# CHAPTER 1. General introduction

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## 1.1. Description of mycorrhizal symbiosis

In temperate forests, one of the most common mutualistic symbioses is formed by association of tree root tips with mycorrhizal fungi forming mycorrhiza. Mycorrhizas are important for plant nutrient uptake (Tuomi *et al.*, 2001). The mycorrhizal symbiosis is a trading system, in which the mycorrhizal fungi through their external hyphal network take up and deliver nutrients to the host plant, in return, they obtain from the plant the carbohydrates they need for essential organic compound synthesis (Smith & Read, 2008). Mycorrhizas increase the surface for nutrient uptake and overcome the nutrient-depletion area around roots for immobile nutrients (Agerer 2001). They connect nutrient fluxes between trees and soil and contribute significantly to plant phosphorous (P) and nitrogen (N) supply (van der Heijden *et al.*, 1988). Since forest trees have about 100% of the root tips colonized by mycorrhizal fungi (Lang *et al.*, 2011; Lang & Polle, 2011), almost all N and P present in plants has been taken up via mycorrhizas (van der Heijden *et al.*, 1988; Högberg *et al.*, 2006; Lambers *et al.*, 2009). Therefore, mycorrhizal symbiosis acts as a major active force of the forest ecosystem processes (Read *et al.*, 2004). Five types of mycorrhizal association have been described, and among them, two are of major economic and ecological importance: arbuscular mycorrhizas (AM) and ectomycorrhizas (EM) ( Smith & Read, 2008).

### *1.1.1. Arbuscular mycorrhizas*

Approximately 70-90% of terrestrial plants are colonized by arbuscular mycorrhizal (AM) fungi (Smith & Read, 2008). AM typically dominate in grasslands, shrublands, and tropical rainforests (Read, 1991). AM comprises the plant root, and the fungal structures, arbuscules or vesicles and the extraradical mycelium (Figure 1). The arbuscules exist within the cortical cells and are the main sites of nutrient exchange between mycorrhizal fungi and symbiotic plant (Parniske, 2008).Vesicles are located within or between the cells of the root, and they act as the nutrient storage structures

(Smith & Read, 2008). The extraradical mycelium in the soil is a capacious structure, which can be in excess of 100 meters per cubic centimeter of soil. (Miller et al., 1995).

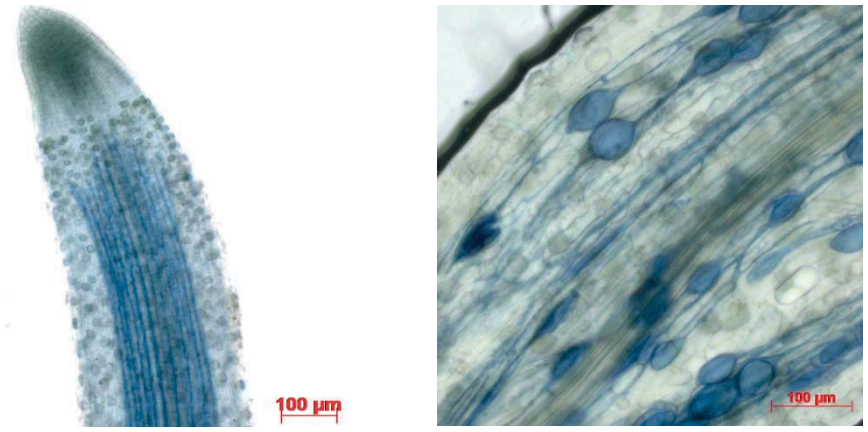


**Figure 1.** Ash (*Fraxinus excelsior* L.) arbuscular mycorrhizal root tip containing vinegar-ink stained fungal structures (left) such as hyphae and vesicles (right).

