CHAPTER 1: Mycorrhizal community structure and nutrient supply

1.1. Mycorrhizal mutualism

Mycorrhiza is the mutualistic association between certain soil fungi and plant roots: while the fungus improves plant mineral nutrition, the plant supplies the fungus with carbohydrates, which are ultimately derived from photosynthesis (Smith and Read 2008). More than 90% of the world's plants have mycorrhizal roots (Trappe 1987). There are seven types of mycorrhizal associations: arbuscular Mycorrhiza (AM), ectomycorrhiza (EM), ectendomycorrhiza, arbutoid mycorrhiza, monotropoid mycorrhiza, ericoid mycorrhiza, and orchid mycorrhiza (Smith and Read 2008). EM associations are the predominant form in temperate forests due to the dominance of members of *Pinaceae*, *Fagaceae*, *Betulaceae* and *Salicaceae,* forming preferentially EM (Brundrett 2004). Other kinds of mycorrhizas such as ericoid, ectendomycorrhiza and AM exist also in temperate forest ecosystems, but they will not be considered in this thesis, since the research here is focused on European beech (*Fagus sylvatica* L.).

1.1.1. Ectomycorrhizas: functional characteristics

There are three characteristic structures of EM symbiosis: the mantle, consisting of hyphae ensheating the root tips (Figure 1.1C), the hyphal net (Harting net) formed in the intercellular space of root epidermis and cortex cells, which maximizes the contact between plant and fungus (Figure 1.1D), and the external mycelium formed by single or aggregated hyphae that extend into the surrounding soil from the surface of the mantle (Figure 1.1C). In contrast with EM root tip, non-ectomycorrhizal (NM) root tips show root hairs (Figure 1.1A, B).

The emanating hyphae of EM increase the potentially absorbing surface area of the roots (Smith and Read 2008). In some cases, these hyphae may form vessel-like structures called rhizomorphs that are capable of nutrients and water transport along long distances (Duddridge et al. 1980, Agerer 2001). External mycelia differ greatly among EM fungal species (Agerer 2001). Based on characteristics such as structure, abundance and lengths of external mycelia, Agerer (2001) has classified EM fungi after four main exploration types: contact, short-distance, medium-distance, and long-distance type, respectively. Contact exploration type EMs possesses a smooth mantel with no or very few emanating hyphae; the rhizomorphs are absent. Short-distance exploration types EMs have usually short, but dense emanating hyphae; the rhizomorphs are lacking. Fungal species characterized as medium-distance exploration type may form rhizomorphs and the hyphae are more extended in the surrounding soil than those of the short-distance exploration type. Long-distance type EMs are

characterized by a smooth mantle with highly differentiated rhizomorphs. In beech forests, the most frequent EM species belong to all different classes of exploration types (Table 1.1).

