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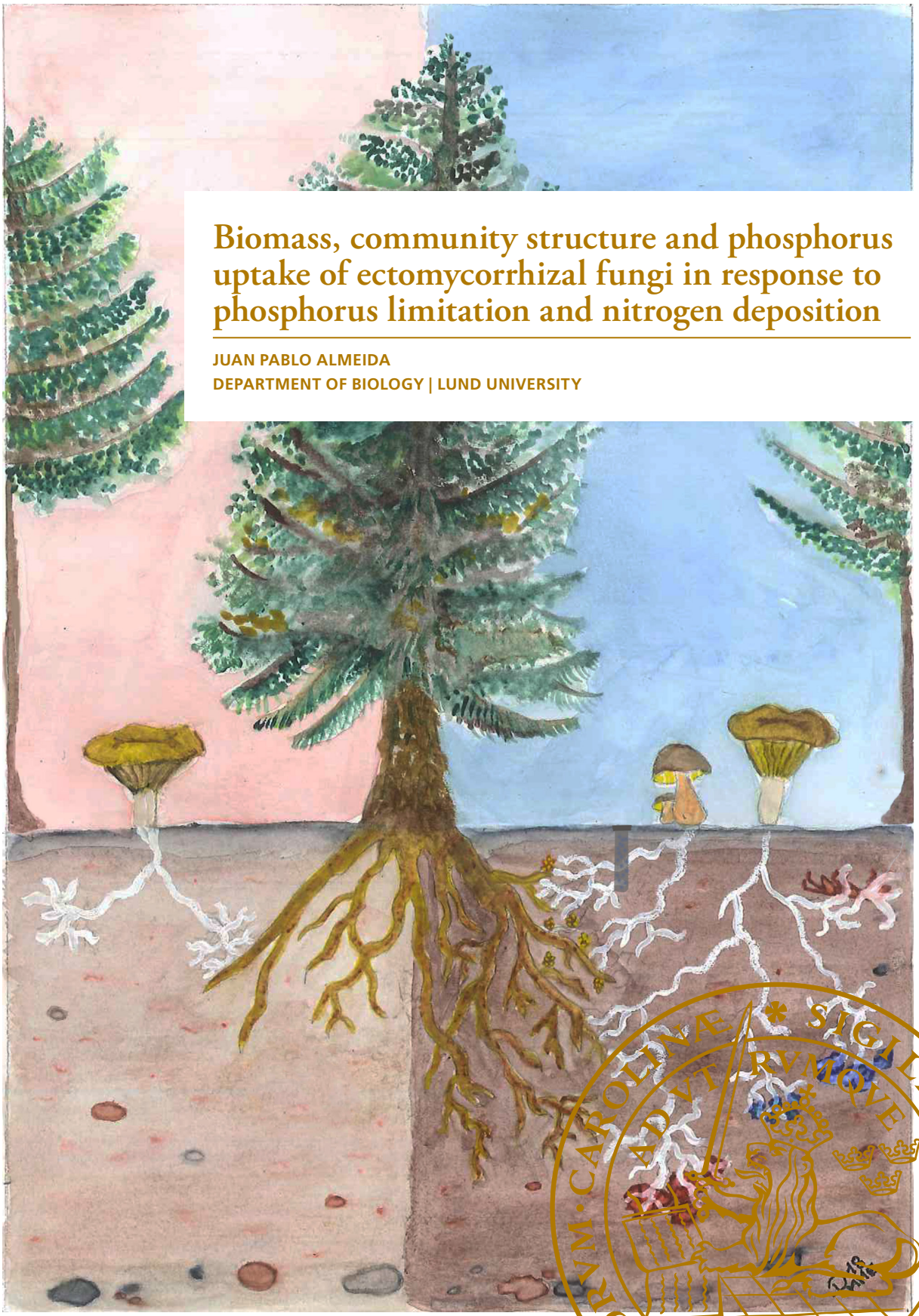

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Juan Pablo Almeida

LUND
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Title and subtitle Biomass, community structure and phosphorus uptake of Ectomycorrhizal fungi in response to phosphorus limitation and nitrogen deposition

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I found that tree production was limited by P. Furthermore, P fertilization reduced EMF production indicating that EMF biomass production was stimulated by P-limiting conditions. Apatite had a positive effect on EMF production when the system was P-limited. P fertilization reduced foraging for nutrients by EMF, also for N rich urea. P had a stronger effect on the composition of EMF communities than N, suggesting that P nutrition had a larger impact on belowground carbon (C) allocation than N in this ecosystem. Furthermore, certain EMF species responded positively to apatite under P limiting conditions, which might have increased mobilization of P from this source.

To enhance my understanding of P mobilization from different P compounds by EMF, I studied one species, *Paxillus involutus,* under more controlled conditions in the laboratory. *P. involutus* is adapted to high N deposition levels and has a documented capability to take up P from poorly soluble sources. I found that *P. involutus* was able to take up P from apatite, P bound to goethite and from phytic acid. Moreover, I found that iron-reducing activity was produced when these sources were provided but not when the fungus was provided with soluble P (phosphate). One possible interpretation to this result was that iron (Fe) reduction is a way for the fungus to prevent that newly liberated phosphate ions are captured by Fe^{3+} and became unavailable for uptake.

In conclusion, the high production of EMF found in P-limited forest decline when P is added, probably due to reduced belowground C allocation when less foraging for P is needed. EMF communities are strongly regulated by P in these forests and species better adapted for P foraging are probably selected for under these conditions.

Key words: Phosphorus and nitrogen limitation, nitrogen deposition, ectomycorrhizal fungi, community composition,apatite, *Paxillus involutus*

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To my family: Micaela, Juan Pablo, Yolita,
Gabriel, Belén y Marita

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Paper II Almeida, J. P., Rosenstock, N. P., Forsmark, B., Bergh, J., & Wallander, H. (2018). Ectomycorrhizal community composition and function in a spruce forest transitioning between nitrogen and phosphorus limitation. *Fungal Ecology*. In press: doi.org/10.1016/j.funeco.2018.05.008

Paper III Almeida, J.P., Ekblad, A., Rosenstock, N., & Wallander, H. Turnover and production of ectomycorrhizal mycelia in an unfertilized and phosphorus fertilized Norway spruce forest (manuscript).

Paper IV Almeida, J.P, Tunlid, A., Persson, P., Johansson, T., & Wallander, H. Phosphorus uptake from poorly soluble phosphorus sources by *Paxillus involutus* in relation to iron reducing capacity and phosphorus starvation. (Manuscript).

Author Contributions

Paper I Responsible for laboratory work, bioinformatics, statistics, interpretation of data. Minor contribution to writing the text.

Paper II Responsible for planning the experiment, fieldwork, laboratory work, statistics, interpretation of data and writing the manuscript.

Paper III Responsible for planning the experiment, field work, laboratory analysis, most of the statistical analysis, interpretation of data and writing the manuscript

Paper IV Responsible for designing the experimental set up, performing all laboratory analysis and statistical tests, interpretation of data and writing the manuscript.

Abstract

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Introduction

Nitrogen and phosphorus nutrition in forested ecosystems

Forested ecosystems are important reservoirs that store carbon (C) either in aboveground or into belowground biomass (Hui *et al.,* 2017). Carbon fixation and partitioning by trees is strongly dependent on other essential macronutrients, such as nitrogen (N) and phosphorus (P) (Gill & Finzi, 2016). N is an important component of amino acids, monomers that forms proteins, while P is a structural part of the phospholipid bilayer of cell membranes (Sterner & Elser, 2002). Both elements are key components of nucleic acids and the energy-transfer compound ATP (Terry & Ulrich, 1973; Sterner & Elser, 2002; Hyland *et al*., 2005). ATP is needed in cellular functions, such as stomatal opening and transfer of organic solutes across membranes (Terry & Ulrich, 1973; Sterner & Elser, 2002). Moreover, biosynthesis and respiration rely on the energy stored in the ATP molecule (Terry & Ulrich, 1973; Sterner & Elser, 2002). Therefore, P and N are critical elements for plant physiology (Sterner & Elser, 2002; Smith et al., 2011). However, N and P are not easily accessible resources in soils; therefore, the proportions required for optimum plant growth are often not sufficiently provided. As a result, tree primary productivity and growth are generally limited by the nutrient present in the lowest supply (von Liebig, 1855).

When a nutrient is limiting tree growth, meaningful nutrient additions are expected to enhance primary productivity and biomass production (Vitousek *et al*., 2010). In forested ecosystems, N fertilization has been shown to have positive effects on tree growth in high latitude forests (boreal and temperate forests) (LeBauer & Treseder, 2008; Schulte-Uebbing & De Vries, 2018), while tropical forests generally respond more to P fertilization (Elser *et al.,* 2007; Li *et al.,* 2016), suggesting a latitudinal difference in N and P limitation.

This latitudinal trend has been attributed to the geological and climatic processes that differentiate tropical and high latitude forests (Vitousek *et al*., 2010; Gill & Finzi, 2016). New N inputs in an ecosystem are the result of the fixation of atmospheric N_2, which is an enzymatic process conducted by free living or root associated N-fixing bacteria (Davidson, 2008; Houlton *et al.,* 2008). For terrestrial

ecosystems, an increase in latitude is associated with a decrease in N fixation (Houlton *et al.,* 2008). Decreasing temperatures at higher latitudes might decrease the efficiency of nitrogenases, which are responsible for the conversion of atmospheric N_2 into biologically available ammonia (Davidson, 2008; Belyazid & Belyazid, 2013). At low temperatures, efficient use of nitrogenases might require more C investment by plants (Rastetter *et al.,* 2001; Houlton *et al.,* 2008). It is predicted that N foraging and uptake from soils in boreal and temperate forests will be more efficient in terms of C cost than supporting the symbiosis with N fixers (Rastetter *et al.,* 2001). As a result, trees in high latitude forests have developed better strategies to forage and mine for N in soil (e.g., symbiosis with ectomycorrhizas) relative to those in low latitude forests, where fixation is less costly and there is a high turnover of nutrients in soils (Rastetter *et al.,* 2001; Gill & Finzi, 2016). Moreover, in boreal forest soils, N is mainly present in recalcitrant organic compounds that reduce the N uptake efficiency by the plant roots (Sponseller *et al.,* 2016). N fixation in low latitude forests (tropical forest) increases because of the higher temperature and abundance of plant N_2-fixing trees (Davidson, 2008; Houlton *et al.,* 2008). The high temperature also increases soil organic matter turnover and plant available N.