There is increasing evidence that AM fungi are involved in uptake of nutrients and enhance the plant growth (Zhu *et al.*, 2003; Smith *et al.*, 2011). Arbuscular mycorrhizal fungi increase plant supply through two pathways. The main one is a massive uptake of immobile resources (particularly inorganic nutrients), via their huge hyphal network. AM can get the nutrients directly beyond the depletion zone around root rhizosphere, translocate them over long distances in the extraradical mycelium, transport them to the intraradical fungal structures, and finally transfer the nutrients across the interfaces to the root cortex cells. Furthermore, AM hyphae can get access to the decomposing patches of organic material and compete with other microbes, thus increase nutrient acquisition (Smith & Read, 2008). Hodge (2001) suggested that AM fungi not only promoted organic N decomposition, but also plant N capture. The importance of AM in plant N uptake in naturally heterogeneous soil is underestimated, particularly, in the areas where decomposing organic patches are crucial N sources (Hodge, 2001).

### *1.1.2. Ectomycorrhizas*

Approximately 5% of terrestrial plant species are colonized with ectomycorrhizal (EM) fungi (Landeweert *et al.*, 2001). Ectomycorrhizas dominate in temperate and boreal forests ecosystems, which are characterized by low availability of plants nutrients (Smith & Read 2008). Ectomycorrhizal symbiosis is critical in biogeochemical cycles in ecosystem where nutrients are the main limitation on plant productivity. There is evidence that about six times as much N is cycling via ectomycorrhizas than via litterfall (Ruess *et al.*, 2003).

The ectomycorrhiza shows three characteristic structures: a sheath or mantle of fungal tissue that covers the root tip, an interface for nutrient exchange between the cortex cells (Hartig net), and an outwardly growing extraradical or external mycelium stretching into the soil (Figure 2). The presence and lengths of hyphae emanating from the mantle are features of distinct EM fungal species and differ between various soil exploration types (Agerer, 2001). Soil exploration by hyphae, is an important aspect for the efficiency of nutrient acquisition (Plassard *et al.*, 2011; Cairney, 2011). EMs increase the nutrient uptake surface, and thus, may overcome the nutrient-depletion zone. The hyphae are mainly confined to the upper 20 cm of the soil profile (Persson, 1983). The research of Ruess et al. (2003) showed that more than 80% of the fine-root production occurred within the top 20 cm of the soil surface.

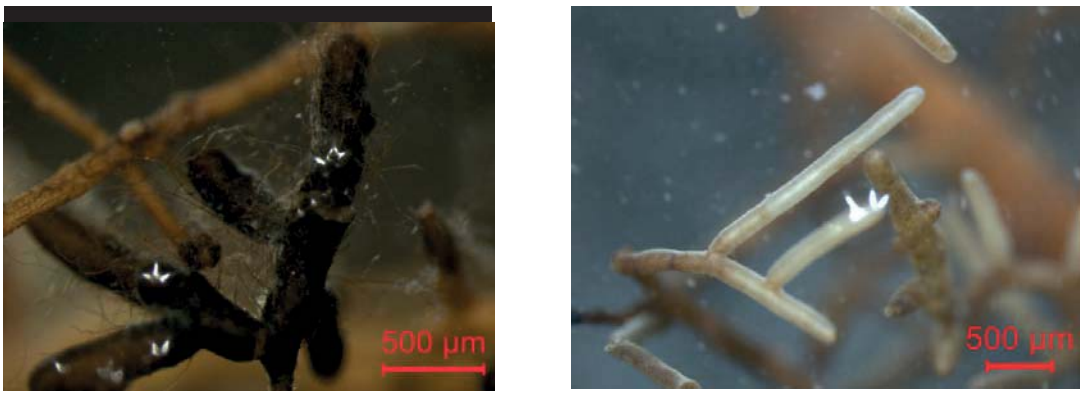


**Figure 2.** Beech (*Fagus sylvatica* L.) ectomycorrhizal root tips colonized by *Tomentella sp.* (left) and *Peziza sp.* (right) fungi. Emanating hyphae forming external mycelia are visible in *Tomentella* ectomycorrhiza.

Although EM symbiosis has a uniform and characteristic suite of structural features, a great diversity of nearly 5000 to 6000 mycorrhizal species has already been found (Smith & Read, 2008). It is important to identify the dominant fungi in a given ecosystem, as is a precondition to study their functional characters (Read *et al.*, 2004). With the application of morphological analysis and advance of molecular methods, there is a growing understanding of the mycorrhizal diversity, both structural and functional. However, both morphological analysis and molecular methods have some disadvantages: morphological analyses are time-consuming, observer dependent and need high skills (Agerer, 1987), while the molecular methods have a high requirement of laboratory facilities. A new method applying Fourier Transform Infrared (FTIR) spectroscopy has been used for the distinction of EMs in field samples with minimum sample preparation (Pena *et al.*, 2014). FTIR spectroscopy has been previously successfully applied to detect and identify fungi growing in wood (Naumann, 2009).