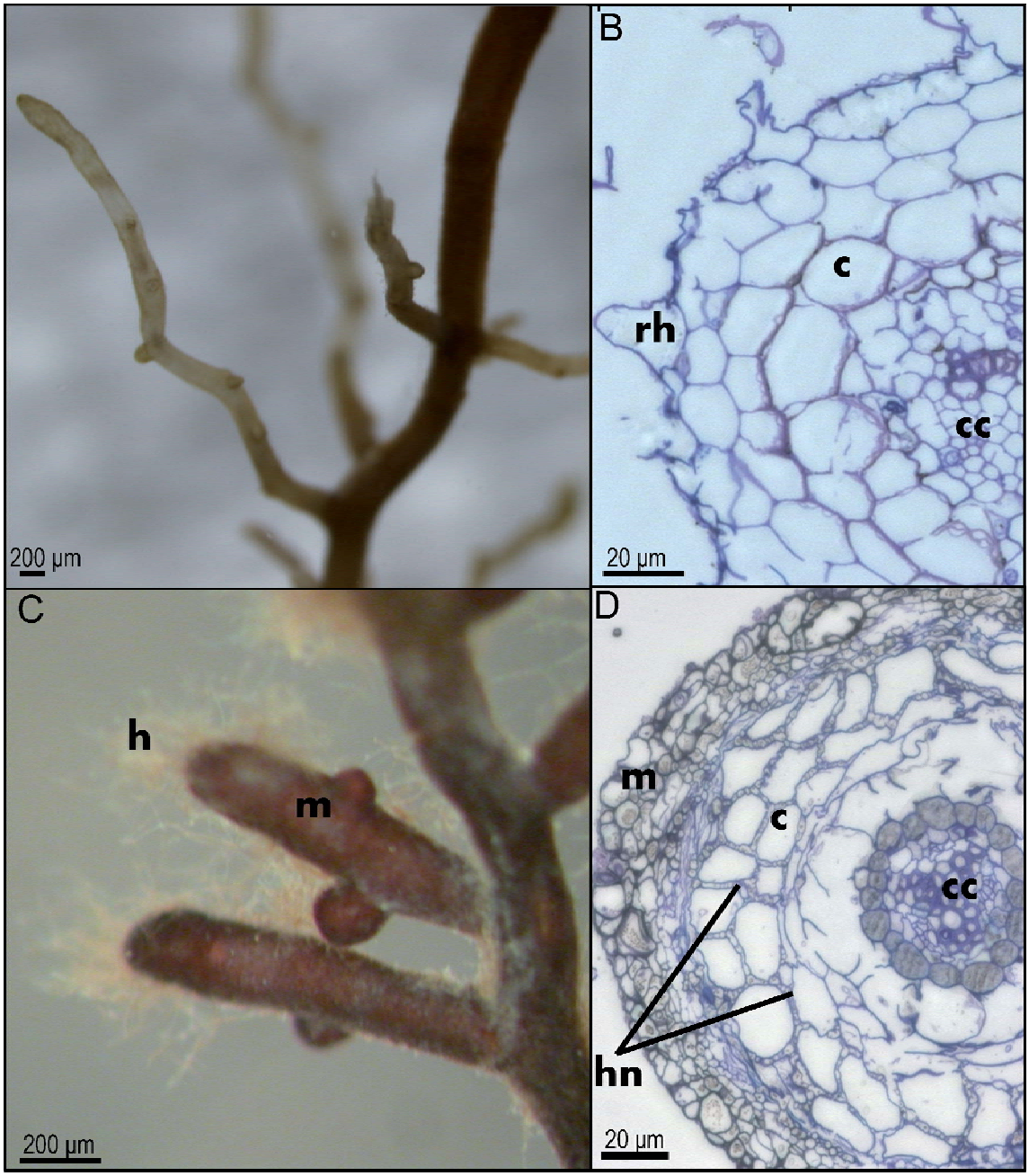

Figure 1.1: Non-mycorrhizal (A, B) and Ectomycorrhizal (C, D) root tips of young beech. Cortex cell (c), central cylinder (cc), hyphae (h), Hartig net (hn) rh, root hair (rh), mantle (m).

Table 1.1: Classification of the most frequent EM fungal species associated with beech roots (Buee et al. 2005, Pena et al. 2010) according to their mycelia system and their putative exploration type (Agerer 2001, Courty et al. 2008)

Species	Exploration type
Lactarius blennius	Contact
Lactarius subdulcis	Contact
Russula sp	Contact
Tomentella lilacinogrisea	Contact
Tomentella subclavigera	Contact
Cenococcum geophilum	Short-distance
Hebeloma crustuliniforme	Short-distance
Clavulina cristata	Medium-distance
Cortinarius sp	Medium-distance
Boletus pruinatus	Long-distance

1.1.2. Ectomycorrhizal communities

A unanimously accepted characteristic of EM fungal communities is their high diversity (Horton and Bruns 2001, Dahlberg 2001, Rinaldi et al. 2008). The EM status has been proven for thousands of fungal species, but exact estimates about EM fungal species richness are still not possible (Rinaldi et al. 2008). In a recent review, Rinaldi et al. (2008) estimated the contemporary known species richness of EM fungi of 7750 species and predicted these numbers to increase to 20000 to 25000 species, with the advent of sequencing of environmental samples.