Unlike nitrogen, for which there is potentially large input from biological fixation, phosphorus inputs derive almost exclusively from rock weathering. As a result, the phosphorus status of an ecosystem is heavily dependent on the soil parent material (Rosenstock *et al*., 2016) and age (Wardle *et al*., 2004). For example, in temperate and boreal forests, where glaciers exposed a great deal of primary mineral surface area less than 15,000 years ago, phosphorus does not generally limit primary production. However, in older ecosystems lacking recent significant geological activity, including much of the world's tropical forests, phosphorus has become the limiting nutrient (Vitousek *et al*., 2010). When phosphate is released through weathering of soil minerals, it follows a cycle in which mineralized phosphorus is taken up by soil organisms and plants and cycled rapidly though uptake and organic matter decomposition (Hyland *et al*., 2005; Belyazid & Belyazid, 2012; Sohrt *et al.,* 2017) and, over time, becomes increasingly less available by binding to secondary mineral surfaces (Turner, 2008; Belyazid & Belyazid, 2012).

Nitrogen and P biogeochemical cycles are dynamic processes that depend on input sources, biological demand and uptake. Changes in the availability and inputs of one of the nutrients can alter the N:P ratios in soils and plants, and thus change the nutrient regime of an ecosystem (Vitousek *et al*, 2010). For example, during early succession stages in some boreal forests, N fixers are abundant and the new N inputs surpass the inputs of P, leading to P limitation (Chapin *et al.,* 1994; Vitousek *et al*., 2010). Moreover, during late successional stages of temperate and boreal forests, an accumulation of N from atmospheric deposition and biological fixation and a decrease in P availability can lead to a declining phase with low

productivity caused by phosphorus limitation (Wardle *et al.,* 2004, 2016; Du & Fang, 2014). Thus, even though P limitation is rare in young ecosystems (northern, glaciated areas), these studies demonstrate that P limitation can occur during both early and late succession in boreal and temperate forests.

Nitrogen deposition and anthropogenic phosphorus limitation

During the post-industrial era, global C, P, and N cycles have changed significantly (Elser *et al*., 2007; Peñuelas *et al.,* 2013) and anthropogenic N inputs from industrial fertilizers and fossil fuel emissions have increased by more than one order of magnitude in comparison with the biologically fixed N (Fig. 1) (Falkowski *et al*., 2000; Galloway *et al*., 2008). This steep increase in anthropogenic C and N inputs relative to P inputs (Fig. 1) can alter plant nutrient stoichiometry and lead to unbalanced nutrition (Peñuelas *et al.,* 2013; Jonard *et al.,* 2015).

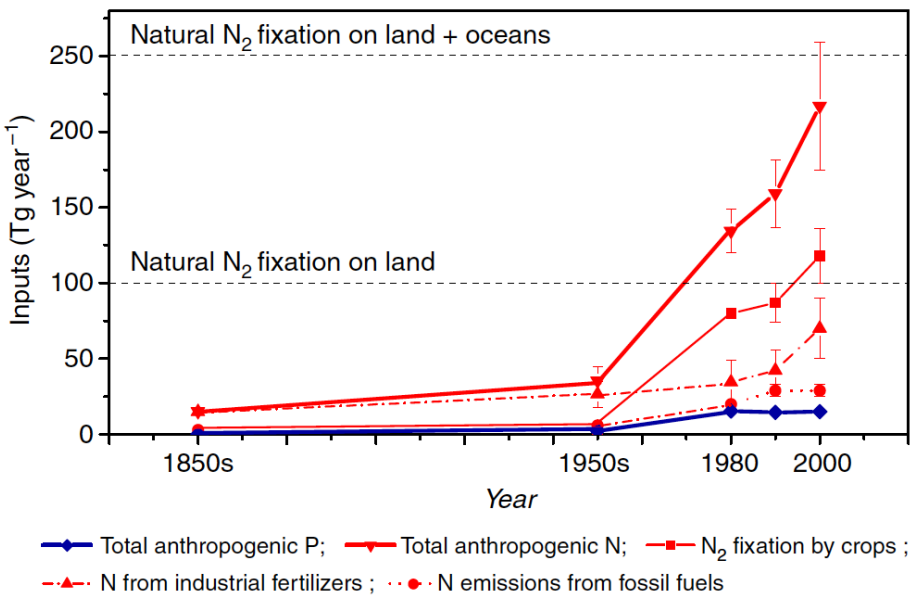

Figure 1: Total anthropogenic N and P inputs on a global scale since the industrial revolution (1860). Error bars indicate the range of data reported. Taken from Peñuelas *et al*. (2013).

The unbalanced N and P inputs in terrestrial ecosystems have increased the N:P ratios in plants and soils, especially in forests from North America and Central and Northern Europe (Peñuelas *et al.,* 2013). For example, in Europe, Jonard *et al.* (2015) analysed foliar nutrient concentrations based on data from six different tree species collected from forests across the entire continent. They reported that, for all tree species, the N:P ratios were above the level in which a detrimental effect (defoliation) can be expected (Veresoglou *et al.,* 2014). It was suggested that some forested ecosystems in high latitudes are P-limited, or transitioning to P limitation due to an increased N inputs.

Many have corroborated this idea by testing the effects of N fertilization on tree growth. Nitrogen addition experiments in temperate forests in North America (Lovett *et al.,* 2013) and in central Europe (Braun *et al.,* 2010) have reported a lack of response of tree growth to N fertilization, suggesting that N is not the main limiting nutrient. However, based on a meta-analysis of data from fertilization experiments in natural forests and forest plantations around the globe, Schulte-Uebbing & De Vries (2018) reported that there was a significant strong response of tree growth to N fertilization in boreal and temperate forests, suggesting that N limitation remains in most of these ecosystems and that P limitation is not yet widespread across high latitude forests.

Very few studies have examined the effects of P fertilization alone (without concomitant N additions) on tree growth in temperate and boreal forests (see Radwan *et al.,* 1991; Finzi, 2009; Mainwaring *et al*., 2014), and adult trees do not generally respond to P fertilization in high latitude forests (Finzi, 2009; Mainwaring *et al*., 2014; Schulte-Uebbing & De Vries, 2018). It has been reported that tree response to fertilization can vary depending on the local soil conditions of a forest stand (Finzi, 2009; Bergh *et al.,* 2014; Mainwaring *et al*., 2014; Schulte-Uebbing & De Vries, 2018). For example, in forest stands with low pH, occlusion of the P fertilizer to iron oxides can dampen the effects of fertilization. However, in sites with high pH, P fertilization is easily available for use in plant growth (Finzi, 2009). In temperate plantations from North America, Mainwaring *et al*. (2014) compared the effects of P fertilization on 16 different Douglas fir (*Pseudotsuga menziesii*) stands with different edaphic characteristics. They found a positive effect of P fertilization on tree growth in stands with a pH higher than 5 that was not found in stands with a pH lower than 5. Therefore, local conditions of a forest stand are important when interpreting the effects of P fertilization in high latitude forests.

Nitrogen deposition and phosphorus limitation in Swedish boreal forests

In Western Europe, boreal forests receive less N deposition than temperate forests (Erisman *et al.,* 2015) and N deposition has decreased in recent decades (Binkley & Högberg, 2016; Pihl Karlsson *et al.* 2017). However, in some forests from Southwest Sweden, the N deposition values currently range from 7.5 to more than 15 kg N^{-1} ha^{-1} yr^{-1} (Erisman *et al.,* 2015; Pihl Karlsson *et al.* 2017). These values exceed the N critical loads above in which negative changes in the function and composition of an ecosystem are expected (Kuylenstierna *et al.,* 1998; Pardo *et al.,* 2011; Pihl Karlsson *et al.,* 2017) (Fig. 2).

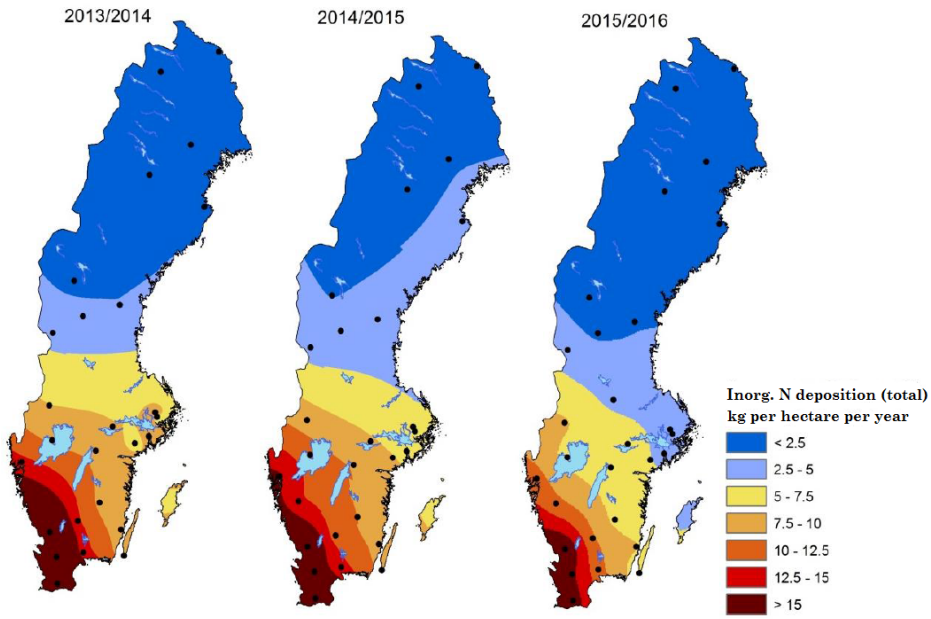

Figure 2: Estimated total N deposition into spruce forest for three years 2013/14, 2014/15, and 2015/16. The estimation has been performed based on 26 locations (illustrated with black dots) (2013/14), 27 locations (2014/15), and 28 locations (2015/16) across Sweden. Modified from Pihl Karlsson *et al*. (2017).