## 1.2. Nutrient limitations in forest ecosystems

Nitrogen (N) and phosphorus (P) are the most common growth-limiting elements in temperate forest ecosystems (Vitousek *et al.*, 2010; Masclaux-Daubresse *et al.*, 2010). Nitrogen is a major constituent of, amino acids, proteins, coenzymes, ATP, nucleic acids, chlorophyll and many plant secondary products, which contribute to the growth and reproduction of plant cells and finally whole plants.

### *1.2.1. Nitrogen*

N is acquired from the soil by the roots with few exceptions (Miller & Cramer, 2005). Plant N availability in temperate forest soil is very low (Gobert and Plassard 2008). Soil nitrogen exists in three general forms: organic nitrogen compounds, ammonium ($NH_4^+$) and nitrate ($NO_3^-$). Organic nitrogen compounds are the dominant N form in the soil (Jones & Kielland, 2012), they are transformed to $NH_4^+$ through mineralization, and $NH_4^+$ may be oxidized to $NO_3^-$ through nitrification. Small organic N such as peptides and amino acids in soil solution can be directly taken up by plants (Näsholm *et al.,* 2009). However, this fraction represents a small percentage of soil total N. The main part of the organic N is found in recalcitrant forms, which first need

to be depolymerised to plant available monomers via soil microbes or some mycorrhizal fungi before being taken up by plants (Schimel & Bennett, 2004; Näsholm et al., 2009).

In contrast to AM, which proliferate in organic patches but rely on inorganic nutrition, EM fungi can get access to organic and inorganic nutrition sources (Hodge & Fitter, 2010). A large number of EM fungal species can use proteins as N source (Abuzinadah and Read 1986; Abuzinadah *et al*. 1986; Nygren *et al*. 2007) and transfer N from this sources to the host (Finlay *et al.*, 1992). However, the extent to which EM fungi degrade organic matter is rather low in comparison with that of saprotrophs (Kohler *et al.*, 2015).

### *1.2.2. Phosphorus*

Phosphorus is a key component of macromolecules such as nucleic acids, phospholipids, or energy-rich phosphate compounds (Schachtman *et al.* 1998). These organic forms of P ($P_{org}$) or the inorganic phosphate ($P_i$) in plant tissues affect multiple aspects of plant metabolism, such as nutrient transport and photosynthesis (Turnbull *et al.* 2007).

Plant P is completely derived from soil (Ulrich *et al.* 1986). The majority of soil P exists in following general groups of compounds: organic P, calcium (Ca) bound $P_i$ and iron (Fe), or aluminum (Al) bound $P_i$ (Barber 1984). The availability of P in forest soils is commonly very low due to the low solubility of bound P and the lack of easily accessible P supply for plant uptake (Montti *et al.* 2011; Brady & Weil, 1999). In natural ecosystems, orthophosphate in the soil solution is the major form they can be accessible to plants (George *et al*., 2011; Jansa *et al*., 2011). Phosphate is very strongly adsorbed by soil particles, and P uptake by plant roots through diffusion is very slow. In most soils, the amount of P available to plants from the soil solution at a given time point is less than 0.01% of the total P in the soil (Brady & Weil, 1999). Together with increased forest soil acidification, atmospheric N deposition, and climate change, a trend towards reduced P nutrition in forests is formed at ecosystem level (Aber 1992; Duquesnay *et al*. 2000; Gradowski and Thomas 2006, 2008; Prietzel

and Stetter 2010). P deficiency causes an array of negative responses of plants (Lynch & Brown, 2006) such as decreased leaf numbers, low photosynthetic rates (Turnbull *et al.*, 2007), stunted growth and delayed maturity (Ma *et al.*, 2002; Danyagri & Dang, 2014).