In typical boreal and temperate forest ecosystems, fine roots of trees are almost 100% colonized by EM fungi (Smith and Read 2008). About 60 to 90 EM fungal species were found in a single stand of mature beech trees (Buee et al. 2005, Pena et al. 2010). EM fungi colonize rapidly, within days after their emergence, all orders of lateral roots formed at the axis of long roots with unlimited growth (Smith and Read 2008). If the laterals emerge from an already colonized parent root, they will be usually colonized with the same fungus. In contrast, if laterals originate from a non-colonized long root or if they belong to a recently germinated seedling with a completely new root system, competition between the available fungi in the soil occurs. In the latter case, EM formation depends on events such as recognition and compatibility, combined with direct inter- and intra-specific competition (Koide et al. 2005).

The structure of the EM community is influenced by abiotic and biotic factors (Bruns 1995, Koide et al. 2005). Soil properties like stratification, moisture, temperature, or fertility, but also the interactions among species can contribute to EM fungal diversity and may limit a species to a certain niche. Differences in substrate preference might be related to different functionality or life strategies of EM fungal species (Bruns 1995).

To date, knowledge on functional diversity of EM fungi in forests are scarce (Jones et al. 2009). In this thesis, functions of EM communities and individual EM species in field communities and experimental systems will be addressed.

1.1.3. Measuring EM community structures

Until the last decade, EM communities were almost exclusively described by the occurrence and abundance of sporocarps (Gardes and Bruns 1996). It was assumed that the production of sexual structures mirrors the relative abundance of vegetative structures of different species (Smith and Read 2008). But it became apparent that this approach did not represent complete EM communities, because very important EM fungi, e.g., *Cenococcum geophilum* and species of the *Corticinaceae* and *Thelephoraceae* have no or inconspicuous sporocarps (Gardes and Bruns 1996, Taylor and Bruns 1999, Peter et al. 2001a, Peter et al. 2001b). Therefore, the study of EM fungal communities at the level of root tips was necessary. To characterize EM communities, morphological description of mantle and extraradical mycelium, so-called morphotyping, has been and is being applied (Agerer 1987-1989, Agerer 1991). However, this technique has critical limitations because it is time consuming and requires an experienced investigator. Furthermore, because of high morphological similarity among different EM species, morphotyping is sometimes inaccurate and one EM species can have different morphotypes depending on age or host plant (Agerer 1987-1989, Peter et al. 2001b).

The precision of fungal identification has been greatly improved through the development of molecular techniques and the use of DNA sequence databases (Taylor and Bruns 1999, Horton and Bruns 2001). Sequences of the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA are currently most used as barcoding target for fungal identification (Ryberg et al. 2009). The ITS region is situated between the small subunit (SSU) and the large subunit (LSU) ribosomal RNA (rRNA), contains two non-coding spacer regions separated by 5.8S rRNA, and has a size of 650-900 bp (Gardes and Bruns 2001). These non-coding spacer regions are characterized by a fast rate of evolution, resulting in high sequence variation between closely related species (Anderson and Cairney 2004). White et al. (1990) designed the first PCR primers for amplification of ITS regions

from fungal DNA. The primer pair ITS1 and ITS4 (White et al. 1990) are still widely used today, but they display a lack of specificity for fungal template DNA and co-amplifiy angiosperm DNA. Thus from a mixed plant-fungal DNA sample, plant DNA can be also amplified. To improve specificity, several taxon-specific primers such as ITS1f or ITS4b that allow selective amplification of basidiomycete DNA from mixed DNA samples extracted from mycorrhizal root tips have been described (Gardes and Bruns 1993).

To date, identification of EM fungi is achieved by comparison of ITS sequences with known sequences deposited in public databases (Koljalg et al 2005, Ryberg et al. 2009). As DNA extraction and amplification may strongly vary amongst fungal species (Tedersoo et al. 2010), currently the most reliable procedure to study EM community composition is a combination of morphotyping and Sanger sequencing. More recent studies describe and compare complex soil fungal communities using high-throughput sequencing techniques (Buee et al. 2009). However, these methods still suffer from a relatively poor accuracy and require careful selection of molecular tools (Tedersoo et al. 2010).

In the present thesis, a combination of morphotyping and ITS sequencing was employed to investigate EM communities. This has the advantage that in addition to an accurate identification, the frequency of the fungi can also be reliably determined. These measures are required to determine not only the species richness but also diversity indices such as the Shannon-Wiener or Evenness (Dahlberg 2001).