Several studies have reported N excess in southern Swedish forests. In a study of the effects of nitrogen fertilization on floor vegetation, Hedwall *et al.* (2013) found that N enrichment had a significant effect on the forest ground flora species composition in central and northern Sweden. In southern Swedish forests, the effects of fertilization were smaller. Forest floor flora is normally sensitive to N addition and has been used to test the effects of N deposition (Hedwall *et al.,*

2013; Binkley & Högberg, 2016). The lack of N fertilization effects on forest floor vegetation in southwest Sweden indicates that the region is probably N saturated.

In a N deposition gradient across Sweden, Akselsson *et al.* (2010) measured nitrate concentrations and C:N ratios in soil water and modelled nitrogen accumulation based on N inputs (N deposition and N fixation) and outputs (N leaching and tree harvesting) of the system. They found that southwest Sweden, which receives the highest N deposition, showed the highest levels of N accumulation and had the highest risk of N leaching. In addition, Akselsson *et al.* (2008) analysed how much N and P would be lost if the trees were harvested using data collected from 14,550 sites in Swedish forests. Based on N and P inputs (N and P deposition, N fixation and mineral weathering) and outputs (N and P leaching and tree harvesting), they modeled N and P accumulation and losses after tree harvesting. The results revealed that tree harvesting would result in net losses of P from forests. However, some forests in southwest Sweden will accumulate N in soils even after tree harvest. Based on these findings, the authors concluded that forests in southwest Sweden are transitioning to P limitation.

Despite this evidence, the transition from N to P limitation of some forests in Sweden is still the subject of debate since there has not been evidence of a positive effect on tree growth of P fertilization without concomitant N addition in upland Swedish forests (Binkley & Högberg, 2016). In the experiment described in the second manuscript from this thesis (**Paper II),** we fertilized a Norway spruce forest (*Picea abies*) in southwest Sweden with P, and found a significant increase in tree stem growth in P fertilized plots (Fig. 3A). Needle nutrient analysis revealed that the P content in the unfertilized control plots were below the deficiency levels reported by Thelin *et al*. (1998) (Fig. 3B). Moreover, the N:P ratios in the unfertilized controls plots were above the threshold level at which P is considered to be limiting growth according to Linder (1995) (Fig. 3C). We also found a reduction of acid phosphatase activity after P fertilization in a pilot study in the same research forest (Fig. 3D).

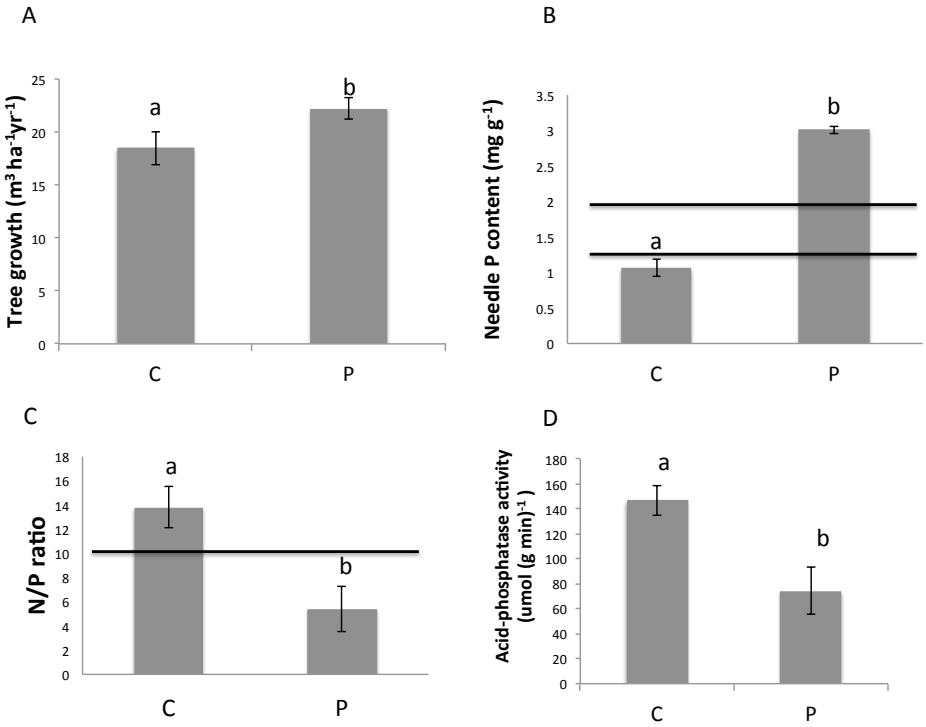

Figure 3: (A) Tree growth (n=6); (B) Neddle P concentration (n=3) (the lower and upper lines represent P deficiency and optimal fertilization levels respectively); (C) N:P ratio in needles (n=3) in the fertilization experiments. The line represent the optimal N:P ratio reported by Linder (1995). Modified from Almeida *et al*. (2018). (D) Acid phosphatase activity in the P fertilized plots (n=3). Bars represent the average value per treatment and error bars correspond to two standard errors. P and C are abbreviations corresponding to the P fertilized plots and the unfertilized (control) plots, respectively.

The tree growth response to P fertilization plus the foliar nutrient concentrations suggest that the trees at this stand are P-limited. The reduction in phosphatase activity suggests that when fertilization alleviated limitation, there was a reduction in the tree and soil microorganism efforts to acquired P from organic compounds. These findings support the case for a transition to P limitation in areas with strong anthropogenic N deposition. However, the effects of N deposition in southwest Sweden have been shown to be variable on a local scale (Akselsson *et al.,* 2010) and the response to fertilization can be dependent on tree species (Bergh *et al.,* 2014). Therefore, this evidence cannot be extrapolated to other forest stands in the region. Nevertheless, this forest is a valuable site for investigation of the transition from N to P limitation and its effects on tree nutrition. In this thesis, I used this forest as a case of study for **Papers I, II, and III** to investigate the effects of this

transition on several aspects of ectomycorrhizal fungi (EMF), which are root symbionts crucial for nutrition in forested ecosystems (Smith & Read, 2010).

Mycorrhizal associations and nitrogen and phosphorus nutrition

Nitrogen and P limitation have important implications for plant nutrient acquisition and trees have developed different strategies to improve N and P uptake (Smith and Read, 2010). For example, most tree species rely on mycorrhizal associations to obtain N and P from soils (Gadd, 2006; Smith & Read, 2010). Mycorrhizal associations are symbiotic relationships between filamentous fungi and plant roots, in which, under most conditions, the fungal symbiont provides nutrients to their plant hosts in exchange for photosynthetically fixed carbon (Finlay, 2008).

Different kinds of mycorrhizal associations have appeared during different times in the phylogeny of filamentous fungi (Strullu-Derrien *et al.,* 2018), with ectomycorrhizas and arbuscular mycorrhizas being the most abundant (Perez-Moreno, 2003; Bonfante & Genre*,* 2010; Tedersoo et al., 2014). Despite relatively similar properties concerning plant nutrition (exchange of nutrients for photo assimilates), both mycorrhizal types differ in their physiological structures, their main distribution, and their nutrient uptake strategies (Bonfante & Genre*,* 2010).

The main structural difference between EMF and arbuscular mycorrhizal fungi (AMF) is the features associated with the root: fungus interphase. For EMF, fungal tissue does not penetrate the root cell walls, but instead surrounds them, forming a layer called the Hartig net. For AMF, the fungal tissue goes inside the root cell wall to form vesicles or arbuscules inside the cell (Bonfante & Genre*,* 2010) (Fig. 4).

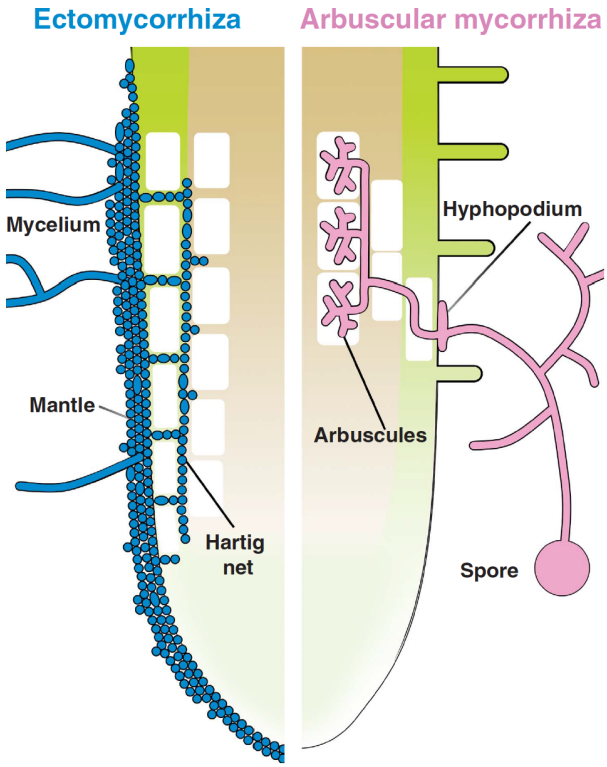

Figure 4: Anatomical comparison between an ectomycorrhizal and arbuscular mycorrhizal fungus:root interphase. Taken from Bonfante & Genre (2010).

In temperate and boreal forests, EMF are the dominant mycorrhizal type (Read & Perez-Moreno, 2003; Tedersoo *et al*., 2014), while AM can be present in the understory of some temperate forests (Read & Perez-Moreno, 2003). However, in tropical forests, AMF is the dominant mycorrhizal type and very few EMF genera are present (Tedersoo *et al*., 2014; Mafla, 2018).

In addition to the structural differences, EMF and AMF have different nutrient acquisition strategies. EMF are more specialized to take up N from soils where turnover is low and nutrients can be locked in organic compounds (Marschner & Bell, 1994; Read & Perez-Moreno, 2003). Some EMF species are able to produce different enzymes to release N from soil organic matter (Bödeker, 2012). Other species have versatile capabilities similar to those of saprotrophic fungi. For example, *P. involutus* for example, can produce secondary metabolites to induce a Fenton reaction and produce free radicals that will ultimately act on organic matter and release N (Shah *et al.,* 2015; Op de Beeck *et al.,* 2018). Enzymatic capabilities to release N from organic compounds are absent from AMF, probably because in tropical forests, where the turnover of organic N is fast and N fixation is high,

there is enough available N for fungal and plant consumption (Read & Perez-Moreno, 2003). Despite being adapted to ecosystems generally limited by N, EMF are able to take up P from different sources present in soils (Antibus *et al*., 1992; Rosling, 2009; Cairney, 2011; Plassard *et al*., 2011). These findings indicate that EMF have great potential to improve P nutrition in high latitude P-limited forests.

