Chapter 10
Plants and Their Ectomycorrhizosphere: Cost and Benefit of Symbiotic Soil Organisms

R. Agerer, A. Hartmann, K. Pritsch, S. Raidl, M. Schloter, R. Verma, and R. Weigt

10.1 Introduction

"Most higher plants have no roots — they have mycorrhizae". This is one of the most challenging statements in ecology (Begon et al. 1986). This entry holds true till now, since many publications corroborate the crucial importance of this mostly mutualistic symbiosis of tracheophytes with fungi. Mycorrhizae are no homogeneous entity. They differ in their modes of interaction with the plant and of nutrition. These features are used to distinguish mycorrhizal classes (Agerer 1993; Smith and Read 2008). One of the best known and most frequently studied mycorrhizal classes are ectomycorrhizae (ECM), which we focus on in this chapter.

R. Agerer (✉) • S. Raidl • R. Verma
Department of Biology I and GeoBio-Center, Organismic Biology, Mycology, Ludwig-Maximilians-Universität München, Menzinger Str. 67, 80638 München, Germany
e-mail: reinhard.agerer@bio.lmu.de

A. Hartmann
Research Unit Microbe-Plant Interactions, Helmholtz Zentrum München, German Research Center for Environmental Health, Ingolstädter Landstrasse 1, 85764 Neuherberg, Germany

K. Pritsch
Institute of Soil Ecology, Helmholtz Zentrum München, German Research Center for Environmental Health, Ingolstädter Landstr. 1, 85764 Neuherberg, Germany

M. Schloter
Environmental Genomics, Helmholtz Zentrum München, German Research Center for Environmental Health, Ingolstädter Landstr. 1, 85764 Neuherberg, Germany

R. Weigt
Department of Biology I and GeoBio-Center, Organismic Biology, Mycology, Ludwig-Maximilians-Universität München, Menzinger Str. 67, 80638 München, Germany

Department of Ecology and Ecosystem Management, Ecophysiology of Plants, Technische Universität München, Hans-Carl-von-Carlowitz-Platz 2, 85354 Freising, Germany

R. Matyssek et al. (eds.), *Growth and Defence in Plants*, Ecological Studies 220,
DOI 10.1007/978-3-642-30645-7_10,

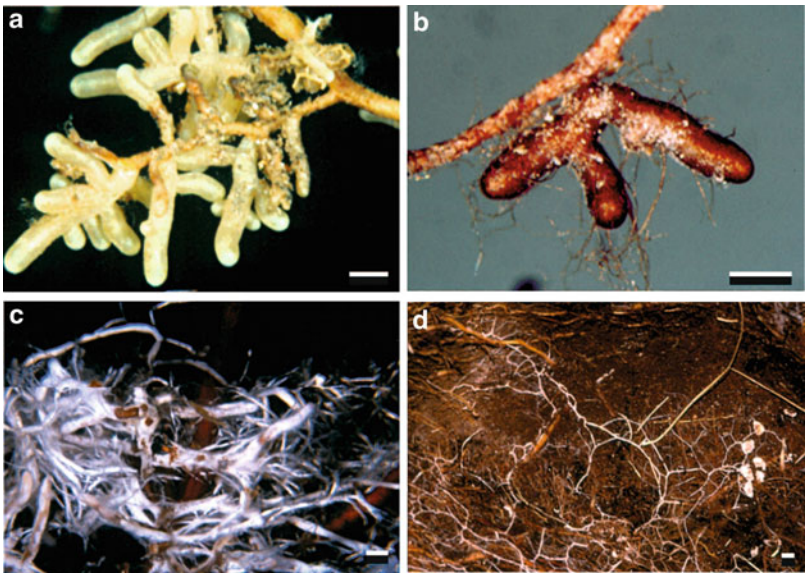

Fig. 10.1 Four different exploration types (ET). (**a**) Contact ET (smooth mantle surface, no rhizomorphs, hydrophilic, *Lactarius* cf. *uvidus*), (**b**) short-distance ET (hairy mantle surface due to long emanating hyphae, no rhizomorphs, hydrophilic, *Genea hispidula*), (**c**) medium-distance fringe ET (with many filamentous rhizomorphs with fringy margins, hydrophobic, *Cortinarius alboviolaceus*), (**d**) long-distance ET (tuberculate ECM systems with very far-reaching, ramifying, interconnected rhizomorphs, hydrophobic, *Suillus plorans*). *Bar* represents for **a**–**c**: 0.5 mm; **d**: 5 mm

ECM are characterised by intercellular growth of hyphae in the root cortex (Agerer 1991) and a hyphal mantle that envelopes the root. It cuts off the root from the surrounding soil in a physical but not physiological manner. The mantle and, if present, its extramatrical mycelium (EMM) explore and exploit the soil, and acquire, take up, transport and transfer water and nutrients from the soil to the tree roots. These fungal structures represent the almost exclusive aid of the tree to get access to the necessary nutrients from natural soil. The fungus in reverse earns carbohydrates for growth and reproduction (Smith and Read 2008). Economically spoken, the tree invests carbohydrates, obtained through photosynthesis, into its partner to receive essential nutrients. Costs for the tree depend upon the amount of carbohydrates used by the fungus for the production of mycelium. The benefits depend on the amount of nutrients and water the tree receives for its carbohydrate investment.

A generalising classification of ECM regarding putative ecologically and functionally important characters has been established by Agerer (2001), and refers to the extent, differentiation and amount of EMM that emanates from the hyphal mantle of the ECM (Fig. 10.1). The "contact exploration type" (C-ET) forms smooth mantles with only a very limited amount of mostly solitary, not very evident emanating hyphae. This type is predominantly hydrophilic, i.e. water can easily moisten the ECM. Organic as well as inorganic substrate can be directly contacted.

Many emanating hyphae and mostly hydrophily are characteristics of the "short-distance ET" (SD-ET). The emanating hyphae grow to a rather restricted distance, but frequently as a dense mycelium into the surrounding soil (Agerer and Raidl 2004). The "medium-distance ET" (MD-ET) forms additional rhizomorphs which can reach considerable distances in the soil. The most frequent subgroup of this type is the "fringe subtype" (MDf-ET) with many rhizomorphs that are often frequently interconnected by thinner rhizomorphs and by variably dense emanating hyphae; the fringe subtype is hydrophobic (Agerer and Raidl 2004). In contrast, the "smooth subtype" (MDs-ET) forms smooth rhizomorphs and is mostly hydrophilic. In the "mat subtype" (MDm-ET), ECM and their emanating hyphae and rhizomorphs are so densely aggregated that there is apparently no space for any other ECM species to colonise roots within these mats. This subtype is otherwise very similar to the MDf-ET. The "long-distance ET" (LD-ET) is characterised by very long, highly differentiated rhizomorphs with vessel-like hyphae (Agerer 1987–2008, 1991, 1995; Agerer and Rambold 2004–2011). These rhizomorphs can obtain a length of several decimetres (Schramm 1966), and are, including their ECM, generally hydrophobic. Which exploration type an ECM belongs to is species- and fungal relationship-dependent (Agerer 2006, 2007).

Rhizomorphs, particularly those of the LD-ET, are highly appropriate for transport of solutes (Duddridge et al. 1980). Only a limited number of studies regarding transport function and efficiency are available yet. Based on the rhizomorphs' anatomical differentiation (Agerer 1991, 1999, 2001, 2006), it might be concluded on their differential function (Kammerbauer et al. 1989). Apart from being suitable devices for uptake and transport, mycorrhizal hyphae, rhizomorphs and hyphal mantles can provide valuable support for bacterial growth. Bacteria apparently influence the formation of ECM or they may contribute to dissolve minerals or may be responsible for nitrogen/nutrient acquisition (Calvaruso et al. 2007; Frey-Klett et al. 2007; Korkama et al. 2006; Mogge et al. 2000; Poole et al. 2001; Schelkle et al. 1996; Timonen et al. 1998). Nutrient uptake by ECM fungi often occurs via enzymatic activities by degrading organic nutrient-rich material. Thereby, a range of enzymes specific to certain molecules or chemical bonds are available to ECM (Agerer et al. 2000; Pritsch et al. 2004). Differences in degradation specificities and capacities are proven for a diversity of ECM (Courty et al. 2006, 2007; Pritsch et al. 2004).

ECM species assemblages are often composed of different ET and can occupy different ecological niches (Agerer and Göttlein 2003; Agerer et al. 2002; Dickie et al. 2002; Genney et al. 2006). A change in species composition, often coinciding with changes in proportions of ET, can be caused by environmental changes (Brand et al. 1992; Godbold and Berntson 1997; Rey and Jarvis 1997; Weigt et al. 2012).

Based preferentially on the results obtained during 12 years of ECM research in the SFB 607 "Growth and Parasite Defence – Competition of Resources in Economic Plants from Forestry and Agronomy", this chapter focuses on (a) ectomycorrhizal space occupation and potential space exploitation, (b) niche occupation and potential nutrient mobilisation and (c) carbon costs of ECM under a changing environment due to elevated within-tree crown concentrations of carbon dioxide (CO_2) and ozone (O_3).

10.2 Space Occupation and Potential Space Exploitation by Ectomycorrhizal Fungi

Space occupation is an important issue regarding competition for resources, well known above-ground, e.g. for light and space within tree crowns (Chaps. 8, 11–13). In contrast, within the soil, space occupation is less apparent. As almost all finest roots (≤1 mm) of European woodland and forest tree species (e.g. *Fagus*, *Picea*, *Pinus*, *Quercus*) are converted to ECM (Smith and Read 2008), space occupation for nutrient acquisition in forest soils is prevailingly identical with space occupation by ECM.

The importance of extramatrical mycelia of ECM, particularly of rhizomorphs, has already been evidenced several times. Read (1992), who was the first to point out the ecological and functional importance of the mycelium, calculated 200 m g^{-1} dry forest soil for *Suillus bovinus* (Pers.) Roussel grown in root chambers. The pioneering synthesis experiments with *Suillus bovinus* ECM on pine seedlings provided first insights into space occupation capacity of ECM fungi. Similar experiments highlighted the extent of the extramatrical mycelia repeatedly. Although quantifications of mycelia in soils have been performed (Schubert et al. 2003; Wallander et al. 2001), there were no investigations available to compare ECM fungi for their capacity to occupy space in the soil, until Raidl (1997) characterised several species regarding differentiation and range of their extramatrical mycelia. This path-breaking study in combination with the high diversity of thoroughly documented ECM types (comp. Agerer 1987–2008), triggered the distinction of exploration types (Agerer 2001) and studies focussing on space occupation of extramatrical mycelia.

Agerer and Raidl (2004) showed for ECM of *Cortinarius obtusus* (Fr.) Fr. and *Tylospora asterophora* (Bonyord.) Donk, synthesised with Norway spruce (*Picea abies* (L.) Karst.) seedlings in flat rhizotrons, that mycelial range and density of *C. obtusus* (MDf-ET) and *T. asterophora* (SD-ET) differed considerably. Biased by the limited space of the rhizotrons used, the range of *T. asterophora* mycelium was approximately 12 mm that of *C. obtusus* 19 mm. Identical investigations on the MDf-ET *Piloderma croceum* J. Erikss. & Hjortstam and the LD-ET *Rhizopogon roseolus* (Corda) Th.Fr. supported on the one hand the values obtained for *C. obtusus* and resulted on the other hand in a mycelial range of at least 400 mm for the LD-ET. Compared to the C-ET with almost no EMM (Agerer 2001, 2007) as a negligible mycelial space occupation, the mycelium of the SD-ET covers 89 ± 18 (SE) mm cm^{-1} ECM, the MDf-ET 165 ± 39 mm cm^{-1} ECM and the LD-ET 1,336 ± 354 mm cm^{-1} ECM (Table 10.1 and Weigt et al. 2012). When calculating the potential space occupation per unit of ECM length, i.e. the complete area that is covered by the extramatrical mycelial systems, irrespective of the density of the hyphal layers within the mycelial systems (Weigt et al. 2012 and "Erratum"), the values between the SD- and the MDf-ET are not much different with 321 ± 104 mm cm^{-1} ECM and 271 ± 69 mm cm^{-1} ECM, respectively. The LD-ET, however, differs strongly with a 15- to 17-fold higher potential space occupation of 4,787 ± 1,322 mm cm^{-1} ECM. The different space ranges are compared as

Table 10.1 Specific values of ECM based on a length of 10 mm and exploration types