1.2. Functions of EM in C and N exchange between plant and soil

1.2.1. Carbon transfer to EM fungi

A vast range of EM fungi show saprotrophic abilities (Koide et al. 2008, Courty et al. 2005) and can exist in the absence of the host plants freely in the soil. Formation and sustainability of EM symbiosis strongly depend on plant derived carbon (C) supply to the associated fungi (Smith and Read 2008). The transfer of current assimilates to soil microorganisms has been estimated using different approaches such as short pulse ^{13}C labelling (Högberg et al. 2008, Rühr et al., 2009), concomitant measurements of ^{13}C isotopic signature of CO_2 respired and newly assimilated organic matters pools (Gessler et al. 2007, Kodama et al. 2008), as well as by different experiments, e.g. by disruption of the photosynthate flux by tree girdling (Högberg et al. 2001, Högberg et al. 2002, Bhupinderpal-Singh et al. 2003. Pena et al. 2010) or by application of free air carbon dioxide enrichment (FACE), with a different isotopic signature to identify new tree derived soil C (Godbold

et al. 2006). All these studies have shown that the recent assimilates are rapidly transferred to the belowground compartment and much of this C flux becomes available to EM fungi. A field-scale girdling experiment in a conifer forest (Högberg et al. 2001) showed that by interruption of C flux, soil microbial respiration decreased in two weeks by 56%, of which 41% was due to the loss of EM mycelia (Högberg et al. 2001, Högberg et al. 2002). In another study, the contribution of EM external mycelium and of roots to forest soil CO_2 efflux have been estimated to account ca. 25 % and 15 %, respectively and the residual 60 % were due to soil heterotrophic respiration (Heinemeyer et al. 2007). Furthermore, CO_2 enrichment in a poplar plantation indicated that EM fungal C constitutes the dominant (62%) C input to soil organic matter (Godbold et al. 2006). Controlled experiments using ^{13}C isotope signatures indicated that as much as 22% of photosynthate was allocated to EM fungi (Hobbie 2006).

Monitoring of EM fungal communities, colonization rate, and formation of fruiting bodies showed negative effects of reduced plant C supply emphasizing the strong C demand of EM fungi. For example, when C flux to roots was interrupted by girdling, or reduced by defoliation, EM fruiting body formation ceased (Högberg et al. 2001, Kuikka et al. 2003). Defoliation of conifers also suppressed EM morphotypes with thick mantels compared to those with thin mantels (Markkola et al. 2004), and increased the number of root tips colonized by low-biomass EM that probably require less plant derived C (Saravesi et al. 2008). Reduction of photosynthesis by long-term shading resulted in a strong diminution of EM root colonization compared with sun-exposed beech trees (Druebert et al. 2009). In conclusion, these studies have shown that EM have important ecological roles in belowground C allocation and that plant C productivity is an essential driver of EM formation. However, large gaps exist regarding the importance of plant C flux and C-storage pools for EM diversity and functions. Since drought is an important environmental factor that has strong effects on photosynthesis and belowground C allocation, it is of particular interest if and how EM fungal communities and functions respond to this stress factor. This question is particularly relevant for beech, a species known to be relatively drought sensitive (Ellenberg and Strutt, 2009). Therefore, in this thesis the C-flux was modulated by girdling, shading and drought to study EM functions and community composition.

1.2.2. Nitrogen forms and plant availability

Nitrogen (N) is an essential constituent of many organic compounds of plant cells, such as amino acids, amides, proteins, nucleic acids, pigments, coenzymes etc. For growth and optimal physiological functioning, plants require a permanent supply with N. Although the atmosphere

contains about 386 x 10^{13} t (78% by volume) dinitrogen (N_2), N is a limiting factor for plants in most near-natural forest ecosystems (Rennenberg 1998, Chen et al. 2000), since N_2 cannot be directly assimilated by plants (Semoka 2008). In soil, N occurs in complex organic compounds such as humic acids, which also are not available for most plants. Only a small fraction of total N in the soil is present in forms such as ammonium (NH_4^+), nitrate (NO_3^-), or amino acids that can readily be taken up by plant roots. Apart from anthropogenic N pollution, the most important source of N in forest ecosystems is leaf litter (Chen et al. 2000). In the first phase after shedding, the litter is decomposed by soil microorganisms, mainly saprotrophic fungi (Figure 2). Thereby, N is transferred into the dissolved organic N pool and then mineralized to NH_4^+ by other microorganisms, mainly bacteria (Scharenbroch and Lloyd 2004). NH_4^+ can either be taken up by plant roots, by soil microorganisms, including EM fungi, or by chemoautotrophic nitrifiers (Figure 1.2). Nitrifying bacteria, *Nitrosomonas* spp and *Nitrospira* spp, oxidize NH_4^+ to nitrite (NO_2^-) and *Nitrobacter* ssp further oxidize NO_2^- to NO_3^- through the process of nitrification (Figure 1.2, Scharenbroch and Lloyd 2004).