Since EMF are essential for N and P uptake (Gadd, 2006; Smith & Read, 2010) and a significant part of the photo-assimilates goes underground to support the symbiosis (Finlay, 2008), N and P limitation and the transition from one to the other influences C allocation, which can in turn influence EMF growth, nutrient uptake, and the structure of EMF communities. In this thesis, I studied the effects of P limitation in a forest with high N deposition on EMF biomass (**Papers II and III)**, N uptake and leaching (**I and II)**, EMF community composition **(papers I and II),** and P nutrition (**Paper IV)**.

Belowground carbon allocation and EMF biomass in response to phosphorus limitation and high nitrogen deposition

Belowground carbon allocation during nitrogen and phosphorus limitation

In boreal and temperate forests, trees depend on EMF associations to forage and mine for N, which is commonly the limiting nutrient in these regions (Binkley & Högberg, 2016). A substantial amount of C is delivered belowground by trees to support EMF symbiosis (Gill & Finzi, 2016). Gill & Finzi (2016) conducted a meta-analysis in which data pertaining to the annual gross primary productivity, total belowground carbon allocation, and annual N and P mineralization rates were collected from different forests around the globe to test belowground C partitioning across biomes. They found that as available N (as indicated by N:P ratios) in soils decreased, there was an increase in belowground C partitioning. They concluded that to access N, which is locked in the soil organic matter, trees in high latitudes deliver more C to the fungal symbionts to enhance foraging and N uptake.

However, an increase of N in the system is predicted to reduce belowground carbon allocation since a high belowground C investment is not cost efficient when the nutrient is abundant (Treseder, 2004; Högberg *et al.* 2010; Janssens *et al.* 2010; Bae *et al.* 2015). The effect of N availability on belowground C allocation was tested by Bae *et al.* (2015), who estimated belowground C allocation (based on the difference between C respired in the soil and C in the litter fall) in temperate forest stands with different inherent soil fertilities and ages. They found that belowground C allocation was inversely correlated with soil N availability, independent of stand age. This decrease in belowground C allocation is expected to influence EMF, probably by decreasing the mycobiont biomass (Nilsson & Wallander, 2003; Högberg *et al.,* 2007, 2010). In an N fertilization experiment,

Högberg *et al.* (2010) traced the belowground C by providing the plants with ^{13}C labelled CO_2. They found that when N was added the amount of ^{13}C detected in EMF decreased by almost 50% relative to the unfertilized control plots. Högberg *et al.* (2007) tested the effects of N fertilization and tree girdling (to block the passage of C to the roots by cutting off the phloem) on EMF biomass in a boreal forest. They found that fertilization and girdling decreased EMF biomass to almost the same degree, supporting the idea that the reduction in belowground C allocation caused by fertilization reduces EMF biomass as well.

Similar to N limitation, P limitation should be expected to increase belowground C allocation and EMF growth to enhance nutrient forging and uptake of P. Keith *et al.* (1997) showed that P fertilization of a natural eucalyptus (*Eucalyptus pauciflora*) stand significantly increased aboveground C allocation, indicating that the plants were P-limited. However, belowground C allocation was decreased by fertilization. This decline in delivered C was associated with a decrease in EFM biomass. In a study comparing Norway spruce stands with different P availabilities (caused by the soil parent material P), Rosenstock *et al.* (2016) reported that in P limiting stands above ground tree biomass was lower while EMF biomass was higher in comparison with P sufficient stands. Thus, P limitation can affect C tree partitioning by decreasing aboveground growth and allocating more C to the fungal symbionts. These findings are consistent with other P fertilization studies that have shown a negative effect of P addition on EMF hyphal length (Baum & Makeschin, 2000) and EMF root colonization (Pampolina *et al*., 2002).

Below ground carbon allocation during the transition from nitrogen to phosphorus limitation

As mentioned above, evidence suggests that during P limitation, trees will increase belowground C allocation, which will lead to increased EMF production. However, N increase (due to deposition) is expected to have the opposite effect and reduce belowground C partitioning. Therefore, it is less certain how belowground C allocation and EMF growth will be affected when prolonged N addition results in P limitation. The three scenarios described below can be expected (Fig. 5).

First, allocation to EMF decreases as N availability increases until P becomes limiting and C allocation increases again. Wallander & Nylund (1992) tested the effects of nutrient additions on extramatrical mycelium growth of the EMF *Laccaria bicolor* inoculated in *P. sylvestris* seedlings in semi-hydroponic culture systems. They found that when seedlings were fertilized with a solution containing all nutrients except for P, *L. bicolor* produced significantly more extramatrical

mycelium than seedlings growing with full nutrient solution. Moreover, when plants were fertilized with a solution with no P and an excess of N, there was an even higher increase in extramatrical mycelium. These findings could suggest that the excess N exacerbated P limitation, causing more belowground C allocation. A similar effect could be expected in boreal forests transitioning from N to P limitation due to N accumulation from deposition. However, since seedlings respond stronger to fertilization (Mainwaring *et al*., 2014; Schulte-Uebbing & De Vries, 2018) it is less known if this also occurs under field conditions in mature forests.

Second, allocation of C to EMF is more strongly controlled by N limitation than P limitation, resulting in reduced allocation to EMF at elevated N. Most of the N pool in the leaves constitutes immobile N bound to structural proteins in leaf cells. Foliar P is more mobile and can be transferred to new leaves before foliar abscission (McGroddy *et al.,* 2004). McGroddy *et al.* (2004) analysed data pertaining to foliar C, P, and N stoichiometry from different tropical and temperate forests around the globe and found that the C:P ratio in leaf litter was higher than that in fresh foliage, indicating P resorption from senescent foliage. This difference was more marked in tropical forests in which P limits growth. However, differences in C:N ratios between leaf litter and fresh leaves were similar across forests from different latitudes, suggesting that N resorption was not enhanced in N-limited forests. Thus, in N-limited forests, trees probably rely more on soil foraging to increase N uptake, which will increase the need for belowground C allocation. Conversely, P resorption from senescent foliage might alleviate P demand in P-limited forests and moderate the C cost of P acquisition from soils (McGroddy *et al.,* 2004; Gill & Finzi, 2016). This could indicate that belowground C partitioning is more strongly controlled by N limitation than P limitation.

Finally, relative allocation to EMF decreases because of N deposition, but tree productivity also increases. In this scenario, total belowground allocation remains constant.

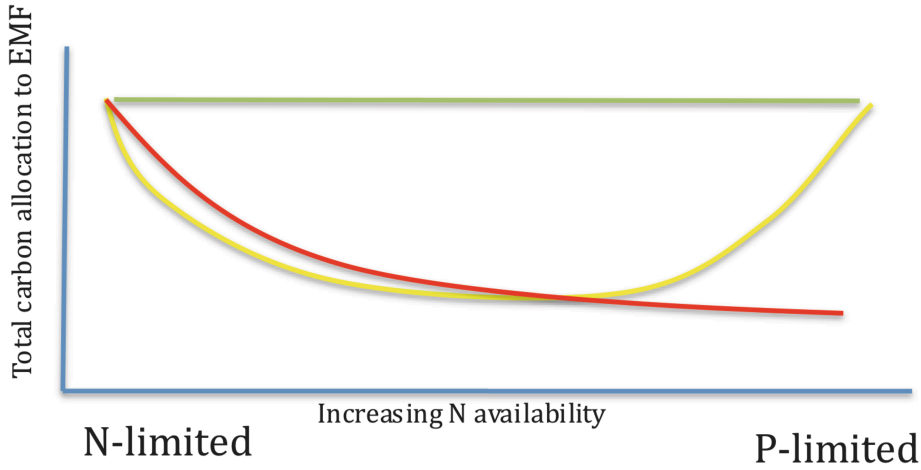

Figure 5: Different scenarios of belowground C allocation to EMF during transition from N to P limitation. First scenario (yellow): allocation to EMF decreases as N availability increases, until P becomes limiting and C allocation increases again. Second scenario (red): allocation of C to EMF is more strongly controlled by N than by P resulting in reduced allocation to EMF at elevated N. Third scenario (green): relative allocation to EMF decreases but tree productivity increases as N availability increases so total belowground allocation remains constant.

To test the effects of P limitation (in a region with high N deposition) on EMF biomass, in **Paper II** we studied EMF biomass from ingrowth meshbags (see below) incubated in a P-limited Norway spruce forest in southwest Sweden (see section 1.3). We added N or P to push the system to further N or P limitation. Two experiments were performed. In the first experimental site (NP experiment), N alone (200 kg N ha^{-1} ammonium nitrate) and a combination of N and P were added (200 kg N ha^{-1} ammonium nitrate and 400 kg P ha^{-1} superphosphate). In the second experimental site (P experiment), P (400 kg ha^{-1} of superphosphate) was added. Non-fertilized plots were used as controls in both experiments. Additionally, P foliar content in the tree needles was measured.

The EMF biomass was estimated by incubating ingrowth meshbags belowground (Fig. 6). This method is useful for measuring fungal biomass, especially from EMF mycelium. This technique employs carbon-free substrate (acid washed quartz sand) covered by a nylon mesh that excludes fine roots and allows mycelium ingrowth (Wallander *et al*., 2001). After 144 days of incubation, EMF biomass was estimated using the fungal membrane component ergosterol as a biomarker. In addition, a visual estimation of the frequency of hyphae counted in the mesh was used as a proxy for mycelial ingrowth (Wallander *et al*., 2001; Bahr *et al*., 2015).

Figure 6: (A) Diagram of the ingrowth meshbag incubated underground. (B) Ingrowth meshbag being installed. (C) Opened meshbag and hyphal growth around the sand substrate (photo by Adam Bahr).