	C-ET	SD-ET	MDf-ET	LD-ET
Mantle biomass (MA-BM)	0.94 µg[a]	0.64 µg[b]	0.55 µg[c]	1.01 µg[d]
Extramatrical mycelial BM (EMM-BM)	0 µg	3.2 µg	6.0 µg	48.7 µg
Ectomycorrhiza BM (ECM-BM)	0.94 µg	3.84 µg	6.55 µg	49.71 µg
EMM length (EMM-L)	0 m[1]	3.7 m	6.9 m	55.9 m
Actual mycelial space occupation (aMSO)	0 mm^{2e}	89 ± 18 mm^2	165 ± 39 mm^2	1,336 ± 354 mm^2
Potential MSO (pMSO)	0 mm^{2e}	321 ± 104 mm^{2f}	271 ± 69 mm^{2f}	4,787 ± 1,322 mm^{2f}
pPO_4-Depletion	8.7 $mm^{2g,a}$	321 mm^{2f}	271 mm^{2f}	4,787 mm^{2f}
pN_{org}-Depletion	4.4 $mm^{2g,a}$	23.1 mm^{2f}	42.9 mm^{2f}	346.6 mm^{2f}
pNH_4-Depletion	62.2 $mm^{2g,a}$	321 mm^{2f}	271 mm^{2f}	4,787 mm^{2f}
pNO_3-Depletion	613 $mm^{2g,a}$	321 mm^{2f}	271 mm^{2f}	4,787 mm^{2f}

MA-BM biomass of mycorrhizal mantle (based on diameter of *Picea*-ECM and on their mantle thickness as obtained from Agerer and Rambold 2004–2009, and on the specific hyphal dry mass of 228.9 mg cm^{-3} according to Bakken and Olson (1983)); *EMM-BM* biomass of extramatrical mycelium (according to Weigt et al. 2010, 2012); *ECM-BM* biomass of ECM comprising mantle and extramatrical mycelium, without Hartig net; *EMM-L* length of extramatrical mycelium (according to Weigt et al. 2010, 2012); *aMSO* actual mycelial space occupation (according to Weigt et al. 2012); *pMSO* potential mycelial space occupation, i.e. the complete area covered by an extramatrical mycelial system, irrespective of the density of individual hyphae (according to Weigt et al. 2012 and "Erratum"); *pPO_4-depletion* potential depletion area for phosphate, assuming a specific depletion zone of 0.2 mm around an uptaking surface (Nye and Tinker 1977) and a mean hyphal density less than 0.05 mm (Weigt et al. 2012 and "Erratum"); *pN_{org}-depletion* potential depletion area of organic nitrogen, assuming 0.002 mm of wood decomposition around a saprotrophic hypha (concluded from Liese (1964, 1970) and Schmid and Liese (1964) indicating 1–2 µm), laccase activity of ECM (Courty et al. 2006), and an uptake of nitrogen bound to this plant cell wall material, and a mean hyphal density of less than 0.05 mm; *pNH_4-depletion* potential depletion area for ammonium, assuming a depletion zone of 2 mm around an uptake surface (according to Chapin et al. (2002): 1–2 mm) and a mean hyphal density less than 0.05 mm; *pNO_3-depletion* potential depletion area for nitrate, assuming a depletion zone of 10 mm around an uptake surface (according to Chapin et al. (2002): 6–10 mm) and a mean hyphal density less than 0.05 mm

[a]Mean of $n = 15$ species
[b]Mean of $n = 12$
[c]Mean of $n = 7$
[d]Mean of $n = 5$
[e]Possibly too low, as ECM of the C-ET often possess a few solitary emanating hyphae, too
[f]Due to the dense arrangement of the extramatrical hyphae with a mean distance of <0.05 mm (Weigt et al. 2012 and "Erratum"), the whole area occupied by the extramatrical mycelium (pMSO) and not the actual occupied area (aMSO) determines the space of acquisition
[g]Based on the mean diameter of C-ET ECM of 0.44 mm

shown in Fig. 10.2, where mycelia were squeezed between two perspex plates containing peat as growth substrate (Agerer and Raidl 2004; Weigt et al. 2010, 2011). In view of these growth conditions, transfer of the results to natural soils appears not unproblematic, but as the litter in the Of fraction of the organic soil is layered, too, due to periodic litter fall, and as ECM and extramatrical mycelia are preferentially squeezed horizontally between such natural layers (Agerer, pers. obs.), an application of results obtained in flat rhizotrons to natural soil appears acceptable. The obvious individual variability of space occupation mirrors ontogenetical differences, and, therefore, the mean value may rather well represent ET-dependent space occupation in nature.

Space occupation is crucial for resource exploitation and uptake (see also Chap. 11). Colpaert and van Tichelen (1996) pointed out that "probably one of the best ways of studying the effect of environmental stress factors on mycorrhizas is to focus on the growth of the external mycelium". A rhizotron filled with natural soil (organic rich mineral soil of the A horizon), and a root connected to a living mature spruce tree, formed many systems of *Xerocomus badius* (Fr.) Kühner ECM (LD-ET), Weigt et al. (unpubl.). Porter roots of the ECM possessed a projection area of 13 cm^2, and the ECM of 1.8 cm^2. If the depletion zones for phosphate (0.2 mm; Nye and Tinker 1977) are added, then the potential space to be exploited by the ECM increases to 9 cm^2. Assuming that the rhizomorphs and the hyphae between the rhizomorphs are less than 0.4 mm apart from each other, which seems reasonable (Weigt et al. 2010), the complete space covered by the mycelial complex can be considered as the potential exploitation surface with an area of 94 cm^2, i.e. approximately 82 % of the rhizotron used (data not shown). If the ECM had been formed by a C-ET, the potential exploitation area, as represented by the projected area of the smooth ECM, would be only 9 cm^2 with regard to phosphate, roughly 10 % of the space potentially occupied by the LD-ET. This highlights the importance of the EMM for tree nutrition and the possible influence of the ET within an ECM community. The potential space occupation as calculated for the LD-ET *X. badius* corresponds well with the data provided by Leake et al. (2004) who calculated the EMM of the LD-ET *Pisolithus tinctorius* (Agerer and Rambold 2004–2011) to representing 75 % of the absorptive area of the whole soil compartment occupied by roots.

The ET-specific values of space occupation (Table 10.1) can be directly applied to the stand to evaluate the mycelial extension and biomass when knowing the species community and ECM length (Weigt et al. 2012). In a first application, the ECM community under mature Norway spruce trees fumigated with twice-ambient O_3, as compared to an untreated control (Häberle et al. 1999), resulted in a shift from MD-ET and LD-ET to C-ET and SD-ET after 5 years, reducing the actual and potential mycelial space occupation area by 77 % (Weigt et al. 2012). Similar values were obtained in a more detailed investigation following 8 years of O_3 treatment. There, the reduction was even more pronounced and decreased to 19% of the control plot under ambient O_3 (unpubl. data). This decrease is somewhat diminished, when the space occupation of the ECM without EMM is added, i.e. only the mantle surface without EMM is considered, since the absolute abundance of ECM in the plot under twice-ambient O_3 was found to be higher than in the control plot (data not shown).

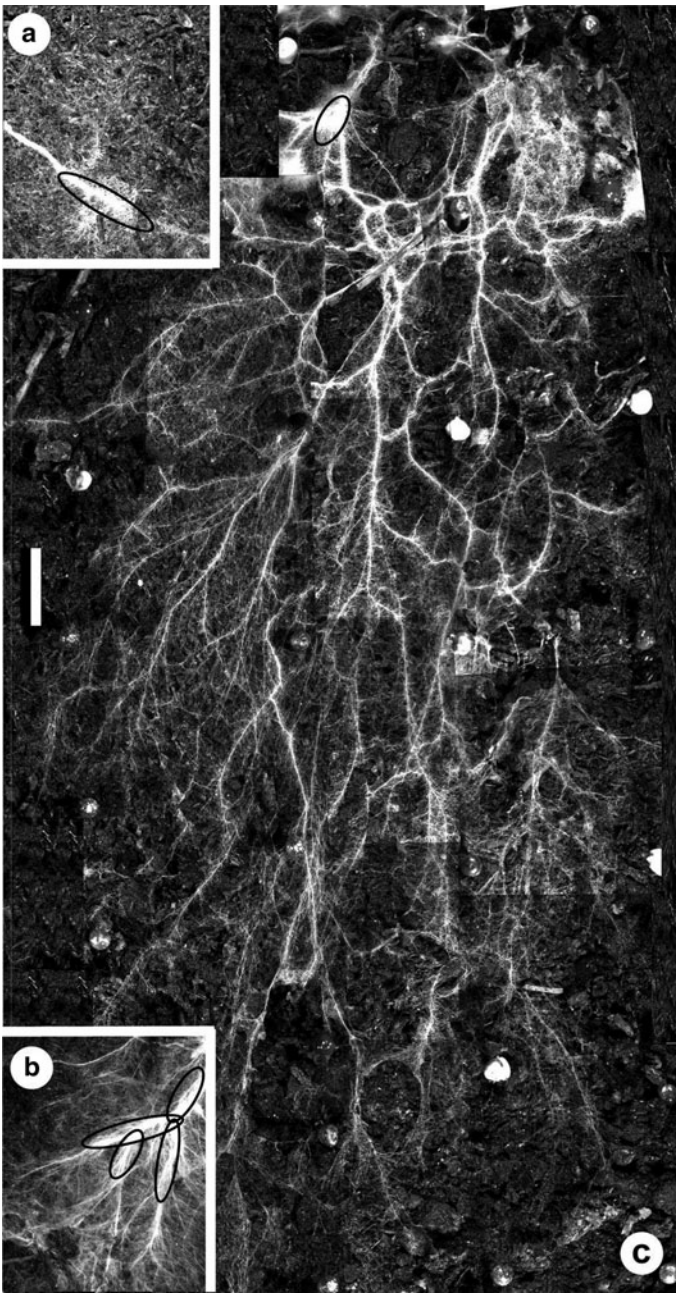

Fig. 10.2 Ectomycorrhizae and EMM on peat surface of a rhizotron. (**a**) *Tylopsora asterophora* (SD-ET; *white line* attached to the *left* side of the ECM is the supporting root; ellipse indicate ECM). (**b**) *Cortinarius obtusus* (MDf-ET; the *ellipses* indicate the position of the ECM within the extramatrical mycelium). (**c**) *Rhizopogon roseolus* (LD-ET; *ellipse* indicate ECM, filamentous structures represent hyphae and rhizomorphs; *small round* patches in **c** are pins used for orientation during photography). *Bar* represents for all figs. 10 mm

10.3 Carbon Costs of ECM Under Elevated Concentrations of Carbon Dioxide and Ozone

The calculation of carbon costs of ECM that have to be invested by the tree necessitates a quantification of the standing biomass, turnover and respiration rate of ECM including their EMM, and the biomass of fruitbodies. Very limited data are available for these issues and allow only rough estimations about the carbon allocation at ECM community level, ending up with 12.5–15 % of annual primary production (Smith and Read 2008). Other estimations ranged between 9 and 50% of the trees' net primary production (Markkola 1995; Rillig et al. 2002; Simard et al. 2002).

Such estimations suffer for various reasons. Standing biomass of ECM or their total lengths or projection areas under natural conditions are sporadically measured at best or only based exclusively upon very limited data sets (Dahlberg et al. 1997; Fogel and Hunt 1979; Wöllecke 2001). The proportion of mantle and Hartig net biomass on ECM total biomass remained almost unconsidered. Vogt et al. (1982) generalised the fungal proportion of ECM as being 40 %. With respect to total carbon investment, however, the quantification of fungal biomass via hyphal mantle alone is misleading as the mycelium in proportion to the mantle biomass may dramatically differ between species (Table 10.1). Since the establishment of the phospholipid fatty acid (PLFA) method by Wallander et al. (2001), quantifications of extramatrical mycelia are more frequent now, but remain in most cases non-compared to absolute abundance or biomass of ECM, and may still underestimate the total mycelial biomass, as external mycelia production was shown to be 300 % higher in natural soil than in acid-washed sand (Hendricks et al. 2006). This method can roughly discriminate between saprotrophic and mycorrhizal hyphae, but is not able to distinguish the contribution of mycelia of different ECM, which would be necessary to calculate species-specific contributions of ECM to soil mycelia (Agerer 2001; Weigt et al. 2012).