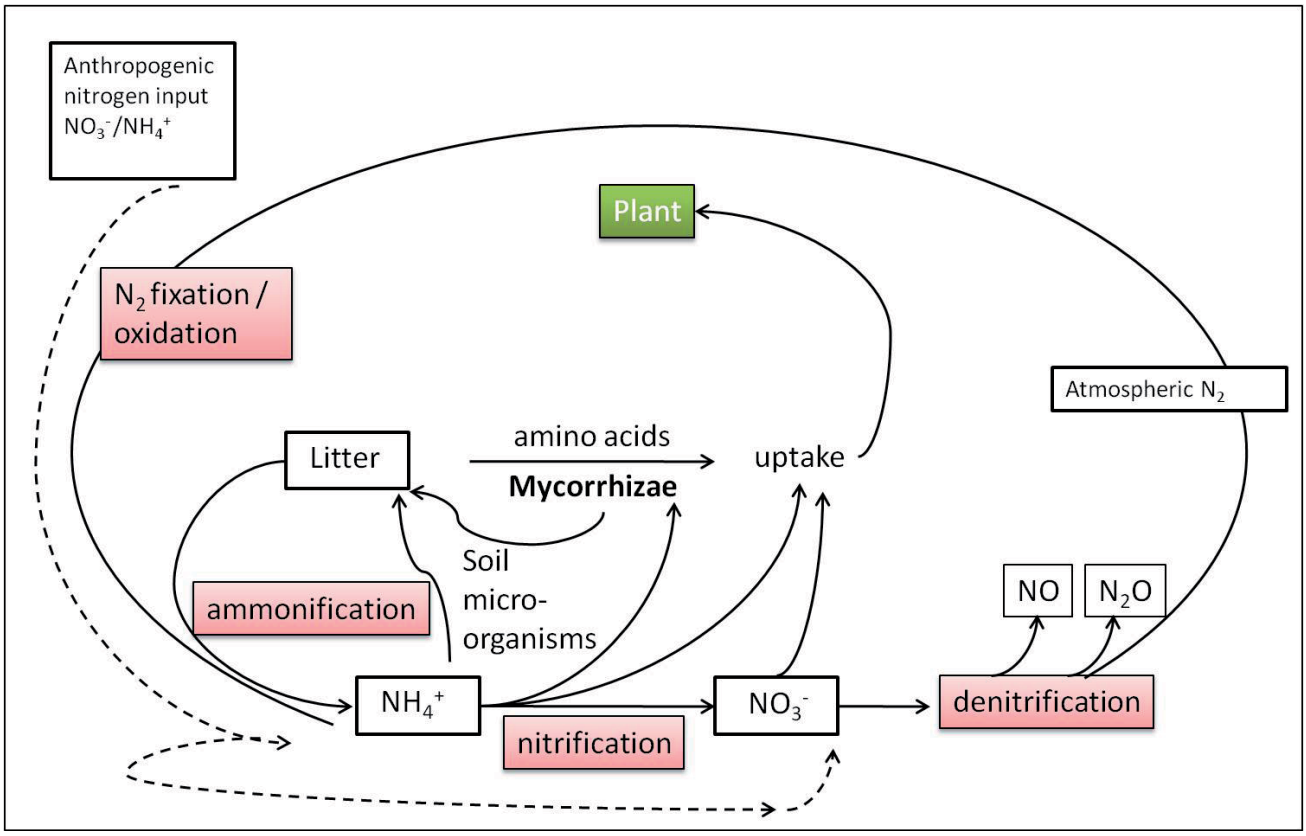

Figure 1.2: Major pathways of nitrogen cycle in forest ecosystems were displayed. Adapted after Schulze (2000).