The NP experiment described in **Paper II** did not reveal an effect of N fertilization on ergosterol, although the hyphal visual frequency tended to decrease. However, when the plots were fertilized with both N and P, there was a significant decrease in both ergosterol and visual hyphal frequency. The foliar P concentrations and N:P ratios in the unfertilized controls and the N fertilized plots suggest that this forest is P-limited. The significant decrease in EMF biomass after N+P fertilization was probably caused by alleviation of the host P demand. This is supported by the significant increase in foliar P concentrations and the reduction of foliar N:P ratios to optimal levels after N+P fertilization. The significant EMF biomass reduction in the N+P fertilization treatment in comparison with the

control plots and the N plots suggests that P limitation increases EMF biomass even under high N levels as shown by Wallander & Nylund (1992) for seedlings. However, Bahr *et al.* (2015) studied EMF biomass in the same forest and found that the effect of N+P additions was not higher than the effect of N alone. This might indicate that EMF growth is still sensitive to N additions. Also, it is possible that by the time the experiment described in **Paper II** was performed, the effect of N fertilization had diminished. The measurements conducted in **Paper II** were done 3 years after fertilization while measurements conducted by Bahr. *et al.* (2015) were done 1 year after fertilization.

In the P experiment, although there was a significant effect of P fertilization on foliar P concentrations, foliar N:P ratios, and tree growth, no effect on EMF biomass was detected after P fertilization, contradicting the previous conclusion that P limitation increased EMF biomass.

In Paper II, the EMF biomass measurements are an estimation of the standing biomass in the meshbags after a given incubation period. The standing biomass might not reflect the total EMF biomass production because the turnover (mortality of the mycelium) has not been considered. It has been shown that increases in soil fertility can influence EMF turnover rates and total EMF biomass production (Ekblad *et al.,* 2016; Hendricks *et al*., 2016). It is possible that the standing biomass measured in **Paper II** underestimated the effect of P fertilization. To overcome this problem, in **Paper III** we aimed to estimate EMF turnover to test the effects of P fertilization on the total EMF biomass production

To estimate turnover and EMF biomass production **in Paper III**, we used a combination of short sequential meshbag incubations overlapped with longer incubations times (Fig. 7). The ergosterol data obtained from the different incubations were used in an exponential decay model in which the turnover rates were calculated.

Briefly, the sum of the biomass produced during two sequential short incubation periods is expected to exceed the biomass produced in an overlapping longer incubation period. This difference can be accounted for by dead biomass (necromass) during the long period. The biomass produced in the long incubation period should then be equal to the sum of the shorter incubation periods less the amount of biomass that died (Fig. 7).

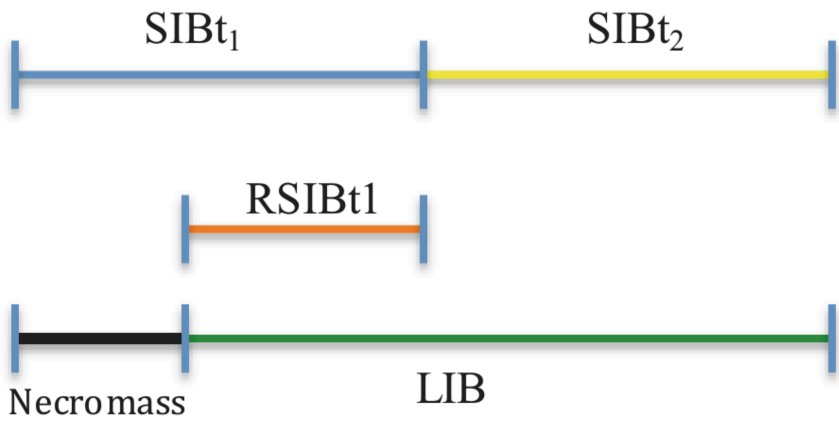

Figure 7: Scheme of the rationale behind the model used to calculate the turnover rates. Two sequential short incubation periods SIBt1 and SIBt2 are overlapping with a longer incubation period LIB. LIB is equal to the sum of SIBt2 and RSIBt1 (the biomass remaining from SIBt1).

Assuming that EMF biomass is lost at a constant exponential rate, the turnover (k) can be calculated using the following equation:

$$k = \frac{-ln\frac{LIB - SIBt2}{SIBt1}}{t2 - t1}$$

Once the turnover rate k is calculated, total biomass production can be estimated using the function of k and the standing biomass of a given time (for further details see **Paper III**).

For a five-month period starting in July 2015 and ending in November 2015, the meshbags were incubated for variable lengths of time (30, 60, 90, 120, and 150 days) in the same fertilized plots that were used for the P experiment described in **Paper II.**

The results from **Paper III** showed that P fertilization had a negative effect on the standing biomass in most incubation times in contrast with what was observed in **Paper II**. The reason for the stronger effect of P fertilization in **Paper III** is not known; however, the fact that more incubation periods and a larger number of bags were used makes the present study more reliable. Thus, the standing biomass of one given incubation time might not truly reflect the effects of fertilization.

The turnover rates did not significantly differ between the P fertilized and control plots. However, the EMF biomass production was decreased by P fertilization as expected from a P-limited forest. This reduction in ECM biomass production as a result of P fertilization can be interpreted as a decreased carbon allocation by the tree when limitation is relieved. The decrease in EMF biomass production in the P fertilization treatment shown in **Paper III** supports the hypothesis that EMF growth increases when P becomes limiting as a result of N deposition. Accordingly, these results might suggest that C allocation to EMF in forests with high N deposition decreases until P becomes limiting and C allocation to EMF increases again (Fig. 5, first scenario).

EMF biomass production and nutrient amendments

Ingrowth meshbags have been intensively used to capture EMF growth (Wallander & Nylund, 1992; Bahr *et al.,* 2015; Ekblad *et al.,* 2016; Rosenstock *et al.,* 2016; Almeida *et al.,* 2018) because roots and most saprotrophic fungi can be excluded (Wallander *et al*., 2001; Wallander *et al.,* 2013). However, the main advantage of the use of ingrowth meshbags is the potential to add the nutrient rich substrates into the bags to assess preferential colonization of different nutrient sources. If nutrient limitation in the forest increases EMF production as a strategy to enhance nutrient foraging and uptake, nutrient rich substrates should enhance EMF production depending on the nutrient status of the forests.

There is evidence of an increase in EMF biomass in the presence of P rich sources, such as apatite in P deficient forests (Hedh *et al.,* 2008; Berner *et al.,* 2012; Rosenstock *et al.,* 2016). Rosenstock *et al.* (2016) reported that the difference in EMF biomass in meshbags between P-limited and P sufficient stands was exacerbated in the apatite amendment meshbags. Bahr *et al.* (2015) found that ingrowth meshbags amended with apatite presented higher EMF biomass than unamended meshbags. However, in N+P fertilized plots, the effect of apatite on EMF biomass disappeared as P limitation was alleviated.

As mentioned above, biomass estimates obtained from a given incubation time are the result of the total biomass production and turnover in the mycelium colonizing the meshbag. Experiments analysing EMF colonization on nutrient substrates have shown that the residence time of the mycelium is affected by the nature of the substrate analysed (Bending & Read 1995; Rosling *et al.,* 2004; Ekblad *et al.,* 2016). In **Paper III**, we analysed the effects of nutrient amendments on EMF biomass production and turnover. Apatite and urea-amended meshbags were incubated along with pure-quartz bags as described in the previous section.

We found that mycelial turnover was not affected by the nutrient amendments but total EMF production was. As expected, apatite amendment only increased EMF biomass production (in relation to the pure-quartz bags) in the control plots, where P limits growth. In the fertilized plots, where limitation was alleviated, there was no effect of apatite on EMF production (Fig. 8).

Phosphorus fertilization significantly decreased EMF production in the urea-amended meshbags (Fig. 8). These findings suggest that the decrease in EMF biomass production caused by P fertilization also affects foraging for N, which would be expected if P fertilization resulted in a general decline in belowground carbon allocation. The fact that P fertilization had such a strong effect on EMF production in N amended bags further supports that P fertilization reduces EMF production. Moreover, the strong effect of N amendment on EMF production, even when N is not limiting growth, suggests that the EMF production (as a measurement of EMF production in soils) is underestimated in the pure quartz meshbags, probably because they are void of nutrients. This potential limitation in the ingrowth meshbag method could be overcome by adding ion exchange resin to improve the nutrient holding capacity in the meshbags as done by Wallander *et al.* (2011) and increase EMF colonization.

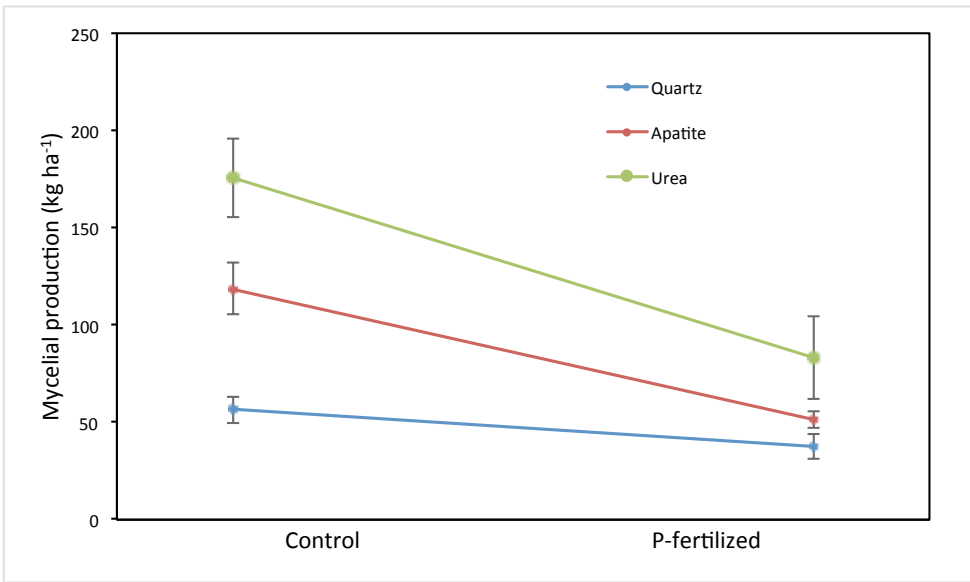

Figure 8: EMF biomass production estimates between the control and P-fertilized plots and between the meshbag amendments. The bars correspond to the standard deviation of the mean.