Turnover and respiration rates represent further restrictions in estimating the carbon transfer of trees to the fungal partners. The turnover rate of EMM is assumed as being 7 days (Smith and Read 2008). Longevity, and consequently turnover of ECM, is species- and site-dependent and varies between 51 and 139 days (Rygiewicz et al. 1997; Sittig 1999). Fine roots themselves have much longer lifetimes (Smith and Read 2008), and their biomass cannot be used as a basis of ECM biomass calculations. The measurement of respiration of ECM and separately of the extramatrical mycelia are suggested as amounting to 60 % of the carbon allocated to the fungus or 4.3 % of total carbon assimilated (Rygiewicz and Andersen 1994). Soil respiration appeared to be predominantly influenced by ECM and particularly by those species that produced rhizomorphs (Hasselquist et al. 2010). Weigt et al. (2010) estimated for 7-month-old Norway spruce seedlings ECM with *Piloderma croceum* that between 5.9 and 8.3 % of seedling dry mass was transferred to the fungal partner. But as respiration of ECM mycelium and ECM is known to be temperature sensitive (Koch et al. 2007), climate, weather conditions, seasonality and species-dependence make generalisations ambitious. When

fruitbody dry matter was included in carbon budget estimations, it referred either mostly to epigeous or occasionally to hypogeous fungi (Agerer 1985; Dahlberg et al. 1997; Fogel and Hunt 1979; Markkola et al. 1995).

These limitations also apply to treatment-specific influences on ECM. Positive impacts of elevated CO_2 concentrations, applied to trees or seedlings on ECM biomass, have been shown several times (e.g. Godbold et al. 1997; Parrent et al. 2006; Weigt et al. 2010). It is generally concluded that elevated CO_2 causes increases in ECM colonisation and the amount of produced EMM (Alberton and Kuyper 2009; Parrent et al. 2006; Tingey et al. 2000). For example, Weigt et al. (2010) found a slight but not significant increase in total ECM length by 25 and 61 % of the MDf-ET *Piloderma croecum* and the MDs-ET *Tomentellopsis submollis*, respectively, in response to twice ambient CO_2 and moderate nitrogen amendment. Simultaneously, the EMM of *P. croceum* ECM increased slightly from approximately 50.8 to 75.5 μg g^{-1} soil. This increment was caused by higher numbers of ECM, as the hyphal biomass and length per cm ECM remained rather stable at approximately 6 μg cm^{-1} and 6.9 m cm^{-1}, respectively.

With respect to the influence of tree crown O_3 exposure on root systems, only the changes in general root parameters have often been investigated, such as longevity and turnover (e.g. King et al. 2001; Phillips et al. 2009). At the study site "Kranzberger Forst", altered C allocation to below-ground compartments of mature trees under elevated crown O_3 exposure was shown by increased soil respiration and fine root production, and a shift in vertical fine root distribution (Nikolova et al. 2010 and Chap. 11). Studies regarding the performance of ECM often concentrate on seedlings and only a very limited number on older trees. For mature trees, Haberer et al. (2007) found an increase in the density of vital ECM and a change in ECM community structure of European beech (*Fagus sylvatica* L.) at the "Kranzberger Forst", as well as a reduced specific N uptake under twice-ambient O_3 and concluded on a change in nitrogen nutrition of the trees by ECM under O_3 treatment. Species-specific changes in nitrogen acquisition could not be documented. Reduced N uptake under elevated O_3 by mature beech and, less pronounced, in spruce was also shown by a soil-^{15}N-labelling study (Weigt 2010, see Chap. 11). At the same site, Grebenč and Kraigher (2007) found a significantly increased number of vital ECM on beech under twice-ambient O_3 concentrations. Although not consistently from year to year, the number of ECM types and species richness increased in addition. Kasurinen et al. (2005) concluded from their studies that increasing tropospheric O_3 levels can represent an important stress factor in northern birch forests, as they might alter mycorrhizal morphotype assemblages, mycorrhizal colonisation rates and sporocarp production.

Changes in ECM diversity have never been reported with respect to their ET-related amount of EMM, although it can be regarded as an important functional unit (Agerer 2001; Leake et al. 2004). Our recent experiments apply the ET-classification to analyse the influence of O_3 treatments (Weigt et al. 2010, 2012) and focus on potential changes in ET-specific biomass of the EMM in natural Norway spruce stands.

Although it appears generally difficult to calculate absolute carbon allocation to ECM, treatment-dependent relative changes might be successfully compared. Weigt et al. (2010, 2012) calculated generally the EMM biomass of SD-ET, MDf-ET and LD-ET as 3.2, 6 and 48.7 μg cm^{-1} ECM (Table 10.1), respectively. Applying these ET-specific values to the Norway spruce stand at the "Kranzberger Forst" (Häberle et al. 1999, Chap. 11), the total mycelial biomass under twice-ambient O_3-fumigated trees decreased considerably as compared to an untreated plot after a 5 years (Weigt et al. 2012) and, similarly, after 8 years of O_3 treatment (unpubl. results, see above). As C-ET generally form thicker ECM with thicker mantles (Table 10.1), the above-mentioned biomass decrease is partially but inconsiderably diminished when mantle biomass is added, since absolute abundance of ECM increased under twice-ambient O_3 (unpubl. data).

10.4 Niche Occupation and Nutrient Mobilisation

ECM preferentially occur in the organic layer, especially in the Of-horizon (Meyer 1962). In addition, the quality of the organic substrate plays an important role for ECM assemblage and abundance (Tedersoo et al. 2003) as well as decomposition status of organic litter (Aneja et al. 2006). Fine-scale studies suggested that ECM species are not evenly distributed vertically and horizontally (Agerer et al. 2002; Baier et al. 2006; Dickie et al. 2002; Gebhardt 2005; Genney et al. 2006; Scattolin et al. 2008; Wöllecke 2001). Site occupation patterns on micro-scale appeared to be often dependent upon soil nutrient contents (Agerer and Göttlein 2003; Rosling and Rosenstock 2008).

10.4.1 Distribution in a Heterogeneous Soil Environment

Some recent approaches demonstrated species-specific niche occupation of ECM (Gebhardt 2005), but only a few ECM types occasionally showed a stand-dependent correlation with soil nutrients (e.g. *Cenococcum geophilum* Fr., *Piloderma croceum*, *Tomentella* sp.). The rather few positive results were possibly because of the large cores for ECM studies (5 cm diameter) and the even larger cores (10 cm diameter) surrounding the 5-cm-cores taken before, for ECM studies. They likely levelled off heterogeneities in nutrient contents and ECM distribution that could be expected at micro-scale level (comp. Agerer and Göttlein 2003). Genney et al. (2006) found an ECM morphotype-specific vertical distribution and differences in the corresponding extramatrical mycelia reminiscent of the EMM patterns of ECM exploration types. Although nutrient status of the soil horizons was not analysed, a correlation of some ECM types to deviating soil conditions was evident, at least concluding from the provided soil profile pictures. The method "micromapping of ECM" (Agerer et al. 2002) related mycorrhizal abundances to

nutrient contents of micro-soil cores of 8 mm diameter (Agerer and Göttlein 2003). Some of the studied nutrients were heterogeneously distributed, showing considerable differences at distances of 2.5 cm, e.g. for Ca (193 vs. 565 mg kg^{-1}), K (93 vs. 260 mg kg^{-1}), Mg (29 vs. 93 mg kg^{-1}) and Fe + Mn (94 vs. 141 mg kg^{-1}), along with a different pH (4.8 vs. 5.2). Abundances of ECM were partially significantly correlated with these soluble nutrients (Agerer and Göttlein 2003): *Cortinarius obtusus* positively with NH_4^+ and Mg, *Lactarius theiogalus* positively with NH_4^+, K, Na, Mg, Fe + Mn, and negatively with pH. Wallander et al. (2003) found evidence for different colonisation patterns of apatite and mineral use by a diversity of ECM fungal mycelia.

Leaves of O_3-treated trees may reveal an altered quality as indicated by leaf litter colonising microbial communities (Chung et al. 2006), although mineralisation rates of beech and spruce litter were similarly independent of the O_3 treatment (Aneja et al. 2007). The amounts of lignin and cellulose in O_3-treated versus control spruce needles and beech leaves, however, did not differ after 8 weeks of exposition to microbial communities (Aneja et al. 2007). As litter from O_3-treated spruce and beech plots had higher contents in starch at unchanged *C/N* ratio, a preferential colonisation by saprotrophic fungi can be hypothesised, possibly outcompeting ECM fungi (Gadgil and Gadgil 1975; Lindahl et al. 1999). The results obtained by Aneja et al. (2007), showing a shift from basidiomycote dominated fungal communities to ascomycotous ones, indicate that the ECM fungi, predominantly basidiomycotes (Rinaldi et al. 2008), are possibly disadvantaged in comparison to saprotrophic Ascomycota. The shift from ECM species with high amount of extramatrical mycelia to those with less under O_3-treated Norway spruce, considerably less pronounced in beech (see Sect. 10.3), might, apart from a decreased sugar availability in roots of O_3-treated plants, be the result of hyphal competition between saprotrophic and mycorrhizal fungi for carbohydrates and nutrients in the litter. An apparent direct access to phosphate from a saprotroph by the LD-ET fungus *Paxillus involutus* (Batsch) Fr. was evidenced by Lindahl et al. (1999), an additional indication that the decrease of LD-ET ECM under elevated O_3 could favour saprotrophic fungi. Additionally, also changing nutrient contents under elevated O_3 treatment (Haberer et al. 2007) and their influence on leaf-inhabiting microbial communities are important. Since changes in bacterial assemblages have also influenced the results obtained by Aneja et al. (2007), interpretation of litter quality influences on ectomycorrhizal communities, particularly in a heterogeneous environment, is difficult.

10.4.2 Nutrient Acquisition and Transport

Carbon transfer from trees to their ECM partners for support of the extraradical and particularly of the extramatrical mycelia are with no doubt crucial investments for getting access to nutrients, especially to the macronutrients nitrogen (N) and phosphorus (P), and likely to potassium (K), magnesium (Mg) and possibly calcium (Ca) (Smith and Read 2008). Nitrogen limitations are typical for many ecosystems

dominated by ECM plants in temperate and boreal forests and woodlands (Smith and Read 2008) and strongly influence the productivity of trees. Most of the nitrogen (N) is organically bound in the organic matter (Smith and Read 2008), and this organically bound N is preferentially the target of ECM fungi (Read 1992). Apart from differential enzymatic activities of the fungi (Pritsch et al. 2004), mineral weathering by lowering the pH, often caused by exudation of organic acids like oxalate (van Schöll et al. 2006) frequently deposited as crystals on the hyphae, and the extent of the extramatrical mycelia (Read 1992; Rousseau et al. 1994; Wallander et al. 2003) play important roles for nutrient acquisition. Transport capacities are particularly important for those ECM that form extended and long-reaching rhizomorphs (Duddridge et al. 1980; Kammerbauer et al. 1989; Rousseau et al. 1994).

10.4.2.1 Enzymatic Activities of Ectomycorrhizae

Potential enzyme activities of ECM are indicators of traits related to functioning of ECM in the ecosystem (Pritsch and Garbaye 2011). Extracellular enzymes are expressed by soil organisms to mobilise not only nutrients bound in nitrogen or phosphorus-containing polymers such as e.g. chitin, proteins, phospholipids, or DNA, but also mineral nutrients contained in dead plant material. Ectomycorrhizal species show a range of enzyme activities that can be displayed as their enzyme activity profiles obtained by using excised ECM tips in a series of enzyme assays in microplate assays under laboratory conditions (Courty et al. 2005; Pritsch et al. 2004, 2011). An enzyme activity profile indicates the potential activity of a given number of enzymes measured at individual mycorrhizal tips and can be used to compare profiles of different species (Courty et al. 2005) or of species and ECM communities under different treatments or environmental scenarios. The method has been successfully applied in many studies and revealed for example a strong influence of the plant host species (Pritsch et al. 2006), host genotype (Courty et al. 2011), soil conditions i.e. liming (Rineau and Garbaye 2009) or heavy metal contamination (Pritsch et al. 2006).