N mineralization rate depend on various abiotic and biotic factors such as temperature, humidity, physical and chemical characteristics of the soil, microbial populations, and quantity and quality of

litter (Yang et al. 2003). For instance, the rate of mineralization increases at higher temperatures and intermediate soil water content (Brüggemann et al. 2005). Low soil water content inhibits microbial activities by reducing diffusion of soluble substrates (Schjonning et al. 2003), microbial mobility (Killham et al. 1993), and intracellular microbial water potential (Stark and Firestone, 1995). C and N contents of the organic material are also factors that influence N mineralization. At a high C:N ratio, the microorganisms are N-limited, while below a critical value they are C-limited (Kaye et al. 1997). In the latter case, N immobilization in heterotrophic microorganisms is restricted, resulting in formation of inorganic N (Kaye et al. 1997). In the present study, we have modulated belowground C availability by girdling, drought and sugar feeding (Winkler et al. 2010, see appendix 2). Girdling resulted in a significant increase of N mineralization, an effect that was combated by glucose application. C limitation facilitated in our study high soil NO_3^- concentrations, and by application of glucose, microbial NH_4^+ immobilization was promoted. Soil NH_4^+ concentrations increased in response to drought, also.

Organically bound N may represent an N source for many plant species in arctic, boreal, temperate, Mediterranean shrubland and alpine ecosystems (Näsholm et al. 2009). Organic N sources that are directly accessible for plants are free amino acids in soil solution, whereas the organic N pool is mainly formed by peptide- and protein-bound amino acids (Näsholm et al. 2009). Therefore, an intermediate step in plant organic N nutrition is depolymerization of organic macromolecules to plant available monomers (Schimel and Bennett 2004). The main pathway for this is the proteolysis of peptides and proteins. The involved proteolytic enzymes are mainly produced by soil microorganisms including mycorrhizal fungi (Näsholm et al. 2009). Although it is known that EM fungi produce different exoenzymes for degradation of organic nutrients (Read and Perez-Moreno 2003), we have little information if this results in functional diversity with respect to mycorrhizal N –accumulation. To address this question in the present study, beech leaf litter, which is degraded very slowly, was used to follow N uptake by different EM species in an old-growth forest.

1.2.3. Nitrogen in forest soils and uptake by ectomycorrhizal fungi

In forest soils, NH_4^+ is the dominant form of inorganic N (Nadelhoffer et al. 1984, Semoka 2008), because nitrification is usually suppressed by low soil temperature, low pH, and allelopathic soil conditions (Kronzucker et al. 1995). However, soil NH_4^+ to NO_3^- ratios vary strongly with forest type, time point, and environmental conditions (Nadelhoffer et al. 1985). In undisturbed forest soils the ratio is about 10:1, but it might be higher in deciduous (33:1) than in coniferous forest (about 5:1, Jerabkova et al. 2006). Over a range of deciduous forest soils, NH_4^+ concentrations varied between

2.4 and 12.5 mg kg^{-1} and NO_3^- from <0.1 to 2.8 mg kg^{-1} (Vitousek et al. 1982). Soil NO_3^- concentrations increased after disturbances such as fire, wind throw, and clear cutting (Kronzucker et al. 1995).

The study site in this thesis was a beech forest located in a low mountain range in southern Germany, Tuttlingen forest. In the investigated soil, NH_4^+ was the dominant form of inorganic N. NH_4^+ concentrations ranged from 4 to 8 mg kg^{-1}, while NO_3^- were less than 2 mg kg^{-1} (Dannenmann et al. 2009, see appendix 1). DON concentrations ranged between 20 and 40 mg kg^{-1}(Dannenmann et al. 2009, see appendix 1). All these N sources of variable sizes may be accessed by beech trees via their fungal symbionts, since the roots of European beech, like those of all *Fagaceae*, are almost 100% colonized by EM fungi (Smith and Read 2008).