Nitrogen leaching and EMF nitrogen uptake in response to phosphorus limitation and high nitrogen deposition

Nitrogen uptake and demand

In temperate and boreal forests, organic N is the main N source for nutrition (Näsholm *et al.,* 2009); however, increased N availabilities are expected to shift N demand from organic to mineral N compounds (Zhou *et al.,* 2019; Allison *et al.,* 2008). It has been reported that increased N availability enhanced ammonium uptake in comparison with amino acid uptake by EM plants (Wallander *et al.,* 1997; Zhou *et al.,* 2019). In a fertilization study of a boreal forest in Alaska, Allison *et al.* (2008) found that N fertilization reduced soil enzymatic activity related to chitin and protein breakdown. It was suggested that N deposition in boreal forests could have negative effects on organic N cycling because of the decrease in N enzymatic activity by EMF and other soil organisms.

To test the effects of N fertilization on EMF N uptake from organic matter, in **Paper I,** we amended ingrowth meshbags with composted maize leaves and incubated them in the same plots from the NP experiment described in **Paper II**. After 17 months of incubation, the chemical changes in the maize amendment resulting from fungal colonization were characterized using infrared and X-ray absorption spectroscopy.

We found that in meshbags incubated in the non-fertilized plots, there was a reduction in heterocyclic N compounds from the maize compost material relative to non-incubated compost material, suggesting that fungi had utilized these compounds and probably transferred them outside the meshbags. In the N-fertilized plots, the reduction of heterocyclic N compounds was lower. Heterocyclic N compounds are components of organic molecules, such as nucleic acids, carbohydrates, and chlorophyll, which comprise up to 35% of the total organic nitrogen in natural soils (Schulten &Schnitzer 1997; Talbot & Treseder

2010). This higher reduction of heterocyclic N compounds in the non-fertilized plots support previous findings showing that elevated N might reduce N fungal mining from soil organic matter. However, it was also found that there was an increase in the C:N ratios in the maize amendment from meshbags relative to the C:N ratios of the non-incubated compost material, irrespective of N fertilization. This could suggest that N was transferred out of the meshbag by EMF and probably transported to the host trees. Moreover, there was an increase in the amounts of carboxylic compounds in the meshbag compost material in comparison with the non-incubated compost material, suggesting enhanced oxidative degradation of the organic material. The N uptake from the organic matter even after N fertilization might indicate that the N demand in this forest persists.

An increase in N availability caused by N deposition can lead to N leaching from the system. Areas with high levels of N deposition are reported to have enhanced N losses through leaching (Stevens *et al.,* 1993; Gundersen *et al*., 1998; Högberg *et al.,* 2010; Bahr *et al.,* 2013). Gundersen *et al*. (1998) analysed data regarding N fluxes (N inputs and outputs) from more than 300 different forest stands across Europe and found that those receiving more than 10 kg N ha^{-1} $year^{-1}$ as deposition and with a soil C:N ratio less than 25 C:N have a high risk for N loss through nitrate leaching. Enhanced N leaching can be a result of less N uptake and retention by EMF (Hogberg *et al.,* 2010). In forests transitioning to P limitation, a decrease in N demand and an increase P demand by trees and EMF are expected to lead to diminished N uptake and to increased N leaching (Stevens *et al.,* 1993; Blanes *et al.,* 2012). Indeed, P fertilization has been shown to be able to enhance N demand, increase N uptake by trees (Blanes *et al.,* 2012; Mayor *et al.,* 2015), and reduce nitrate concentrations in soil solution (Stevens *et al.,* 1993). In a Spanish fir (*Abies pinsapo*) forest receiving 12 kg N ha^{-1} $year^{-1}$ of throughfall N deposition, Blanes *et al.* (2012) reported that P fertilization increased above growth biomass production and reduced phosphatase enzymatic activity in the mycorrhizal roots as a result of alleviated P limitation. Moreover, P fertilization increased N retention in the roots and litter layer (as measured by ^{15}N recovered). As described in **Paper II**, we found an increase in N concentration in the needles following P fertilization, suggesting that an increase in tree growth generated N demand and increased N uptake. However, the fungal colonization in urea-amended meshbags reported in **Paper III** was not enhanced, and was actually significantly decreased by P fertilization, which suggests that fungal demand for N was not enhanced by P fertilization. Blanes *et al.* (2012) found that P fertilization decreased EMF root colonization but increased fine root production. It was suggested that an increase in P availability would reduce the dependence of plants on EMF, and that the increased N uptake observed was a result of direct plant uptake. Thus, P fertilization seems to increase tree N demand, enhance N uptake and decrease N

leaching. However, because P fertilization also has a negative effect on EMF, the enhanced N uptake could be accomplished directly by the plant.

Nitrogen retention and EMF biomass

In addition to the great capacity of EMF to take up N in different forms (Näsholm *et al.,* 2009), the large underground mycelial networks can contribute to N retention in boreal forests (Read *et al.,* 2004). Therefore, a decrease in EMF biomass as a result of N excess might also enhance N leaching (Bahr *et al.,* 2013).

Bahr *et al.* (2013) analysed the correlation between EFM biomass from ingrowth meshbags and environmental data, such as throughfall N deposition, soil water N content, and humus C:N ratio from 29 Norway spruce stands distributed across southern Sweden. The forests stands had varying N deposition levels and soil water N contents. The N deposition ranged from 0.95 to 24.6 N ha^{-1} $year^{-1}$. Multivariate correlation analysis revealed that the EMF biomass (assessed as the visual estimation of hyphal growth) was negatively correlated with soil water N. However, a direct correlation between EMF biomass and leaching could not be found.

In **paper II**, we tested the correlation between N leaching and EMF biomass. We investigated N incorporation and leaching through the fungal mycelia colonizing the meshbags from the NP and P experiments described in previous sections.

Using a syringe, we injected ^{15}N as ammonium-nitrate on the top of the meshbags two days before meshbag harvesting. The meshbags contained ion resin beads at the bottom to collect the ^{15}N that had leached through the ECM mycelia (Fig. 9).

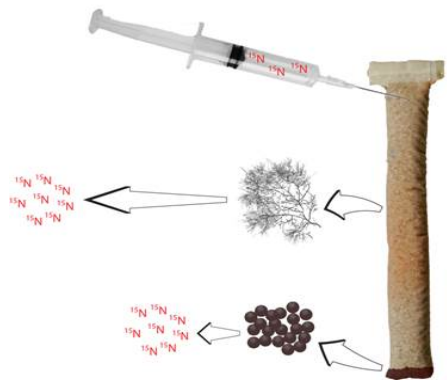

Figure 9: Scheme of the ^{15}N addition to the ingrowth meshbags from Paper II (Made by Adam Bahr).

After harvesting, the mycelium was isolated from the sand inside the meshbags and the ^{15}N incorporated in the ECM mycelium was quantified as the fraction of the total amount of tracer added that was recovered in the mycelium. To estimate the amount of ^{15}N that had leached through the ECM mycelium, the amount of ^{15}N tracer recovered in the resin beads was also measured and quantified (see **Paper II** for details regarding the methodology).

We found that the amount of ^{15}N leaching to the resin beads in the bottom of the meshbags was negatively correlated with fungal biomass in the meshbag (ergosterol) and with the total ^{15}N incorporated in the ECM mycelia, suggesting that the increase in N leaching was related to a decrease in biomass (Fig. 10).

The total ^{15}N incorporated in the ECM mycelia and the ^{15}N concentration in the mycelia biomass was not affected by N fertilization. We expected a decrease in the concentration of ^{15}N in the meshbag mycelium in the N fertilization treatment because of a lower expected N demand. However, these results suggest that leaching in the meshbags was caused by a reduction in ECM biomass rather than reduced N demand and uptake by the fungi.

Therefore, we predict that leaching can be increased in response to a reduced EMF biomass in N saturated forests. However, if N deposition induces P limitation, an increase in EMF biomass production might help reduce N leaching in these forests.

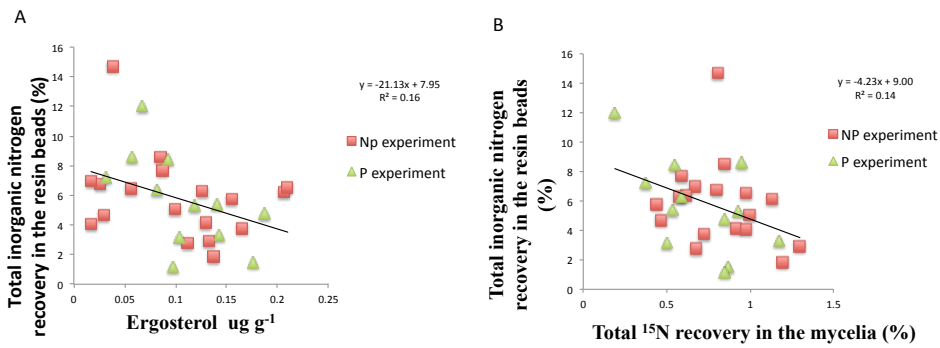

Figure 10: Correlation between A) ergosterol and leaching (^{15}N recovered in the resin beads) B) total recovery of ^{15}N in the mycelia and leaching (^{15}N inorganic nitrogen recovered in the resin beads). The red squares correspond to the NP experiment. The green triangles correspond to the P experiment. Modified from Almeida *et al*. (2018).

EMF community structure in response to phosphorus limitation and high nitrogen deposition

Effect of phosphorus limitation and high nitrogen deposition on the community structure

Different ectomycorrhizal species differ in their abilities to utilize N and P, in the amount of C needed from the host and in the tolerance to the excess or deficiency of a nutrient (Lilleskov *et al.,* 2002a; Simard *et al.,* 2015; Zavišić *et al.,* 2018). Therefore, changes in the amount of C delivered by the host under different nutrient conditions can alter the structure and composition of the EMF communities (Allison *et al.,* 2008). Indeed, several studies have shown that N deposition significantly influenced EMF community structure (Lilleskov *et al.,* 2002a; Allison *et al.,* 2008; Kjøller *et al.,* 2012 Suz *et al.,* 2014). **In Paper II**, we tested the effect of N and P addition on EMF communities in a region with high N deposition. A meta-barcoding survey of EMF was performed in the fertilized plots described in the previous sections. Community analyses in meshbags were conducted using apatite-amended and pure-quartz meshbags incubated in the NP experiment (N and N+P fertilization treatments) only. Community analyses in soils were conducted using soil samples collected from both NP and P experiments (see details in **Paper II**).