To study the influence of elevated ozone concentrations on below-ground functioning of ECM, enzyme activity profiles were determined for communities of ECM collected underneath O_3-treated mature Norway spruce trees and a corresponding group of non-treated trees at the "Kranzberger Forst" free-air ozone fumigation site. A set of eight enzyme activities was tested including leucine amino peptidase (EC 3.4.11.1), β-xylosidase (EC 3.2.1.37), β-glucuronidase (EC 3.2.1.31), cellobiohydrolase (EC 3.2.1.91), *N*-acetylglucosaminidase (EC 3.2.1.14), β-glucosidase (EC 3.2.1.3), acid phosphatase (EC 3.1.3.2) and as oxidative enzyme laccase (EC 1.10.3.2). Almost all hydrolytic enzyme activities except for glucuronidase were decreased under elevated O_3, while laccase activity, an oxidative enzyme involved in degradation of lignin and other phenolic substances, significantly increased (Jana Ernst, unpubl. results). Thus the above-mentioned differences in carbon allocation to the mycorrhizal communities with a drastic

decrease in carbon costly exploration types in spruce also resulted in differences in the enzyme activity profiles of the ECM communities. Stimulated laccase activity may point towards the necessity to access more recalcitrant nutrients for example an increase in protein–phenol complexes (Theuerl and Buscot 2010). This may either be due to altered litter quality or a higher demand of nutrients for the plants under stress conditions thus requiring associated organisms with more efficient potential for mobilising nutrients.

Insights into the complexity of plant stress reactions and their influence on mycorrhizosphere functioning were also obtained from experiments under more controlled conditions in phytotron experiments of young spruce and beech trees grown in containers (see Chap. 12). In a phytotron study with juvenile spruce and beech grown in mixture, soil samples collected in the mycorrhizosphere of the plants were used to measure enzyme activities as functional parameter (Pritsch et al. 2005). The mycorrhizosphere is enriched in active microorganisms because of the priming effect of plant carbon input (Kuzyakov 2002) and therefore was assumed to reflect altered carbon allocation from stressed plants (Andersen 2003). Enzyme activities in mycorrhizosphere soil integrate activities of all present contributors such as roots, ECM fungi, associated microorganisms and free-living microorganisms in this compartment. In the phytotrone study of Luedemann et al. (2005, 2009), O_3 stress was combined with the root pathogen *Phytophthora citricola* as a second stressor. This study showed that the competitive behaviour of spruce and beech was modified by the combined stress (Luedemann et al. 2005, 2009) and that altered enzyme activity patterns indicated stress reactions in the mycorrhizosphere (Pritsch et al. 2005). For example, increased activities of phosphatase and chitinase in the mycorrhizosphere of spruce were interpreted as a consequence of the plant reaction towards this combined stress that weakened spruce more than beech. This study revealed the complexity of interactions with co-occurring factors such as competition between plant species and interaction with a pathogen and their influence on enzyme activities in the mycorrhizosphere. It also showed that reactions in the mycorrhizosphere of young plants with an increase of some enzyme activities can differ from that of older plants with a decrease of the same enzyme activities. Thus, the link between plant stress due to ozone and enzyme activities in the mycorrhizosphere can be masked by other factors such as plant age and tree species.

10.4.2.2 Nutrient Mobilisation and Transfer

Soil densely occupied by ECM and extramatrical mycelia contained significantly higher concentrations of labile N, PO_4, SO_4, H, Al, Fe, Cu, Mn and Zn compared to soil lacking ECM (Aguilera et al. 1993; Griffiths et al. 1991,1994). This indicates an evident influence of mycorrhizal hyphae on free soil chemistry by dissolving more nutrients than have been taken up by the mycelium (Smits et al. 2008). ECM mycelia have been shown to grow towards mineral grains and direct energy flow towards the minerals (Hedh et al. 2008; Paris et al. 1996; Rosling and Rosenstock

2008; Smits et al. 2008; Wallander 2000; Wallander et al. 2003). The amount of removal is depending upon the fungal species involved (Bending and Read 1995; Perez-Moreno and Read 2000). This is supported by studies that could correlate hyphal length occupying the substrate with phosphate nutrition and growth parameters of seedlings (Ekblad et al. 1995; Rousseau et al. 1994). ECM communities comprising species with higher amount of extramatrical mycelia and with a greater proportion of mycorrhizal total lengths, thus able to occupy larger soil areas, should have higher capabilities for transfer of nutrients to the trees. Although species- and strain-specific differences are known (Bending and Read 1995; Gorissen and Kuyper 2000; Wallander 2000), the dramatic decrease in potential soil occupation of the ECM communities under O_3-treated mature spruce, as seen in the "Kranzberger Forst" (see Sect. 10.2) (Weigt et al. 2012, and unpubl. data), should be mirrored by lower nutrient contents of the trees, not necessarily expressed simply by nutrient concentrations, because lower standing biomass of the trees may have accumulated lower total amounts of nutrients in spite of similar concentrations.

As shown by Weigt et al. (2010, and "Erratum") the mean distance of hyphae in a potential mycelial space occupation (pMSO) area, i.e. the complete area that is covered by the extramatrical mycelial systems, irrespective of the density of individual hyphae, is less than 0.05 mm and therefore, the complete area can be exploited in PO_4 (Table 10.1), as the PO_4-specific depletion zone is approximately 0.2 mm (Nye and Tinker 1977, see above). Since the potential depletion zones for NH_4 and NO_3 are even larger (Table 10.1), the occupied area can potentially be impoverished of nitrogen, too.

When ECM are mapped in their natural position and their potential mycelial space occupation area is depicted (Fig. 10.3), the range and intensity of mycelial influence on soil compartments become evident. Both *Tylospora fibrillosa* (SD-ET; Fig. 10.3c) and *Cortinarius obtusus* (MDf-ET; Fig. 10.3d) occupy rather large areas, being of a wider range and higher density at least close to the ECM in the latter species (Weigt et al. 2012). There is a zone of intermixture of both mycelia (Fig. 10.3e), but the distribution of the ECM indicates also separate regions. The MDs-ET *Piceirhiza internicrassihyphis* and the C-ET *Russula ochroleuca* (Agerer and Rambold 2004–2011) are just within both space occupation areas. This arrangement appears at a first glance as a severe competition problem between the species, but might not be that serious at least with *C. obtusus,* as the C- and MDs-ET ECM are hydrophilic whereas the mycelium of *C. obtusus* is hydrophobic (Fig. 10.3b, f). However, hydrophobic ECM are generally hydrophilic at the periphery of their EMM (Raidl 1997). As *T. fibrillosa* is hydrophilic, its hyphae possibly can function even in the close vicinity of the hydrophobic ones of *C. obtusus* without being crucially competitive, as hydrophily is a prerequisite for intimate contact with the substrate and for nutrient uptake. Such a first analysis of an ECM community indicates that hydrophilic and hydrophobic ECM can apparently occupy different ecological niches, even if they are sympatric. *Cortinarius obtusus* with its hydrophilic mycelial front covers the position of the hydrophilic ECM only in earlier ontogenetic stages, when the EMM is not yet such far reaching. The hydrophilic ECM will have the possibility to contact soil particles with their

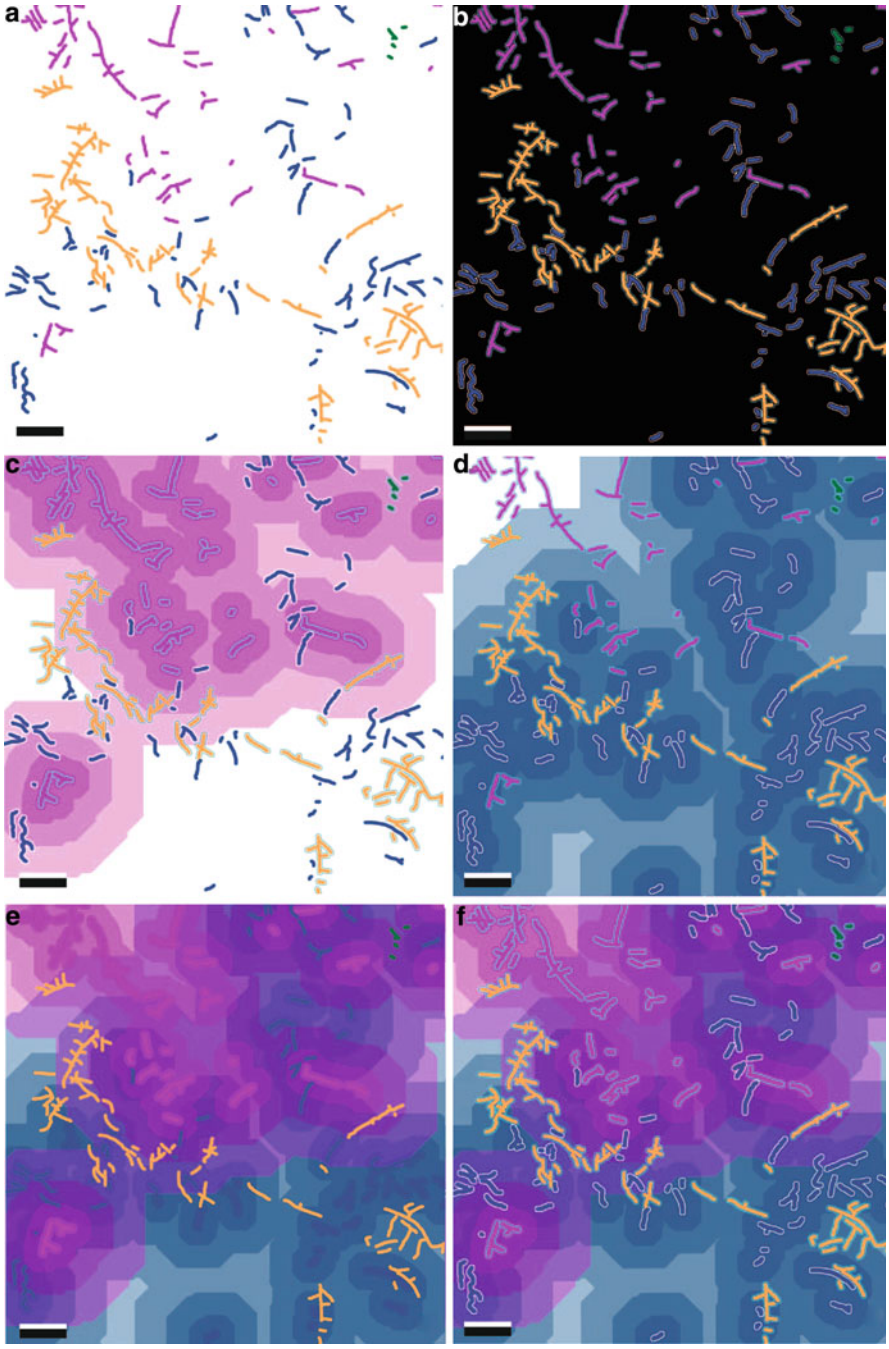

Fig. 10.3 In situ distribution and potential space occupation of ECM: *Cortinarius obtusus* (*blue*), *Tylospora fibrillosa* (*pink*), *Piceirhiza internicrassihyphis* (*ochre*), *Russula ochroleuca* (*green*).

hydrophilic surface, before or after the hydrophilic front of *C. obtusus* hyphae having passed and having become hydrophobic at this spot after ageing. They either can recur on nutrients not completely removed in advance by the hydrophobic competitor, or new nutrients become available, or they are specialised to a different set of nutrients, as shown, e.g. for the hydrophobic *C. obtusus* and the hydrophilic C-ET *Lactarius decipiens* and *L. theiogalus* (Agerer and Göttlein 2003).