## 1.3. Leaf litter and leaf litter decomposition

The decomposition of leaf litter is an essential ecosystem function that is not only required for the input of carbon into the soil food web (Gartner & Cardon, 2004), but also for the release of essential plant nutrients such as N and P. Litter decomposition is the first step of nutrient cycling (Austin *et al.*, 2014), through which the nutrients in leaf litter are converted into available forms for uptake by the vegetation (Gartner & Cardon, 2004). Thus, litter decomposition plays a critical role in vegetation productivity. Leaf litter accumulated on forest floor and root litter in the forest soil potentially act as the main sources of nutrients required for plant growth (Chen *et al*. 2010; Berg and Mc Claugherty 2008; Guo *et al*. 2013).

At regional scales, climatic factors such as the mean annual temperature, precipitation, and soil moisture are found to be positive correlated with litter decomposition (Zhang & Zak, 1995; Trofymow *et al.*, 2002; Powers *et al.*, 2009). Other key factors impacting on the decomposition rate are the chemical properties of leaf litter, especially the concentrations and ratios of nutrients and lignin (Melillo *et al.*, 1982; Berg & McClaugherty, 2008). Decomposition rates are negatively correlated with the ratio of carbon to N (C: N ratio) (Heal *et al.*, 1997). The lignin concentrations of leaf litter is more important at later decomposition rates (Berg, 2000). Lignin is a complex aromatic heteropolymer in cell walls, which is one of the litter components that are most recalcitrant to decomposition (Jacob *et al.*, 2010), increasing lignin concentration restrained biotic decomposition (Austin & Ballare, 2010).

Leaf litter diversity should also be taken into consideration. The decomposition rates of the individual leaf litter mixed with leaf litter of differenr qualities are also influenced by the interactions of different litter types (Gartner & Cardon, 2004). In

mixtures, the higher litter quality (low C: N ratio) can stimulate the decay rate of recalcitrant litter by releasing nutrients (Briones & Ineson, 1996). On the other hand, the release of inhibitory compounds such as phenolics and tannins can also slow down the decomposition rate of leaf litter in mixture (Salamanca *et al.*, 1998). Different leaf quality and leaf structure from divergent species alter the chemical environment, thus, influence the community and activities of the decomposers. The microbial community can adapt to different litter types, but little attention has been paid to the effect of leaf litter identity and diversity on the nutrient uptake strategies of ectomycorrhizal fungi.

## 1.4. Earthworm impact on plant nutrition

Earthworms (EWs) as major detritivorous macroinvertebrates in soil, can dramatically influence the leaf litter decay dynamics, nutrient cycling and soil organic matter dynamics (Seeber *et al.*, 2006; Heneghan *et al.*, 2007; Hedde *et al.*, 2007). They can consume the entire leaf fall in deciduous forests (Bohlen *et al.*, 2004), and release nutrients by their metabolic activities (Lee, 1985). Thus, they stimulate nutrient turnover, and mobilize nutrients from the forest floor (Scheu & Parkinson, 1994). The mineral nutrients will be reused by the plants, affecting, thus, overall plant nutrition and growth (Scheu, 2003; Barot *et al.*, 2007; van Groenigen *et al.*, 2014). EWs increase the nutrient mineralization also indirectly through alterations in soil physical properties and fragmentation of organic material.

Divided by the food strategy and the general environmental conditions, there are three basic ecological categories of EWs: *Epigeic* species, are of small size, live in the fresh surface litter and create casts at the soil surface that influence its roughness and the distribution of macropores (Lee, 1985). *Anecic* species feed on fresh litter pulled from soil surface to their burrows, live in permanent vertical burrows, linked with the soil surface (Lee, 1985). *Endogeic* species, have a large size, feed on the mineral soil associated with organic matter, make horizontally or randomly oriented burrows in the mineral soil, which are considered as tentative structures because they are seldom re-used (Lee, 1985).