EM fungi may endow plants access to organic forms of N, inducing a shortcut in the N cycling. The abilities of EM fungi alone or, in symbiosis, to use different organic N sources such as amino acids or proteins were extensively investigated in laboratory studies (Harley and Smith 1983, Abuzinadah and Read 1986, Baar et al. 1997, Chalot et al 1998, Gobert and Plassard 2008). In the field, proteolytic capacities of EM fungi were indirectly assessed by measuring the natural abundance of ^{15}N in fungal fruiting bodies (Gebauer and Dietrich 1993, Taylor et al. 1997). Greater ^{15}N contents in EM fruiting bodies indicate higher abilities to access organic than mineral N sources (Lilleskov et al. 2002). By employing ^{15}N and ^{13}C labeled amino acids, the ability of EM fungi to take up amino acids was shown (Schimel and Chapin 1996, Näsholm et al. 1998, McKane et al. 2002). We have shown recently a positive correlation of *Cenococcum geophilum* abundance with glutamine uptake by mature beech (Dannenmann et al. 2009, see appendix 1). Under field and also under the green house conditions beech trees preferred amino acids over inorganic N (Dannenmann et al. 2009, Winkler et al. 2010, see appendix 1,2). Same EM fungi prefer NH_4^+ to NO_3^- (Finlay et al. 1989, Ek et al. 1994, Rangel-Castro et al. 2002, Guidot et al. 2005). For example, uptake and assimilation rates were two fold higher for ^{15}N-labelled NH_4^+ than for NO_3^- in *Fagus sylvatica* colonized by *Paxillus involutus* (Finlay et al. 1989). But, there are also EM fungi, such *Hebeloma cylindrosporum* that grow better with NO_3^- as source of N than with NH_4^+ (Scheromm et al. 1990).

Despite of the well known involvement of EM fungi in N uptake, there are scarce data that build a link between plant C status, EM fungi and N nutrition. Usually, the questions about functionality of EM fungi were addressed in laboratory studies, where the aspect of functional diversity has been overlooked.

1.3. Occurrence and N requirements of European beech

Fagus sylvatica L. is the most important deciduous species of the potential natural vegetation in forests of Central Europe (Ellenberg and Strutt, 2009). Beech dominates the vegetation in the submontane and montane belt from 200 m above the sea level (asl) in the northern and from 500 asl in the southern part of Europe up to an altitude of 1200 to 1400 m asl. At the montane level, beech trees mix with conifers (Ellenberg and Strutt, 2009). European beech requires a suboceanic to weakly subcontinental climate. It grows on all types of bedrocks, but a preference for calcareous soil of pH 5.7 – 7.4 was observed (Ellenberg and Strutt, 2009).

In the juvenile phase, beech trees are very shade–tolerant (Gansert and Sprick 1998), but when increased irradiance is available, e.g. in forest gaps, the growth of young beech plants is stimulated (Tognetti et al. 1994, Johnson et al. 1997, Tognetti et al. 1998, Collet et al. 2001, Aranda et al. 2004, Parelle et al. 2006). Ellenberg and Strutt (2009) identified water availability as the most decisive factor for the habitat requirements of beech. Compared with other temperate broad-leaved species, European beech is more sensitive to drought. Growth and the competitive ability of beech decrease strongly during intensive drought periods that may occur during the growth phase (Peuke et al. 2002, Gessler et al. 2007). When water availability is moderate, beech prevents strong reduction in turgor, leaf water potential and in photosynthesis by stomatal regulation (Backes and Leuschner 2000). However, during severe drought stomatal closure is not sufficient to avoid decreases in predawn leaf water potential, photosynthetic capacity, hydraulic conductivity (Gessler et al. 2001), canopy conductance (Granier et al. 2000, Gessler et al. 2004) and growth (Braun and Flückiger 1987, Lebourgeois et al. 2005). Drought influences directly the water status of the beech and impairs pedospheric N uptake, thus affecting N metabolism (Gessler et al. 2004).

The nutritional status of trees is generally characterized by their foliar nutrient concentrations. In field grown beeches in North and Central Europe, leaf N concentrations range from 24 to 28 mg g^{-1} dry mass (Balsberg-Pahlsson 1988, Duquesnay et al. 2000). High N concentrations in leaves appeared in the spring, summer months during leaf growth, and decreased due to N retranslocation in autumn (Santa Regina and Tarazona 2001). Drought treatment had no significant effect on N concentrations in leaves and roots of young plants (Fotelli et al. 2002b, Peuke and Rennenberg 2004), but reduced N concentration in stems (Peuke and Rennenberg 2004).