10.4.3 Competition and Interaction

Ectomycorrhizal fungi are facing a great diversity of organisms as agents and targets of interactions. Since the investigations by Leake et al. (2002), Lindahl et al. (1999, 2001), and Werner and Zadworny (2003), it is obvious that physical and functional interactions occur between mycelia of ECM and of saprotrophs. Prokaryotes of the domain Bacteria isolated from fruitbodies, the so-called mycorrhiza helper bacteria, promote ECM formation (Duponnois and Garbaye 1991). Bacteria can also be included within tuberculate ECM (Li et al. 1992) and live on mantle and hyphal surface of ECM (Bomberg and Timonen 2009; Mogge et al. 2000; Schelkle et al. 1996; Timonen and Hurek 2006). Timonen and Hurek (2006) conclude that external mycelia can expand the habitat favourable for common rhizosphere bacteria into the soil far from the immediate rhizosphere. More important, it is suspected that prokaryotes play a role in nitrogen nutrition and mineral dissolution (Smith and Read 2008; Timonen and Hurek 2006). Even soil mesofauna and microfauna can influence fungi and ECM formation (Chakraborty et al. 1985; Ingham and Massicotte 1994; Mitchell and Parkinson 1976; Timonen et al. 2004). Since Marx and Davey (1969), ECM are regarded as protecting agents against root parasites, e.g. *Phytophthora cinnamomi*. *Castanea sativa* stands infected with *Phytophthora cambivora* revealed less severe reactions (Branzanti et al. 1994, 1999), and ECM with less extramatrical mycelia are formed as compared to a healthy stand, indicating

Fig. 10.3 (continued) (**a**) Micromap of ECM of a Norway spruce in Of-layer drawn in their natural position, shape and dimensions (taken from Agerer et al. 2002). (**b**) *Lines* around ECM indicate hydrophoby (*white*) and hydrophily (*blue*); only ECM of *C. obtusus* are hydrophobic. (**c**) ECM of *T. fibrillosa* (SD-ET) shown with their potential space occupation by the EMM; intensity of *pink colour* indicates density of mycelium as shown by Weigt et al. (2011); *boderlines* between intensity changes indicate distances from ECM surface in multiples of their diameter (0.3 mm), ×1, ×4, ×9, ×16 (the outmost). (**d**) ECM of *C. obtusus* (MDf-ET) shown with the potential space occupation of the EMM; intensity of *blue colour* indicates density of mycelium as shown by Weigt et al. (2011); *boderlines* between intensity changes indicate distances from ECM surface in multiples of their diameter (0.3 mm), ×1, ×4, ×9, ×16, ×25 (the outmost). (**e**) Combination of **c** and **d**, indicating the position of *P. internicrassihyphis* (MDs-ET) in *ochre* and *R. ochroleuca* (C-ET) in *green*; *R. ochroleuca* without space-occupying EMM; potential space occupation area of *P. internicrassihyphis* (MDs-ET) not known and therefore not shown. (**f**) The same as **e**, but hydrophoby/hydrophily of ECM indicated; only ECM of *C. obtusus* are hydrophobic. *Bar* = 5 mm

a shift in ECM community (Blom et al. 2009). Although all such interactions contribute to the multivariate relations within the soil, to ecology and function of trees, most of the recent ECM studies relevant to this topic focus on the influences of bacteria on ectomycorrhizal fungi.

10.4.3.1 ECM-Associated Bacteria

The close association of bacteria with mycorrhizal structures and the localisation of bacteria on the surface of ECM mantle and hyphae have been demonstrated repeatedly with several techniques, including cultural techniques (Timonen et al. 1998), fluorescence in situ hybridisation (FISH) and confocal laser scanning microscopy (Mogge et al. 2000). In several cases, it could be demonstrated that bacteria may even enter mycorrhizal fungi, as in the case of *Laccaria bicolor*, when retrieved from forest soil (Bertaux et al. 2005). Figure 10.4 demonstrates the colonisation of the surface of mycorrhizal mantles of different ECM from forest soil using FISH analysis and epifluorescence microscopy in a quantitative manner. The bacterial community of the mature Norway spruce and European beech stand at the "Kranzberger Forst" was analysed using different phylogenetic probes directed against α-, β- and γ-proteobacteria (Gram-negative bacteria), which are usually very frequently found on the surface of ECM. The major environmental parameter differing between the samples as presented in Table 10.2 was the water content of the soil, which was extremely low for several months until the sampling in December 2003, and wet to even water saturated in April 2004. It is clearly visible that the total number of bacteria, as determined with the DNA-staining dye DAPI (diamidino-phenylindole), was dependent both on the ECM fungus and the environmental conditions. In the case of *Lactarius subdulcis* the number of mycorrhizae-associated bacteria was much higher under wet than under dry conditions. In contrast, the number of bacteria were reduced under wet conditions in *R. ochroleuca* and *X. chrysenteron*. Only in the case of *Fagirhiza pallida*, the number of bacteria was extremely high under both conditions (Table 10.2). Interestingly, the occurrence of Gram-positive HighGC- and LowGC-bacteria were more frequent under dry conditions in the case of *F. pallida* and (almost) not present at wet conditions particularly on the surface of *X. badius* (Fr.) Kühner, *R. ochroleuca* (Pers.) Fr. and *L. subdulcis*. The latter two ECM represent hydrophilic ECM, whereas *X. badius* is hydrophobic. Beta- and gamma-proteobacteria are usually most frequently found in biofilms located on the surface of ECM, while α-proteobacteria were lower in number (Table 10.2). Although a high diversity of different genera and species is contributing to these large families, differences in the colonisation of mycorrhizal surfaces by bacteria can be envisaged. More detailed cloning and sequencing studies revealed that another huge class of soil bacteria, the *Acidobacterium* lineage is very frequently present on the mycorrhizal surface — in same cases amounting to 40–50 % of all bacteria (C. Kellermann, unpubl.). Calvaruso et al. (2007) reported on a *Scleroderma citrinum* Pers. ectomycorrhizosphere that it significantly structures the culturable bacterial

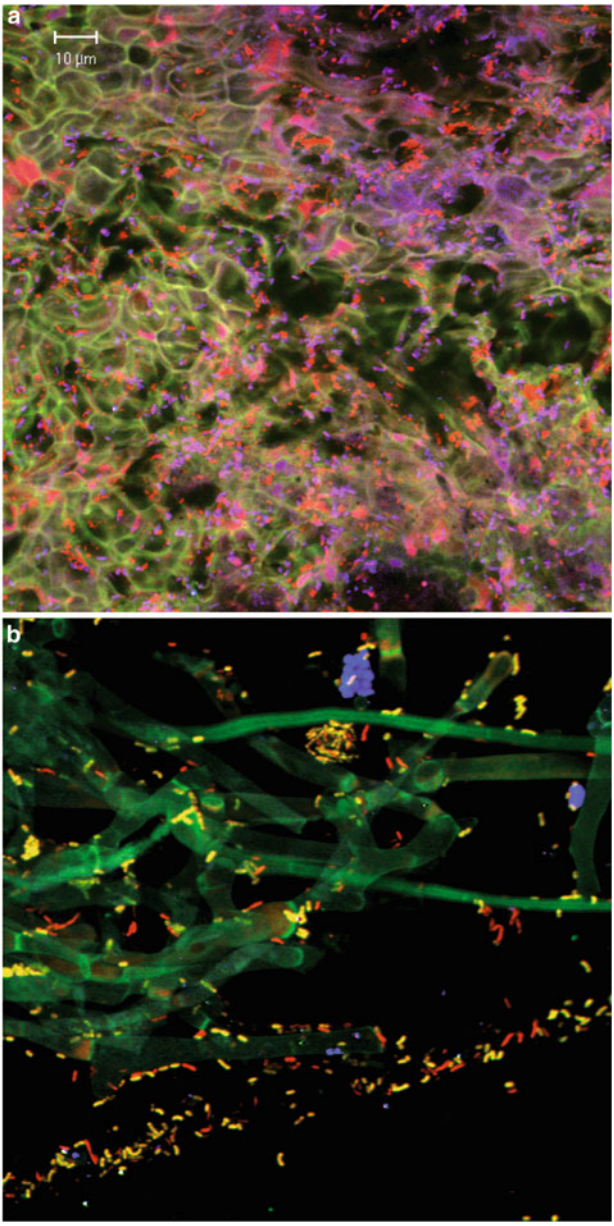

Fig. 10.4 Confocal laser scanning microscopic images of ectomycorrhiza–bacteria associations after specific visualisation of the associated bacteria using FISH analysis: (**a**) ectomycorrhizal mantle of *Lactarius subdulcis* in surface view; (**b**) emanating hyphae and cystidia of *Fagirhiza pallida*. FISH analysis was performed each with a combination of two oligonucleotide probes: EUB388-Cy3 and Alf1B-FLUOS in **a** and EUB388-Cy3 and BET43-FLUOS in **b**. Using these combinations of probes and fluorescent dyes, α-proteobacteria or β-proteobacteria appear *yellow-coloured*. All other cells are labelled *red*. *Bar* for both figs. 10 µm (Claudia Kellermann, unpubl.)

Table 10.2 In situ identified bacterial groups associated with ectomycorrhizae from forest soil (FISH analysis and epifluorescence microscopy)

Ectomycorrhizae	Bacterial numbers (cell counts per mm^2)	α-Proteobacteria	β-Proteobacteria	γ-Proteobacteria	High GC-bacteria	Low GC-bacteria
Xerocomus chrysenteron	5,000–12,000	–	++	+	(+)	–
	1,000–5,000	+	++	+	(+)	–
Fagirhiza pallida	>12,000	+	+++	++	(+)	(+)
	>12,000	++	+++	++	–	–
Xerocomus badius	1,000–5,000	–	++	+	(+)	–
	1,000–5,000	–	++	–	–	–
Russula ochroleuca	1,000–5,000	–	++	+	–	–
	Up to 1,000	+	+	+	–	–
Lactarius subdulcis	0–100	–	–	–	–	–
	5,000–12,000	+	+++	++	–	–

First sampling date (*upper row*; December 2003; very dry soil conditions!) and second plus third sampling date (*lower row*; April 2004; after rewetting during winter). – = lacking, (+) = found only occasionally (5–10 %), + = present in minor amounts (10–20 %), ++ = present in major amounts (20–40 %), and +++ = present in dominant amounts (around 60 %). 100 % = all bacteria stained with general DNA-stain DAPI; Claudia Kellermann, unpubl.

communities in the two soil horizons studied by selecting very efficient strains for phosphorus and iron mobilisation. Thus, bacteria are regularly associated with the surface of mycorrhizal hyphae. According to their diverse physiological potentials, bacteria certainly contribute substantially to the impact of mycorrhiza on soil–plant interactions and the performance of plants.

10.4.3.2 ECM Fungi as Competitors and as Potential Parasites

Environmental stress or changes in growth conditions, whether soil-derived or via above-ground plant components, may trigger impacts on the balance of organismic dependences within the soil, and could also influence mutualistic systems (Andersen 2003). Stress scenarios that change carbohydrate allocation into the root system and alter carbon availability for the fungal partner can influence competition, too (Alberton et al. 2007).

Some ECM species apparently associate with or exclude each other when tested in nature at micro-scale dimensions in squares of 2.5 × 2.5 mm (Agerer et al. 2002). Although statistically not significant, *Cortinarius obtusus* and *Piceirhiza internicrassihyphis* ECM preferentially occurred in such a close neighbourhood, whereas the latter species did not associate with *Russula ochroleuca*. *Russula ochroleuca*, in turn, preferred *Xerocomus badius* (Fr.) Kühner ECM (Agerer et al. 2002). Co-inoculation of seedlings under artificial or semi-natural conditions with two or several ECM species evidenced a preferential formation of a subset of ECM species (Kennedy and Bruns 2005; Parladé and Alvarez 1993), indicating that competition may play an important role during ECM formation. Mycelia of a non-identified ECM isolate (Wu et al. 1999) showed an aggressive replacement of the mycelium of *P. tinctorius* and a progressive formation of ECM in areas where *P. tinctorius* had resided earlier in the experiment. Direct evidence of hyphal interaction between two different ECM fungi was obtained by Agerer (2002).

Changes in carbon allocation to roots and ECM via altered photosynthetic capacities due to negative influence of O_3 or enhanced carbohydrate allocation due to increased CO_2 concentrations might influence ECM communities (Andersen 2003; Weigt et al. 2010). A striking example has been found in spruce under twice-ambient O_3 at the "Kranzberger Forst". ECM communities in the O_3-treated spruce experienced a dramatic increase in ECM of *Hygrophorus olivaceoalbus* (Fr.) Fr. (Agerer 2011). Apparently the roots react strongly against this fungus, as the cells produce extraordinarily high amounts of tannins. The hyphae form a Hartig net, but in older root regions, colonise cells and form microsclerotia within the root cells that apparently function as asexual propagules. Finally, the meristem decomposes and in old ontogenetic stages the ECM break open (Agerer 2011). As this phenomenon is not restricted to O_3-treated spruce but occurs similarly in higher altitudes of the National Park Bavarian Forest, different stressors have to be considered for this peculiar ECM features.

