems have evolved to overcome low nutrient availability by producing vast external mycelia exploiting the soil and by evolving mechanisms to store and accumulate elements. At low deposition of acidifying pollutants, binding of, for instance, small amounts of heavy metals to polyphosphates might not disturb the nutrient supply to the host; indeed, in the case of physiologically essential metals, it might even be useful. On the other hand, if deposition of pollutants is high, binding to the polyphosphates means a

detoxifying mechanism which prevents damage to the host trees. If this mechanism is important for the tree, a decrease of ectomycorrhizas and external mycelia due to pollutants will have negative impacts on tree pollutant tolerance, on tree nutrition and possibly on tree anchorage. In this context, it has been shown that Norway spruce seedlings react sensitively to forests soils with low pH resulting in reduced biomass and reduced Ca/Al molar ratios (Brunner *et al.* 1999). The concentrations of Al or the Ca/Al molar ratio in fine roots or in ectomycorrhizas, therefore, might be valuable indicators in the assessment of the ecological risk of soil acidification (Cronan & Grigal 1995; Zysset *et al.* 1996).

Advances in the past few years using molecular techniques have greatly increased our ability to identify the fungal partners of ectomycorrhizas (Mehmann *et al.* 1995; Karen & Nylund 1997; Jonsson *et al.* 1999; Peter *et al.* 2001). However, many questions still remain open and much research is required. The following issues will be particularly important for future progress: (i) to measure and quantify the external fungal hyphae emanating from ectomycorrhizas into the soil, (ii) to discover how different ectomycorrhizas vary in their sensitivity, (iii) to determine the physiological consequences for the trees being colonised by different ectomycorrhizal fungi, and (vi) to assess the importance of a high biodiversity of ectomycorrhizas compared to a low biodiversity. Acidifying pollutants remain a problem in many countries, despite international attempts to control pollutants such as S dioxides and N oxides within the programs of the UN/ECE Convention on Long-Range Transboundary Air Pollution. It is evident that in future calculations of critical loads (N, acidity, heavy metals) as the scientific rationale for the development of an effects-based new protocol on the further reduction of emissions in the ECE countries, ecological processes below ground – including ectomycorrhizas – must receive the attention they deserve.

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