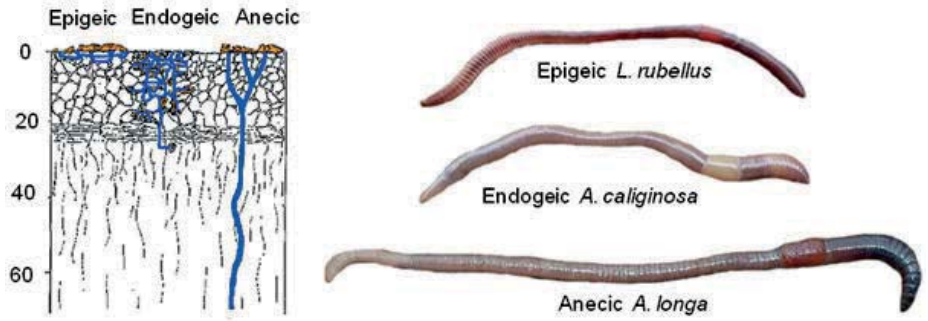


**Figure 3.** Earthworm functional groups and their distributions. *Epigeic* earthworms (i.e. *Lumbricus rubellus*), *Endogeic* earthworms (i.e. *Aporrectodea caliginosa*), *Anecic* earthworms (i.e. *Aporrectodea longa*) (Sources: Figure adapted from Fraser and Boag 1988, photos of common earthworms courtesy of R. Gray.)

The interaction between EWs and mycorrhizal fungi likely affects plant nutrient acquisition. EWs destroy the hyphae and hyphal network in the process of producing casts and burrows in the root zone and rhizosphere. Plant nutrient acquisition might be affected by the disruption of EWs activities. However, an increased amount of N mobilized from straw litter and taken up by maize plants was found in the presence of both EWs and AM fungi in a field experiment (Li *et al*., 2013). In experiments using grass litter, no significant differences were detected for the effects of EWs on plant N uptake in the presence or absence of AM (Wurst *et al.*, 2004; Eisenhauer *et al*., 2009). Apparently, the factors influencing the interaction between EWs and mycorrhizal fungi on nutrients uptake by plants are still ambiguous.

## 1.5. Objectives of the present thesis

European beech (*Fagus sylvatica* L.) and European ash (*Fraxinus excelsior* L.) are two common temperate tree species that are co-occurring in many temperate deciduous forests (Ellenberg & Leuschner 2010). However, the two species differ in mycorrhizal type and leaf litter decomposability (Emborg, 1998). The beech trees form EMs and produce recalcitrant leaf litter while ashes are colonized by arbuscular AM fungi, and produce a high quality leaf litter, characterized by a low C-to-N ratio.

Given the ecological and economic importance of beech and ash trees, the questions addressed in this thesis refer specifically to these two forest tree species.

The main goals of this thesis were:

I. To investigate the effects of EWs on plant acquisition of N and mycorrhizal community structure. I hypothesised that: (1) Due to the different nutrient uptake strategy of AM and EM, plus different leaf litter qualities, EW differentially affect mycorrhizal colonization, community structure and root tip $^{15}N$ accumulation dependent on leaf litter type, AM or EM fungi (2) EM fungi access organic and inorganic N sources while AM fungi only rely on inorganic N, EM plants acquire more leaf litter N than AM plants in the absence of EWs, while the pattern is reversed in the presence of EWs, because AM colonization may recover faster after EWs disturbance due to the ability of AM fungi to infect roots by spores (3) Based on different properties and nutrient availability of leaf litters, EWs facilitate plant capture of N from fast decomposing ash, but not that from recalcitrant beech leaf litter. To address these hypotheses, beech (*Fagus sylvatica*) and ash (*Fraxinus excelsior*) trees were planted in rhizotrons treated with $^{15}N$ labelled ash and beech leaf litter. The rhizotrons were amended with two species of earthworms (*Aporrectodea caliginosa* and *Lumbricus terrestris*). Four treatments were established in a two-factorial design: ash leaf litter and EWs (AE), ash leaf litter without EWs (A), beech leaf litter and EWs (BE), and beech leaf litter without EWs (B). The results are presented in chapter 2:

"Impacts of earthworms on nitrogen acquisition from leaf litter by arbuscular mycorrhizal ash and ectomycorrhizal beech trees."

II. To examine the impact of tree diversity and litter identity on the biochemical composition of fine roots and root tips colonized by distinct EM fungal species. I hypothesised that: (1) Due to the varying abilities of distinct EM fungal species to access different nutrients, leaf litter quality differentially influences the FTIR chemical profile of EM taxa. (2) Since the leaf litter is the main source of nutrient