10.4.4 Microbial Activities in the Rhizosphere and Root Exudates

The volume of soil influenced by plant roots was first termed "rhizosphere" by Hiltner in 1904 and is specified as a zone of high microbial activity due to large quantities of carbon and other nutrients. Large amounts of carbon (C) are released from roots into the soil in the form of root exudates, containing 5–21% of photosynthetically fixed C (Marschner 1995). This release of C compounds and nutrients into the rhizosphere, the so-called "rhizodeposition", comprises the total C entering the soil in the form of water-soluble exudates, secretions, lysates, gases and mucilage (Grayston et al. 1996). Within rhizodeposits, water soluble exudates, mainly carbohydrates, carboxylic acids and amino acids (Lynch and Whipps 1990), are probably the most attractive components for microorganisms and therefore highly responsible for microbial growth (Lynch and Whipps 1990). As microbial communities are strongly influenced by root exudates (Brant et al. 2006), it has been hypothesised that plants may select beneficial microbial communities in their rhizosphere (Singh et al. 2007). The data published in the literature so far, which support this hypothesis, are mainly related to *Arabidopsis*, where specific mutants were used. For example, there is biochemical evidence that the tricarboxylic acid cycle intermediate L-malic acid secreted from roots of *Arabidopsis* selectively signals and recruits the beneficial rhizobacterium *Bacillus subtilis* FB17 in a dose-dependent manner (Rudrappa et al. 2008). When mycorrhizal fungi are associated with the roots the root vicinity is aptly referred to as "mycorrhizosphere" (Smith and Read 2008).

In a study by Esperschütz et al. (2009a), C fluxes between young European beech trees and soil were investigated following a chilling period of 3 weeks, using a continuous labelling set-up of the plants with ^{13}C labelled CO_2. It has been postulated that due to low concentrations of easily degradable carbon during tree dormancy, growth conditions for microbes in the rhizosphere are limited. Therefore, after chilling, when exudates are released into the mycorrhizosphere, a strong response of microbial communities utilising plant C is expected. Photosynthetically fixed carbon could be traced into plant tissue, dissolved organic carbon and total microbial biomass, where it was utilised by different microbial communities. Results from ^{13}C analyses of different plant parts reflected the allocation and distribution of recently fixed C within the young trees; 3.5 days after the start of labelling, the ^{13}C had been detected in leaves, where the assimilated carbon is used for leaf formation in the initial growth phase (Dyckmans et al. 2002) after dormancy. In leaves, assimilates are transformed into sugars and amino acids, which are transported via twigs and stems to other parts of the plant. Probably due to the length of the translocation pathway and anabolism of carbohydrates of woody plants (Kozlowski et al. 1991), the labelled C was detected in the below-ground plant parts after 20.5 days. Coarse roots basically serve as a storage site of carbon, resulting in a lower amount of incorporated newly assimilated C compared to fine roots. Conversely, fine roots, known as a highly active plant tissue, require high amounts of carbon for growth, resulting in high extents of recently assimilated carbon. Significant incorporation of labelled ^{13}C into the microbial biomass was

observed 10.5 days after the experiment has been started. Due to carbon allocation into the rhizosphere, nutrient stress decreased. It became evident that exudates were preferentially used by Gram-negative bacteria, resulting in an enhanced growth of these microbes (comp. Sect. 10.4.3.1). In accordance with other studies (Arao 1999; Butler et al. 2003), mycorrhizal fungi were also enriched in ^{13}C. Overall, the obtained results indicate a fast turnover of exudates and the development of initial food web structures. Additionally, a transport of assimilated carbon into bulk soil by mycorrhizal fungi was observed. Rhizodeposition may vary in response not only to environmental (water potential, light, soil compaction, temperature) and biological parameters (plant species, stage of development; Baudoin et al. 2003), but also to the presence of microorganisms (Grayston et al. 1996).

Esperschütz et al. (2009b) studied the consequences of increased O_3 exposure of trees on carbon fluxes and microbial community structure in the rhizosphere. Therefore, young beech trees exposed to ambient or twice-ambient O_3 concentrations over a 3 years' period were continuously labelled in the last vegetation period using ^{13}C-labelled CO_2. Harvesting of rhizosphere soil from individual trees was performed on a monthly basis, and analysis of the C fluxes was performed by following the ^{13}C label into the dissolved organic carbon fraction of the rhizosphere soil as well as into the phospholipid fraction of the rhizosphere microbes. It was postulated that an increase in O_3 concentration in the atmosphere would alter the microbial community structure and carbon fluxes in the rhizosphere soil. Results from this study demonstrate the high dynamic of microbial communities utilising plant-derived carbon in the rhizosphere of beech trees over a vegetation period and in response to plant stressors. It could be shown that microbial biomass in the mycorrhizosphere as well as individual microbial communities and their activity pattern in the mycorrhizosphere of young beech trees are mainly driven by seasonal variability. An increase in total microbial biomass as well as in individual microbial communities was detected during the vegetation period from June through September 2006. However, a clear O_3 effect was also visible mainly at the end of the vegetation period. PLFA data indicated earlier induced plant senescence as a response to the twice-ambient O_3 treatment. In particular, Gram-positive bacteria and fungi showed higher incorporation of plant C. Coherently, low incorporation of plant C into Gram-negative bacteria suggested the utilisation of other C sources rather than plant C at these time points. Furthermore, higher microbial biomass and abundance of plant C utilising microbes were observed under the O_3 treatment over the whole vegetation period. Surprisingly, the total amount of rhizodeposits did not change between O_3-treated and untreated plants, indicating that probably the quality of the exudates drives the growth of microbial communities rather than their total amount.

10.5 Conclusions

The ectomycorrhizosphere of roots plays a key role in functional ecology of trees and is a target and responder of environmental stress, irrespective of whether caused above- or below-ground. Space occupation of ECM is an important issue

for function, e.g. resource capture, of trees and fungi and can be influenced by environmental stress. Future studies on ECM should therefore include length measurements of the standing crop of ECM with respect to their mycelial exploration types, calculations of potential space occupation, and of carbon cost investment in ECM including the EMM, as well as of the mantle and of the Hartig net biomass. The presented data for ET-specific EMM biomass and potential space occupation can be used for such estimations, but additional studies are necessary to support the presently applied values. Particular focus has to be laid on the "smooth" subtype of the "medium distance" ET, as for that type biomass, hyphal length and space occupation of its EMM are not known yet. For fully understanding the functional relationships of ECM fungi and plants, turnover rates of ECM and their EMM, as well as ECM respiration at least under laboratory conditions, should be included. In situ mapping of ECM can be combined with ET-specific range and densities of EMM in combination with their hydrophobic or hydrophilic properties to unravel competition phenomena and niche occupation. Physical and functional relations of ECM to saprotrophic and parasitic as well as between ectomycorrhizal fungi, and to bacteria should be intensely studied. Especially the function of the bacteria — hyphal surface bound ones as well as endobacteria — should be in focus regarding ectomycorrhizal nitrogen nutrition, as ectomycorrhizal hyphae can apparently select for bacterial populations efficient for particular nutrient mobilisation. As the soil comprises an assemblage of micro-niches, heterogeneous in their nutrient and ion composition, micro-soil cores as small as possible should be analysed directly where the ECM are growing. Enzymatic activities of ECM communities should be recorded and related to total ECM length in order to obtain an overall picture of the enzymatic capacity of these symbiotic organs in the soil. Diversity studies on ECM can be supported by DNA-sequencing and a subsequent taxon identification. Finally, in order to provide information about ecological relevance, ECM quantification and last but not least consideration of their putatively functionally important features, predominantly revealed by exploration-type specific mycelial features and the organisation of rhizomorphs, need to be focussed on.

References

Agerer R (1985) Zur Ökologie der Mykorrhizapilze, vol 97, Bibliotheca Mycologica. Cramer, Vaduz

Agerer R (1987–2008) Colour atlas of ectomycorrhizae. 1st–14th delivery. Einhorn, Schwäbisch Gmünd

Agerer R (1991) Characterization of ectomycorrhiza. In: Norris JR, Read DA, Varma AK (eds) Techniques for the study of mycorrhiza, vol 23, Methods in microbiology. Academic, San Diego, pp 25–73

Agerer R (1993) Mycorrhizae: ectomycorrhizae and ectendomycorrhizae. Prog Bot 54:505–529

Agerer R (1995) Anatomical characteristics of identified ectomycorrhizas: an attempt towards a natural classification. In: Varma K, Hock B (eds) Mycorrhiza: structure, function, molecular biology and biotechnology. Springer, Heidelberg, pp 685–734

Agerer R (1999) Never change a functionally successful principle: the evolution of Boletales s. l. (Hymenomycetes, Basidiomycota) as seen from below-ground features. Sendtnera 6:5–91

Agerer R (2001) Exploration types of ectomycorrhizae. A proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. Mycorrhiza 11:107–114

Agerer R (2002) The ectomycorrhiza *Piceirhiza internicrassihyphis*: a weak competitor of *Cortinarius obtusus*? Mycol Prog 1:291–299

Agerer R (2006) Fungal relationships and structural identity of their ectomycorrhizae. Mycol Prog 5:67–107

Agerer R (2007) Diversity of ectomycorrhizae as seen from below and above ground: the exploration types. Z Mykol 73:61–88

Agerer R (2011) Asexual reproduction of *Hygrophorus olivaceoalbus* by intracellular microsclerotia in root cells of *Picea abies* – a winner of ozone stress? Mycol Prog. 11:425–435 doi:10.1007/s11557-011-0757-y

Agerer R, Göttlein A (2003) Correlations between projection area of ectomycorrhizae and H_2O extractable nutrients in organic soil layers. Mycol Prog 2:45–52

Agerer R, Raidl S (2004) Distance-related semi-quantitative estimation of the extramatrical ectomycorrhizal mycelia of *Cortinarius obtusus* and *Tylospora asterophora*. Mycol Prog 3:57–64

Agerer R, Rambold G (2004–2011) DEEMY – an information system for characterization and determination of ectomycorrhizae. München, Germany.http://www.deemy.de [first posted on 2004-06-01; most recent update: 2011-01-10]

Agerer R, Schloter M, Hahn C (2000) Fungal enzymatic activity in fruitbodies. Nova Hedwigia 71:315–336

Agerer R, Grote R, Raidl S (2002) The new method 'micromapping', a means to study species-specific associations and exclusions of ectomycorrhizae. Mycol Prog 1:155–166

Aguilera LM, Griffiths RP, Caldwell BA (1993) Nitrogen in ectomcycorrhizal mat and non-mat soils of different-age Douglas-fir forests. Soil Biol Biochem 25:1015–1019

Alberton O, Kuyper TW (2009) Ectomycorrhizal fungi associated with *Pinus sylvestris* seedlings respond differently to increased carbon and nitrogen availability: implications for ecosystem responses to global change. Glob Change Biol 15:166–175

Alberton O, Kuyper TW, Corissen A (2007) Competition for nitrogen between *Pinus sylvestris* and ectomycorrhizal fungi generates potential for negative feedback under elevated CO_2. Plant Soil 296:159–172

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

Aneja MK, Sharma S, Fleischmann F, Stich S, Heller W, Bahnweg G, Munch JC, Schloter M (2006) Microbial colonization of beech and spruce litter - influence of decomposition site and plant litter species on the diversity of microbial community. Microb Ecol 52:127–135

Aneja MK, Sharma S, Fleischmann F, Stich S, Heller W, Bahnweg G, Munch JC, Schloter M (2007) Influence of ozone on litter quality and its subsequent effects on the initial structure of colonizing microbial communities. Microb Ecol 54:151–160

Arao T (1999) In situ detection of changes in soil bacterial and fungal activities by measuring ^{13}C incorporation into soil phospholipids fatty acids from ^{13}C acetate. Soil Biol Biochem 31:1015–1020

Bakken LR, Oisen RA (1983) Buoyant densities and dry-matter contents of microorganisms: conversion of a measured biovolume into biomass. Appl Environ Microbiol 45:1188–1195

Baier R, Ingenhaag J, Blaschke H, Göttlein A, Agerer R (2006) Vertical distribution of an ectomycorrhizal community in upper soil horizons of a young Norway spruce (*Picea abies* [L.] Karst.) stand of the Bavarian Limestone Alps. Mycorrhiza 16:197–206

Brand F, Taylor AFS, Agerer R (1992) Quantitative Erfessung bekannter Ektomykorrhizen in Fichtenversuchsfiächen nach Behandlung mit saurer Beregnung und Kalkung: Die Reakton der

natürlichen Ektmykorrhiza-Population der Fichte auf saure Beregnung und Kalkung. Bericht BMFT-Projekt Nr. 0339175F

Baudoin E, Benizri E, Guckert A (2003) Impact of artificial root exudates on the bacterial community structure in bulk soil and maize rhizosphere. Soil Biol Biochem 35:1183–1192