As outlined above, under field conditions beech trees prefer NH_4^+ uptake to NO_3^-. Even if beech may have the capacity to take up NO_3^-, the ratios NH_4^+ to NO_3^- found in soil of deciduous forests of about 10:1 inhibit NO_3^- uptake (Gessler et al. 1998a). Furthermore, beech trees display a seasonal and a

diurnal course of NH_4^+ net uptake (Gessler et al. 1998a, Gessler et al. 2002). The rates of NH_4^+ uptake have a maximum in midsummer and lower values in spring and autumn and increase with soil temperature (Gessler et al. 1998a). The diurnal patterns of NH_4^+ uptake are 50% higher during the day than during the night and the highest rates are observed during midday and in the afternoon (Gessler et al. 2002). Drought decreases N uptake rates of beech seedlings and the effects are aggravated by the presence of competitors such as *Rubus fruticosus* (Fotelli et al. 2002b).

Gessler et al. (2007) evaluated the potential risks for European beech under climate-change conditions, characterized by weather extremes with frequent drought periods (IPCC 2001). They concluded that growth and competitive ability of beech might be reduced under predicted climate conditions. Mainly seedlings, but also the adult trees may suffer from restricted nutrient uptake capacity under limited water availability (Gessler et al. 2007). Given these interrelationships between drought, C production and N nutrition, the role played by EM fungi has to be better understood.

1.4. Objectives of the present thesis

This thesis focuses on functional diversity of beech ectomycorrhizas. The overarching goal was 1) to investigate the functions of different EM fungal species with respect to N uptake and 2) to estimate the significance of plant C supply to sustain EM diversity.

To address these aims the following hypotheses were tested:

- In a mature beech forest, the EM community is composed of species with different abilities to access liter-derived N. In particular, we assumed that EM of the long distance exploration type accumulate litter-derived N faster than short distance type ones, because they can explore a wider range and reach N resources inaccessible to roots. Differences in ^{15}N accumulation between EM fungal species decrease over time with the increasing availability of litter-released N via the soil.

  To test these hypotheses ^{15}N labeled beech leaf litter was exposed in the top soil of an old growth beech forest. ^{15}N enrichment was regularly measured in soil, fine roots and root tips colonized by different EM fungal species. To distinguish between N uptake from litter patches and from the soil, a set of litterbags were removed after 14 months and the incorporation of the label in EM root tips and roots in the presence and absence of the litterbags was measured **(Chapter 2).**

- EM fungi reveal functional diversity with respect to N transport and turnover. Under environmental conditions such as low irradiance and restricted water availability they reveal complementary behavior rather than competition.

  To test this hypothesis, we conducted a growth chamber experiment with young beech plants, whose root systems were either colonized by typical EM fungal communities, or which were non-mycorrhizal (NM). We expected that variation in the environmental conditions would result in similar, possibly negative effects, on N transport and turnover if the whole EM community showed functional redundancy or, in contrast functional complementary would result in differential behavior of different EM species in response to changing light and water supply **(Chapter 3).**

- N uptake by EM plants is higher than in NM plants under drought stress and lower under shade because of differences in C allocation and C costs.

  To test this hypothesis, the performance of young beech plants, whose root systems were non-colonized or were colonized by typical EM fungal communities, was tested under different light and water supply. It was assumed that N-uptake was maintained in EM under drought stress but that shade would impose C-limitation, thereby preventing maintenance of EM-based N influx **(Chapter 4).**

- EM fungal abundance and diversity are independent of the current photosynthates supply and can be maintained by internal resources.

  To investigate this assumption, old-growth beech trees (*Fagus sylvatica* L.) were girdled to suppress carbon allocation to roots. Tree carbohydrates status was measured repeatedly during one year and related to root demography, EM colonization and EM species abundance **(Chapter 5).**

1.5. References

Abuzinadah R.A., Read D.J. 1989a. The role of proteins in the nitrogen nutrition of ectomycorrhizal plants. IV. The utilization of peptides by birch (*Betula pendula* L.) infected with different mycorrhizal fungi. New Phytologist 112: 55–60.