In the meshbag EMF communities, apatite amendment significantly influenced EMF communities in the meshbags in the control and N fertilized plots; however, the effect of apatite on the communities disappeared when the plots were fertilized with N + P. These findings suggest that the presence of a P-rich mineral source strongly regulates the EMF community composition under P limiting conditions. Species that efficiently take up P from minerals might be rewarded with more C from the trees in P-limited forests. The lack of effect of apatite when P demand is alleviated by N+P fertilization might indicate that the host trees allocate less C to EMF species adapted to P uptake from these sources.

In the soils EMF communities, N+P fertilization had a stronger effect on ECM communities than N fertilization alone, indicating that the soil ectomycorrhizal community composition was more sensitive to changes in P than to N availability. Indeed, P fertilization significantly altered the ECM community composition (Fig. 11).

These results stress the importance of P in this forest and indicate that EMF community assemblage is strongly regulated by this nutrient, suggesting a dynamic interaction between EMF fungi and the nutritional status of forests and soils.

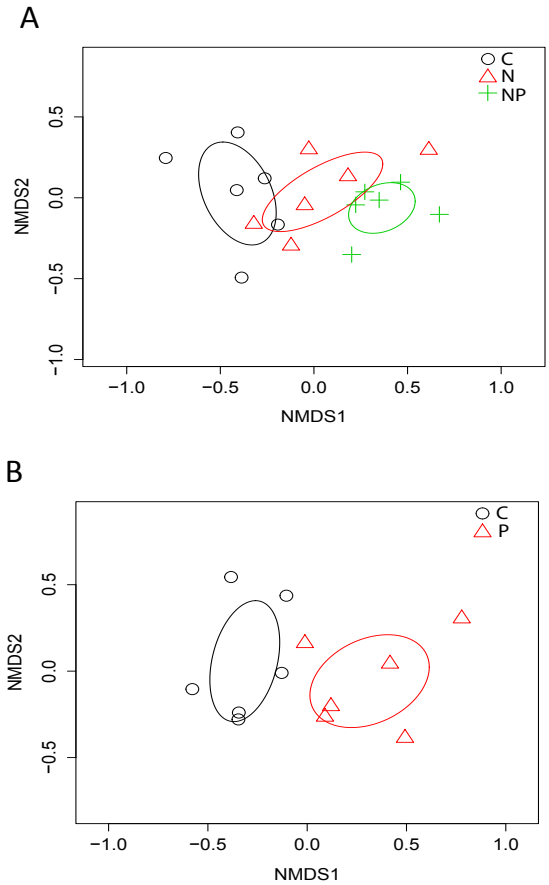

Figure 11: Response of EMF soil communities to fertilization treatments. NMDS ordination analysis of EMF communities in the humic soil for the NP experiment (A) and the P experiment (B). Differences between treatments were calculated based on Bray-Curtis dissimilarity and plotted in non-metric multidimensional scaling plots (NMDS). Modified from Almeida *et al.* (2018).

Effect of phosphorus limitation and nitrogen high nitrogen deposition on individual species

EMF species are diverse in structure and function and form different extramatrical structures (fungal mycelium extending from the root tip into the soil) adapted to soil exploration and nutrient uptake (Agerer, 2001; Hobbie & Agerer, 2010). These structures vary from short hydrophilic emanating hyphae to long hydrophobic rhizomorphs (Agerer, 2001). Based on these features, EMF species can be categorized into exploration types depending on the distance the emanating hyphae extend into the surrounding soil (Agerer, 2001). Different exploration types are expected to differ in the amount of C needed from the host and in their strategies to take up N and P from the soils (Lilleskov *et al.,* 2002b; Allison *et al.,* 2008). Thus, when there is an increase in N in soils and a concomitant reduction in belowground C allocation, EMF exploration types with low C requirements should be benefited and exploration types that require high C investment to produce long distance structures are predicted to be reduced (Lilleskov *et al.,* 2011).

Suz *et al.* (2014) surveyed EMF community structure and species functional traits along N deposition gradients in 22 oak (*Quercus* spp.) temperate forest stands across nine countries in western and central Europe. They found that species with restricted soil exploration ranges, such as *Lactarius quietus,* increased in abundance in response to N deposition, while the abundance of species from the genera *Cortinarius*, *Piloderma,* and *Tricholoma*, which have medium exploration types, were negatively affected by N deposition. In a survey of an N deposition gradient from a boreal forest in Alaska, Lilleskov *et al.,* (2002a) found that *Cortinarius* and *Piloderma* species responded negatively to N deposition and *Lactarius* increased with N deposition, suggesting that some species could be used as bioindicators for N deposition (Suz *et al.,* 2014). However, the distance the mycelium lengthened from the root tips did not always correlate to N deposition, and some short exploration type species have been reported to decline under high N deposition (Lilleskov *et al.,* 2011; Kjøller *et al.,* 2012; Almeida *et al.,* 2018), suggesting that not all EMF short distance species have the same response to decreased belowground C allocation. Allison *et al.* (2008) analysed EMF community structure in another N deposition gradient in Alaska and found that, for some EMF species, the abundance of sporocarps and the relative abundance of the fungus in soils were significantly reduced as an effect of N deposition. However, other species significantly reduced sporocarp formation as an effect of N deposition while the relative abundance of the fungus in soils remained constant. This might be an indication that some species can reduce C investments in reproductive structures and persist under low C allocation.

Long exploration types have also been shown to vary in their response to N deposition (Lilleskov *et al.,* 2011; Suz *et al.,* 2014). In the EMF communities from ingrowth meshbags studied in **Paper II**, the long exploration type *Boletus badius* (currently known as *Imleria badia*) significantly increased in abundance after N fertilization. Considering the evidence regarding P limitation in these stands, this could suggest that N fertilization exacerbated P limitation and that the increase in abundance of this long exploration species is a strategy to enhance P foraging and uptake. Indeed after N+P fertilization, the abundance of this species decreased significantly (Fig. 12). Moreover, when the meshbags were amended with apatite, the abundance of this species was further enhanced, suggesting that the growth of this species is stimulated by apatite and it is possible that superior P uptake from this mineral compared to other species was rewarded by larger C flux to the fungus.

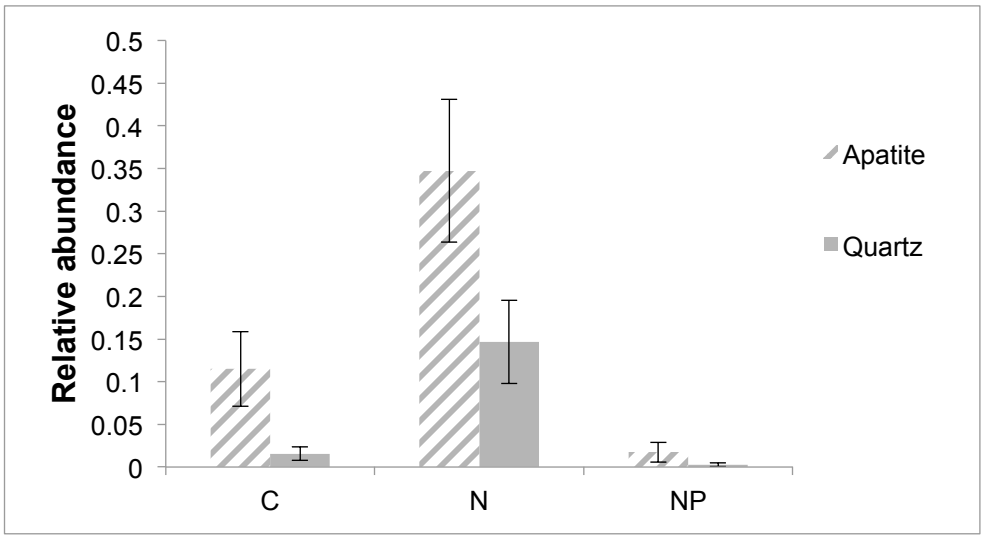

Figure 12: Relative abundance (DNA sequences reads) of *Boletus badius* (currently known as *Imleria badia*) in the apatite and quartz meshbags in the fertilization treatments from the NP experiment. Bars represent average values per treatment (n=12), error bars correspond to two standard errors. Modified from Almeida *et al*. (2018).

It has been postulated that, during the transition from N to P limitation, the EMF community composition will change from nitrophylic taxa to taxa specialized and more efficient for phosphorus uptake (Lilleskov *et al.,* 2002a). In an N deposition gradient in a boreal forest from Alaska, Lilleskov *et al.* (2002a), reported that among the species adapted to excess N, at the end of the gradient *Paxillus involutus* significantly increased in abundance. *P. involutus*, is able to access P from poorly soluble sources in axenic cultures as well as when growing in symbiosis with plants (Wallander *et al.,* 1997; Leake *et al.,* 2008; Rosling, 2009;

Smits *et al.,* 2012). In addition, it has been shown to be efficient at taking up P from mineral compounds (Adeleke *et al.,* 2012) and to have fast phosphate uptake when compared with other EMF species (Van Tichelen & Colpart, 2000). Therefore, Lilleskov *et al.* (2002a) proposed that *P. involutus* is a P efficient species and its increase might respond to an increase in P demand caused by N deposition.

In **Paper II**, we suggest that *B. badius* might be a species efficient at P uptake that responds with higher abundance when P demand from trees increases. Kottke *et al.* (1998) analyzed nutrient storage capacity in 17 different EMF species from root tips collected from the black forest in Germany and found that *B. badius* had high amounts of large intracellular polyphosphate granules and the highest concentration of P in the fungal sheath when compared with the other species analyzed. It was suggested by the authors that *B. badius* was potentially important for tree P nutrition, which supports our finding of increased abundance of this fungus in response to apatite under P-limiting conditions.