Begon M, Harper JJ, Townsend CR (1986) Ecology. Blackwell, Oxford

Bending GD, Read DJ (1995) The structure and function of the vegetative mycelium of ectomycorrhizal plants. V. Foraging behaviour and translocation of nutrients from exploited litter. New Phytol 130:401–409

Bertaux J, Schmid M, Hutzler P, Hartmann A, Garbaye J, Frey-Klett P (2005) Occurrence and distribution of endobacteria in the plant-associated mycelium of the ectomycorrhizal fungus *Laccaria bicolor* S238N. Environ Microbiol 7:1786–1795

Blom JM, Vannini A, Vettraino AM, Hale MD, Godbold DL (2009) Ectomycorrhizal community structure in a healthy and a *Phytophthora*-infected chestnut (*Castanea sativa* Mill.) stand in central Italy. Mycorrhiza 20:25–38

Bomberg M, Timonen S (2009) Effect of tree species and mycorrhizal colonisation on the archaeal population of boreal forest rhizospheres. Appl Environ Microbiol 75:308–315

Brant JB, Myrold DD, Sulzman EW (2006) Root controls on soil microbial community structure in forest soils. Oecologia 148:650–659

Branzanti MB, Rocca E, Zambonelli A (1994) Influenza di funghi ectomicorrizici su *Phytophthora cambivora* e *P. cinnamomi* del castagno. Mycol Ital 1994:47–52

Branzanti MB, Rocca E, Pisi A (1999) Effect of ectomycorrhizal fungi on chestnut ink disease. Mycorrhiza 9:103–109

Butler JL, Williams MA, Bottomley PJ, Myrold DD (2003) Microbial community dynamics associated with rhizosphere carbon flow. Appl Environ Microbiol 69:6793–6800

Calvaruso C, Turpault M-P, Leclerc E, Frey-Klett P (2007) Impact of ectomycorrhizosphere on the functional diversity of soil bacterial and fungal communities from a forest stand in relation to nutrient mobilization processes. Microb Ecol 54:567–577

Chapin SF III, Matson PA, Mooney HA (2002) Principles of terrestrial ecosystem ecology. Springer, Berlin

Chakraborty S, Theodorou C, Bowen GD (1985) The reduction of root colonization by mycorrhizal fungi by mycophagous amoeba. Can J Microbiol 31:295–297

Chung H, Zak DR, Lilleskov EA (2006) Fungal community composition and metabolism under elevated CO_2 and O_3. Oecologia 147:143–154

Colpaert JV, van Tichelen KK (1996) Mycorrhizas and environmental stress. In: Frankland JC, Magan N, Gadd GM (eds) Fungi and environmental change. Symposium of the British Mycological Society. Cambridge University Press, Cambridge, pp 109-128

Courty PE, Pritsch K, Schloter M, Hartmann A, Garbaye J (2005) Activity profiling of ectomycorrhiza communities in two forest soils using multiple enzymatic tests. New Phytol 167:309–319

Courty P-E, Pouysegur R, Buée M, Garbaye J (2006) Laccase and phosphatase activities of the dominant ectomycorrhizal types in a lowland oak forest. Soil Biol Biochem 38:1219–1222

Courty P-E, Bréda N, Garbaye J (2007) Relation between oak tree phenology and the secretion of organic matter degrading enzymes by *Lactarius quietus* ectomycorrhizas before and during bud break. Soil Biol Biochem 39:1655–1663

Courty P-E, Labbé J, Kohler A, Marçais B, Bastien C, Churin JL, Garbaye J, Le Tacon F (2011) Effect of poplar genotypes on mycorrhizal infection and secreted enzyme activities in mycorrhizal and non-mycorrhizal roots. J Exp Bot 62:249–260

Dahlberg A, Jonsson L, Nylund J (1997) Species diversity and distribution of biomass above and below ground among ectomycorrhizal fungi in an old-growth Norway spruce forest in South Sweden. Can J Bot 75:1223–1335

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

Duddridge JA, Malibari A, Read DJ (1980) Structure and function of mycorrhizal rhizomorphs with special reference to their role in water transport. Nature (London) 287:834–836

Duponnois R, Garbaye J (1991) Mycorrhiza helper bacteria associated with Douglas fir - *Laccaria laccata* symbiosis: effects in aseptic and in glasshouse conditions. Ann Forest Sci 48:239–251

Dyckmans J, Flessa H, Brinkmann K, Mai C, Polle A (2002) Carbon and nitrogen dynamics in acid detergent fibre lignins of beech (*Fagus sylvatica* L.) during the growth phase. Plant Cell Environ 25:469–478

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 Phytol 131:443–451

Esperschütz J, Pritsch K, Gattinger A, Buegger F, Winkler JB, Munch JC, Schloter M (2009a) Microbial response to exudates in the rhizosphere of young beech trees (*Fagus sylvatica* L.) after dormancy. Soil Biol Biochem 41:976–1985

Esperschütz J, Pritsch K, Gattinger A, Welzl G, Haesler J, Buegger F, Winkler JB, Munch JC, Schloter M (2009b) Influence of chronic ozone stress on carbon translocation pattern into rhizosphere microbial communities of beech trees (*Fagus sylvatica* L.) during a growing season. Plant Soil 323:85–95

Fogel R, Hunt G (1979) Fungal and arboreal biomass in a western Oregon Douglas-fir ecosystem: distribution patterns and turnover. Can J Forest Res 9:245–256

Frey-Klett P, Garbaye J, Tarkka M (2007) The mycorrhiza helper bacteria revisited. New Phytol 176:22–36

Gadgil RL, Gadgil PD (1975) Suppression of litter decomposition by mycorrhizal roots of *Pinus radiata*. N Z J Forest Sci 5:35–41

Gebhardt S (2005) Räumliche Struktur und zeitliche Dynamik von Ektomykorrhizage-meinschaften in Roteichenökosystemen der Niederlausitz, Cottbuser Schriften. vol 34, Brandenburgische Technische Universität Cottbus, Cottbus

Genney DR, Anderson IC, Alexander IJ (2006) Fine-scale distribution of pine ectomycorrhizas and their extramatrical mycelium. New Phytol 170:381–390

Godbold DL, Berntson GM, Bazzaz FA (1997) Growth and mycorrhizal colonization of three North American tree species under elevated CO_2. New Phytol 137:433–440

Godbold DL, Berntson GM (1997) Elevated atmospheric CO_2 concentration changes ectomycorrhizal morphotype assemblages in *Betula papyrifera*. Tree Physiol 17:347–350

Gorissen A, Kuyper TW (2000) Fungal species-specific responses of ectomycorrhizal Scots pine (*Pinus sylvestris*) to elevated CO_2. New Phytol 146:163–168

Grayston SJ, Vaughan D, Jones D (1996) Rhizosphere carbon flow in trees, in comparison with annual plants: the importance of root exudation and its impact on microbial activity and nutrient availability. Appl Soil Ecol 5:29–56

Grebenč T, Kraigher H (2007) Changes in the community of ectomycorrhizal fungi and increased fine root number under adult beech trees chronically fumigated with double ambient ozone. Plant Biol 9:279–287

Griffiths RP, Ingham ER, Caldwell BA, Castellano MA, Cromack K (1991) Microbial characteristics of ectomycorrhizal mat communities in Oregon and California (USA). Biol Fertil Soils 11:196–202

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

Haberer K, Grebenc T, Alexou M, Gessler A, Kraigher H, Rennenberg H (2007) Effects of long-term free-air ozone fumigation on d15N and total N in *Fagus sylvatica* and associated mycorrhizal fungi. Plant Biol 9:242–252

Häberle K-H, Werner H, Fabian P, Pretzsch H, Reiter I, Matyssek R (1999) 'Free-air' ozone fumigation of mature forest trees: a concept for validating AOT40 under stand conditions. In: Fuhrer J, Achermann B (eds) Critical level for ozone – level II. Swiss Agency for the Environment, Forests and Landscape (SAEFL), Bern, pp 133–137

Hasselquist NJ, Varagas R, Allen MF (2010) Using soil sensing technology to examine interactions and controls between ectomycorrhizal growth and environmental factors on soil CO_2 dynamics. Plant Soil 331:17–29

Hedh J, Wallander H, Erland S (2008) Ectomycorrhizal mycelial species composition in apatite amended and non amended mesh bags buried in a phosphorus poor spruce forest. Mycol Res 112:681–688

Hendricks JJ, Mitchell RJ, Kuehn KA, Pecot SD, Sims SE (2006) Measuring external mycelia production of ectomycorrhizal fungi in the field: the soil matrix matters. New Phytol 171:179–186

Hiltner L (1904) Über neuere Erfahrungen und Probleme auf dem Gebiete der Bodenbakteriologie unter besonderer Berücksichtigung der Gründüngung und Brache. Arb DLG 98:59–78

Ingham ER, Massicotte HB (1994) Protozoan communities around conifer roots colonized by ectomycorrhizal fungi. Mycorrhiza 5:53–61

Kammerbauer H, Agerer R, Sandermann H (1989) Studies on ectomycorrhiza XXII. Mycorrhizal rhizomorphs of *Thelephora terrestris* and *Pisolithus tinctorius* in association with Norway spruce *(Picea abies)*: formation in vitro and translocation of phosphate. Trees 3:78–84

Kasurinen A, Keinänen MM, Kaipainen S, Nilsson L-O, Vapaavuori E, Kontro MH, Holopainen T (2005) Below-ground responses of silver birch trees exposed to elevated CO_2 and O_3 levels during three growing seasons. Glob Change Biol 11:1167–1169

Kennedy PG, Bruns TD (2005) Priority effects determine the outcome of ectomycorrhizal competition between two *Rhizopogon* species colonizing *Pinus radiata* seedlings. New Phytol 166:631–638

King JS, Pregitzer KS, Zak DR, Sober J, Isebrands JG, Dickson RE, Hendrey GR, Karnosky DF (2001) Fine-root biomass and fluxes of soil carbon in young stands of paper birch and trembling aspen as affected by elevated atmospheric CO_2 and tropospheric O_3. Oecologia 128:237–250

Koch N, Andersen CP, Raidl S, Agerer R, Matyssek R, Grams TEE (2007) Temperature-respiration relationships differ in mycorrhizal and non-mycorrhizal root systems of *Picea abies* (L.) Karst. Plant Biol 9:545–549

Korkama T, Fritze H, Pekkanen A, Pennanen T (2006) Interactions between extraradical ectomycorrhizal mycelia, microbes associated with the mycelia and growth rate of Norway spruce (*Picea abies*) clones. New Phytol 173:798–807

Kozlowski TT, Krammer PJ, Pallardy SG (1991) The physiological ecology of woody plants. Academic, San Diego

Kuzyakov Y (2002) Review: factors affecting rhizosphere priming effects. J Plant Nutr Soil Sci Z Pflanzenernahr Bodenkd 165:382–396

Leake JR, Donnelly DP, Boddy L (2002) Interactions between ectomycorrhizal and saprotrophic fungi. In: van der Heijden MGA, Sanders IR (eds) Mycorrhizal ecology. Ecological studies vol 157 Springer, Berlin, pp 346–372

Leake J, Johnson D, Donelly D, Muckle G, Boddy L, Read D (2004) Networks of power and influence: the role of mycorrhizal mycelium in controlling plant communities and agroecosystems. Can J Bot 82:1016–1045

Li CY, Massicotte HB, Moore LVH (1992) Nitrogen-fixing *Bacillus* sp. associated with Douglas-fir tuberculate ectomycorrhizae. Plant Soil 140:35–40

Liese W (1964) Über den Abbau verholzter Zellwände durch Moderfäulepilze. Holz Roh Werkstoff 22:289–295

Liese W (1970) Ultrastructural aspects of woody tissue disintegration. Ann Rev Phytopathol 8:231–258

Lindahl B, Stenlid J, Olsson S, Finlay R (1999) Translocation of 32p between interacting mycelia of a wood-decomposing fungus and ectomycorrhizal fungi in microcosm systems. New Phytol 144:183–193

Lindahl B, Stenlid J, Finlay R (2001) Effects of resource availability on mycelial interactions and ^{32}P transfer between a saprotrophic and an ectomycorrhizal fungus in soil microcosms. FEMS Microbiol Ecol 38:43–52

Luedemann G, Matyssek R, Fleischmann F, Grams TEE (2005) Acclimation to ozone affects host/pathogen interaction and competitiveness for nitrogen in juvenile *Fagus sylvatica* and *Picea abies* trees infected with *Phytophthora citricola*. Plant Biol 7:640–649

Luedemann G, Matyssek R, Winkler J, Grams TEE (2009) Contrasting ozone × pathogen interaction as mediated through competition between juvenile European beech (*Fagus sylvatica*) and Norway spruce (*Picea abies*). Plant Soil 323:47–60

Lynch JM, Whipps JM (1990) Substrate flow in the rhizosphere. Plant Soil 129:1–10

Markkola AM, Othonen R, Tarvainen O, Ahonen-Jonnarth U (1995) Estimates of fungal biomass in Scots pine stands on an urban pollution gradient. New Phytol 131:139–147

Marschner H (1995) Mineral nutrition of higher plants. Academic, London

Marx DH, Davey CB (1969) The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. III. Resistance of aseptically formed mycorrhizae to infection by *Phytophthora cinnamomi*. Phytopathology 59:549–558

Meyer FH (1962) Die Mykorrhiza der Waldbäume. Mitt Deutsch Dendrol Ges 62:55–59

Mitchell MJ, Parkinson D (1976) Fungal feeding of orbatid mites (Acari: Cryptostigmata) in an aspen woodland soil. Ecology 57:02–312

Mogge B, Loferer C, Agerer R, Hutzler P, Hartmann A (2000) Bacterial community structure and colonization patterns of *Fagus sylvatica* L. ectomycorrhizospheres as determined by fluorescence in situ hybridization and confocal laser scanning microscopy. Mycorrhiza 9:271–278

Nikolova PS, Andersen CP, Blaschke H, Matyssek R, Häberle K-H (2010) Belowground effects of enhanced tropospheric ozone and drought in a beech/ spruce forest (*Fagus sylvatica* L./*Picea abies* [L.] Karst). Environ Pollut 158:1071–1078

Nye PH, Tinker PB (1977) Solute movement in the soil-root system, Sudies in ecology. vol 4, Blackwell, Oxford

Parrent JL, Morris WF, Vilgalys R (2006) CO_2-enrichment and nutrient availability alter ectomycorrhizal fungai communities. Ecology 87:2278–2287

Paris F, Botton B, Lapeyrie F (1996) In vitro weathering of phlogopite by etomycorrizal fungi. II. Effect of K^+ and Mg^{2+} deficiency and N sources on accumulation of oxalate and H^+. Plant Soil 179:141–150

Parladé J, Alvarez JF (1993) Coinoculation of aseptically grown Douglas fir with pairs of ectomycorrhizal fungi. Mycorrhiza 3:93–96

Perez-Moreno J, Read DJ (2000) Mobilization and transfer of nutrients from litter to tree seedlings via the vegetative mycelium of ectomycorrhizal plants. New Phytol 145:301–309

Phillips DL, Johnson MG, Tingey DT, Storm MJ (2009) Elevated CO_2 and O_3 effects on fine-root survivorship in ponderosa pine mesocosms. Oecologia 160:827–837

Poole EJ, Bending GD, Whipps JM, Read DJ (2001) Bacteria associated with *Pinus sylvestris-Lactarius rufus* etomycorrhizas and their effects on mycorrhiza formation in vitro. New Phytol 151:743–751

Pritsch K, Courty P, Churin J-L, Cloutier-Hurteau B, Ali M, Damon C, Duchemin M, Egli S, Ernst J, Fraissinet-Tachet L, Kuhar F, Legname E, Marmeisse R, Müller A, Nikolova P, Peter M, Plassard C, Richard F, Schloter M, Selosse M-A, Franc A, Garbaye J (2011) Optimized assay and storage conditions for enzyme activity profiling of ectomycorrhizae. Mycorrhiza 21:589–600

Pritsch K, Garbaye J (2011) Enzyme secretion by ECM-fungi and exploitation of mineral nutrients from soil organic matter. Ann Forest Sci 68:25–32

Pritsch K, Raidl S, Marksteiner E, Blaschke H, Agerer R, Schloter M, Hartmann A (2004) A rapid and highly sensitive method for measuring enzyme activities in single mycorrhizal tips using 4-methylumbelliferone-labelled fluorogenic substrates in a microplate system. J Microbiol Methods 58:233–241

Pritsch K, Luedemann G, Matyssek R, Hartmann A, Schloter M, Scherb H, Grams TEE (2005) Mycorrhizosphere responsiveness to atmospheric ozone and inoculation with *Phytophthora citricola* in a phytotron experiment with spruce/beech mixed cultures. Plant Biol 7:718–727

Pritsch K, Günthardt-Goerg MS, Munch JC, Schloter M (2006) Influence of heavy metals and acid rain on enzymatic activities in the mycorrhizosphere of model forest ecosystems. Water Snow Landscape Res 80:289–304

Pritsch K, Esperschütz J, Haesler F, Raidl S, Winkler B, Schloter M (2009) Structure and activities of ectomycorrhizal and microbial communities in the rhizosphere of *Fagus sylvatica* under ozone and pathogen stress in a lysimeter study. Plant Soil 323:97–109

Raidl S (1997) Studien zur Ontogenie an Rhizomorphen von Ektomykorrhizen, Bibliotheca Mycologica. vol 169, Cramer, Berlin, pp 1–184

Read DJ (1992) The mycorrhizal mycelium. In: Allen MF (ed) Mycorrhizal functioning. An integrative plant-fungal process. Chapman & Hall, New York, pp 102–133

Rey A, Jarvis PG (1997) Growth response of young birch trees (*Betula pendula* Roth.) after four and a half years of CO_2 exposure. Ann Bot 80:809–816

Rillig CM, Treseder KK, Allen MF (2002) Global change and mycorrhizal fungi. In: van der Heijden MGA, Sanders IR (eds) Mycorrhizal ecology, Ecological studies. vol 157, Springer, Berlin, pp 135–160

Rinaldi AC, Comandini O, Kuyper TW (2008) Ectomycorrhizal fungal diversity: separating the wheat from the chaff. Fungal Divers 33:1–45

Rineau F, Garbaye J (2009) Does forest liming impact the enzymatic profiles of ectomycorrhizal communities through specialized fungal symbionts? Mycorrhiza 19:493–500

Rosling A, Rosenstock N (2008) Ectomycorrhizal fungi in mineral soil. Miner Mag 72:127–130

Rousseau JV, Sylvia DM, Fox AJ (1994) Contribution of ectomycorrhiza to the potential nutrient-absorbing surface of pine. New Phytol 128:639–644

Rudrappa T, Czymmek KJ, Paré PW, Bais HP (2008) Root-secreted malic acid recruits beneficial soil bacteria. Plant Physiol 148:1547–1556

Rygiewicz PT, Andersen CP (1994) Mycorrhiza alter quality and quantity of carbon allocated below ground. Nature (London) 369:58–60

Rygiewicz PT, Johnson MG, Ganio LM, Tingey DT, Storm MJ (1997) Lifetime and temporal occurrence of ectomycorrhizae on ponderosa pine (*Pinus ponderosa* Laws.) seedlings grown under varied atmospheric CO_2 and nitrogen levels. Plant Soil 189:275–287

Scattolin L, Montecchio L, Mosca E, Agerer R (2008) Vertical distribution of the ectomycorrhizal community in the top soil of Norway spruce stands. Eur J Forest Res 127:347–357

Schelkle M, Ursic M, Farquhar M, Peterson RL (1996) The use of laser scanning confocal microscopy to characterize mycorrhizas of *Pinus strobus* L. and to localize associated bacteria. Mycorrhiza 6:431–440

Schmid R, Liese W (1964) Über die mikromorphologischen Veränderungen der Zellwandstrukturen von Buchen- und Fichtenholz beim Abbau durch *Polyporus versicolor* (L.) Fr. Archiv Mikrobiol 47:260–276

Schramm JR (1966) Plant colonization studies on black wastes from anthracite mining in Pennsylvania. Trans Am Philos Soc 56:5–189

Schubert R, Raidl S, Funk R, Bahnweg G, Müller-Starck G, Agerer R (2003) Quantitative detection of agar-cultivated and rhizotron-grown *Piloderma croceum* Erikss. & Hjortst. by ITS-based fluorescent PCR. Mycorrhiza 13:159–165

Simard SW, Durall DM, Jones MD (2002) Carbon and nutrient fluxes within and between mycorrhizal plants. In: van der Heijden MGA, Sanders IR (eds) Mycorrhzal ecology, Ecological studies. vol 157, Springer, Berlin, pp 33–74

Singh BK, Munro S, Potts JM, Millard P (2007) Influence of grass species and soil type on rhizosphere microbial community structure in grassland soils. Appl Soil Ecol 36:147–155

Sittig U (1999) Zur saisonalen Dynamik von Ektomykorrhizen der Buche (*Fagus sylvatica* L.). Ber Forsch Waldökosyst 162:1–119

Smith SE, Read DJ (2008) Mycorrhizal symbiosis, 3rd edn. Academic, San Diego

Smits MM, Bonneville S, Haward S, Leake JR (2008) Ectomycorrhizal weathering, a matter of scale? Miner Mag 72:131–134

Tedersoo L, Kõjalg U, Hallenberg N, Larsson K-H (2003) Fine scale distribution of ectomycorrhizal fungi and roots across substrate layers including coarse woody debris in a mixed forest. New Phytol 159:153–165

Theuerl S, Buscot F (2010) Laccases: toward disentangling their diversity and functions in relation to soil organic matter cycling. Biol Fertil Soils 46:215–225

Timonen S, Hurek T (2006) Characterization of culturable bacterial populations associating with *Pinus sylsvestris - Suillus bovinus* mycorrhizospheres. Can J Microbiol 52:769–778

Timonen S, Jørgensen K, Haahtela K, Sen R (1998) Bacterial community structure at defined locations of the *Pinus sylvestris-Suillus bovinus* and *Pinus sylvestris-Paxillus involutus* mycorrhizospheres in dry pine forest humus and nursery peat. Can J Microbiol 44:499–513

Timonen S, Christensen S, Ekelund F (2004) Distribution of protozoa in scots pine mycorrhizospheres. Soil Biol Biochem 36:1087–1093

Tingey DT, Phillips DL, Johnson MG (2000) Elevated CO_2 and conifer roots: effects on growth, life span and turnover. New Phytol 147:87–103

van Schöll L, Hoffland E, van Beeren N (2006) Organic anion exudation by ectomycorrhizal fungi and *Pinus sylvestris* in response to nutrient deficiencies. New Phytol 170:153–163

Vogt KA, Grier CC, Meier CE, Edmonds RL (1982) Mycorrhizal role in net primary production and nutrient cycling in *Abies amabilis* ecosystems in western Washington. Ecology 63:370–380

Wallander H (2000) Uptake of P from apatite by *Pinus sylvestris* seedlings colonised by different ectomycorrhizal fungi. Plant Soil 218:249–256

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

Wallander H, Mahmood S, Hagerberg D, Johansson L, Pallon J (2003) Elemental composition of ectomycorrhizal mycelia identified by PCR-RFLP analysis and grown in contact with apatite or wood ash in forest soil. FEMS Microbiol Ecol 44:57–65

Weigt R (2010) Effects of elevated ground-level ozone on nitrogen acquisition of mature European beech (*Fagus sylvatica*) and Norway spruce (*Picea abies*) trees. Doctoral thesis, Weihenstephan Center of Life Sciences, TU München

Weigt R, Raidl S, Verma R, Rodenkirchen H, Göttlein A, Agerer R (2010) Effects of twice-ambient carbon dioxide and nitrogen amendment on biomass, nutrient contents and carbon costs of Norway spruce seedlings as influenced by mycorrhization with *Piloderma croceum* and *Tomentellopsis submollis*. Mycorrhiza 21:375–391

Weigt R, Verma R, Raidl S, Agerer R (2012) Exploration type specific standard values of extramatrical mycelium - a step towards assessing ectomycorrhizal space occupation and biomass in natural soil. Mycol Prog 11:287–297, 'Erratum'

Werner A, Zadworny M (2003) In vitro evidence of mycoparasitism of the ectomycorrhizal fungus *Laccaria laccata* against *Mucor hiemalis* in the rhizosphere of *Pinus sylvestris*. Mycorrhiza 13:41–47

Wöllecke J (2001) Charakterisierung der Mykorrhizazönosen zweier Kiefernforste unterschiedlicher Trophie, Cottbuser Schriften zu Bodenschutz und Rekultivierung. vol 17, Brandenburgische Technische Universität Cottbus, Cottbus

Wu B, Nara K, Hogetsu T (1999) Competition between ectomycorrhizal fungi colonizing *Pinus densiflora*. Mycorrhiza 9:151–159