Effect of phosphorus limitation on EMF phosphorus uptake from organic and mineral molecules

Even though P can be abundant in soils, its uptake is challenging for plant nutrition because only a small fraction is present as free inorganic phosphates (PO_4^{-3}, $HPO_4^{-2,}$ and $H_2PO_4^-$), which are the primary forms available for plant uptake (Plassard *et al*., 2011). Significant amounts of P may be locked in organic molecules or poorly soluble minerals, or bound to iron oxides (Belyazid & Belyazid, 2012; Nehls & Plassard, 2018). Plants overcome these challenges through EMF associations. EMF can enhance P uptake and plant nutrition by using different strategies in which phosphate is released from minerals and organic molecules (Finlay, 2008). Therefore, it is expected that increases in plant P demand will favour EMF species adapted to P limitation by being efficient for P uptake from the different P sources in the soil (Lilleskov *et al.,* 2002a; Almeida *et al.,* 2018). Understanding the mechanisms responsible for accessing P pools in soils by EMF is important to determining the impacts that N deposition and P limitation have in boreal and temperate forests (Peñuelas *et al.,* 2013). In this chapter, I provide a short physiological insight into the mechanisms EMF use to access P from different pools in soils and briefly mention the factors that regulate P uptake.

EMF organic phosphorus uptake

Organic P compounds constitute a large pool of phosphorus in soils (Cosgrove, 1967; Richardson, 1994; Belyazid & Belyazid, 2012; Nehls & Plassard, 2018) and are expected to contribute significantly to the total phosphorus uptake by trees in forest ecosystems (Rennenberg & Herschbach, 2013; Vincent *et al.,* 2013). For instance, in boreal forests, up to 70–90% of soil phosphorus may be bound to organic substrates (Cosgrove, 1967; Vincent *et al.,* 2013). The most abundant organic source is phosphomonoesters, which can comprise more than 50% of the organic phosphorus pool in soils (Makarov *et al*., 2002). Inositol phosphates, such

as phytic acid (six phosphate molecules bound to an inositol ring) (Mittal *et al*., 2011), are probably the most abundant phosphomonoesters (Gerke, 2015). Phytic acid, which is mainly present in plant seeds as P reserve (Graf *et al*., 1987; Lott *et* al., 1995), is an important P source in natural soils since it accumulates when the seed does not germinate (Cairney, 2011; Becquer *et al*., 2014). The conversion of organic phosphorus into available phosphates depends on phosphatase enzymatic activity (Plassard *et al*., 2011). For example, phytates (phosphate monoesterase enzymes) are responsible for the hydrolysis of phytic acid and the release of phosphate (Plassard *et al.,* 2011; Sanz-Penella & Haros, 2014; Antibus *et al*., 1992). Experiments in axenic cultures have shown that several ectomycorrhizal fungi are able to subsist on phytic acid as the sole P source (Antibus et al., 1992). In an enzymatic essay using beech mycorrhizal roots, Bartlett & Lewis (1973) confirmed the hydrolysis of myo-inositol hexaphosphate and an increase of orthophosphates in the medium. Norisada *et al.* (2006) reported that EM *Pinus densiflora* seedlings provided with inositol phosphate as a P source had comparable P content in the needles as plants provided with phosphate. Several studies have shown that plants have poor capacity to solubilize phytates (Irshad *et al*., 2012; Becquer *et al*., 2014), suggesting that ectomycorrhizal fungi might play an important role in plant nutrition from phytic acid.

EMF phosphorus uptake from mineral compounds

In soils, P can be part of poorly soluble minerals (Cairney, 2011). The most common P-bounded minerals in soils are calcium phosphates, such as apatite (Osman, 2012). Many studies have shown that EMF are able to take up phosphorous from apatite sources when growing in symbiosis with plants or in axenic cultures (Wallander *et al.,* 1997; Leake *et al.,* 2008; Rosling*,* 2009; Smits *et al.,* 2012). When using apatite as the sole phosphorus source, Wallander et al. (1997) showed that the foliar P concentrations were higher in mycorrhizal pine seedlings than in non-mycorrhizal seedlings. Laparye *et al.* (1991) tested the capacity of 11 different EMF species to solubilize P from apatite suspended in solid agar (with or without phosphate) and found that the majority of species were able to solubilize apatite, regardless of the presence or absence of phosphate in the medium.

Among the mechanisms suggested for the release of P from apatite by EMF is the excretion of low molecular weight organic acids (LMWOA). Due to their acidifying and complexing properties, they are important agents for mineral weathering in soils (Landeweert *et al*., 2001). The carboxylic groups of LMWOA donate protons to the soil solution, which acidify the medium, protonate mineral surfaces, and increase P solubility (Gadd, 1999; Rosling *et al.,* 2007). In addition,

organic acid anions interact with calcium from the surface of the mineral and displace phosphate, increasing P release rates (Zhang *et al*., 1997; Landeweert *et al*., 2001). There are a variety of low molecular weight organic acids, including oxalic, citric, and fulvic acid (Van Hees *et al.,* 2000; Plassard *et al*., 2011). Oxalic acid is one of the most studied compounds in phosphorus release from minerals because of its strong chelating and acidifying capacities (Landeweert *et al*., 2001; Bindschedler *et al.,* 2016; Nworie *et al.,* 2017). When oxalic acid is excreted, the oxalate ions bind with calcium (forming calcium oxalate) and release phosphorus (Cannon et al., 1995; Rosling *et al.,* 2007). Accumulation of calcium oxalate crystals in ectomycorrhizal roots and under hyphal mats in forest soils has also been observed (Cromack *et al.,* 1979; Malajczuk & Cromack, 1982), especially in the presence of apatite (Wallander *et al.,* 2003), corroborating the role of oxalic acid in the release of P from minerals.

The production and accumulation of oxalic acid varies greatly between different ectomycorrhizal species (Plassard *et al*., 2011). Species such as *Paxillus involutus* and *Rhizopogon roselous* are known to produce large amounts of oxalic acid. Other species, such as *Hebeloma cylindrosporum* and *Laccaria laccata* (Lapeyrie *et al*., 1991), as well as the genera *Amanita*, *Cenococcum*, *Tylospora,* and *Thelephora,* have been shown to be poor oxalic acid producers (Casarin *et al*., 2004; Plassard *et al*., 2011). The ecological meaning of such interspecific variation remains unclear (Plassard *et al*., 2011). It would be useful to study the EMF community's composition in response to P limitation to determine if species that produce high amounts of oxalic acid are selected for in forests soils with low P availability. It is possible that such species might be rewarded by enhanced carbon allocation by the host in environments in which phosphorus is not readily available and where enhanced phosphorus acquisition is worth the costs of producing and excreting high amounts of oxalic acid.

It is important to consider what concentration of LMWOA is high enough to affect apatite weathering in soils. Oxalate is usually estimated in µM concentrations in soil solution (Van Hees et al., 2005), and efficient apatite dissolution has been measured in the mM range (Rosling *et al*., 2007). However, hyphae could produce enough oxalic acid to reach concentrations as high as 30 mM if the oxalic acid is released in small pores or cavities in the soil (Van Hees *et al*., 2005). Thus, the capacity of EMF to release P from apatite might not only depend on the total amount of oxalic acid released, but also on the ability of the fungi to explore and colonize small cavities in the soil while looking for minerals.

Phosphate can also be tightly bound to secondary minerals, such as iron oxides (Belyazid & Belyazid, 2012; Nehls & Plassard, 2018). Due to its stability, Goethite is the most common iron mineral in soils (Torrent & Schwertmann, 1992; Scheinost, 2005). Phosphate ions can be absorbed on its surface by ligand

exchange between the phosphate and OH groups from the mineral (Celi *et al.,* 2000; Martin *et al.,* 2002; Fink *et al.,* 2016). It has previously been suggested that EMF can access P bound to Fe minerals. Casarin *et al.* (2004) reported improved P nutrition of *P. sylvestris* in symbiosis with *Rhizopogon roseolus* growing on soils containing low amounts of available P because of high P absorption on Fe oxides. Adeleke *et al.* (2012) found that *P. involutus* in symbiosis with *B. pendula* was the most efficient among three EMF species tested at taking up P from Fe minerals containing 90% Fe oxides and several P-bearing minerals, such as apatite.

Different mechanisms to mobilize P from iron oxides have been proposed; namely, surface displacement of phosphate ions from the Fe oxide surface mediated by strongly absorbing molecules, such as LMWOA and siderophores (Casarin *et al.,* 2004; Rosling *et al.,* 2007; Rosling & Rosenstock, 2008), by pH reduction mediated by LMWOA and proton exudation (Rosling *et al.,* 2007; Adeleke *et al.,* 2012), and by reductive dissolution mediated by Fe-reducing compounds (Baldwin & Mitchel, 2000; Chacon *et al.,* 2006; Zhang *et al.,* 2014).

Proton exudation, LMWOA and siderophores production have been proposed and studied as the main mechanisms to release phosphate from Fe-mineral surfaces by EMF (Casarin *et al.,*2004; Rosling *et al.,* 2007; Rosling & Rosenstock, 2008 Adeleke *et al.,* 2012). Fe reductive dissolution as a mechanism to release P has been reported mainly by Fe-reducing bacteria in soils and water sediments (Baldwin & Mitchell, 2000; Chacon *et al.,* 2006; Zhang *et al.,* 2014). However, the role of Fe-reducing activity by EMF in response to P nutrition needs further exploration, although *P. involutus* has been reported to produce Fe-reducing metabolites to induce Fenton reaction and improve N nutrition from organic matter (Shah *et al.,* 2015). In **Paper IV**, we investigated whether the capacity of *P. involutus* to reduce Fe can be also used as a mechanism to improve P nutrition (see below).

Phosphorus uptake under low P availability

Some studies have shown that P availability in medium (either soil or axenic cultures) influences EMF phosphatases activity (Cairney, 2011; Plassard *et al.,* 2011). For example, Alvarez *et al.* (2006) investigated the activity of phosphate monoesterase bound to *P. involutus* hyphae in axenic cultures using enzymatic fluorescent labelling. They found that the monoesterase activity peaked when mycelium was growing in the lowest P concentration. In soils, it has been reported that increased soil P availability reduces EMF phosphatase activity (Alvarez *et al.,* 2006; Blanes *et al.,* 2012), and increased EMF phosphatase activity in soils is expected to enhance P uptake from organic compounds.

In **Paper I,** we amended ingrowth meshbags with maize compost and incubated them in a P-limited spruce forest to investigate chemical changes of the compost material after EMF colonization. C:P and C:N analysis of the maize compost revealed that, after incubation in the soil, the EMF community removed substantial amounts of P in addition to the N removal from maize amended meshbags reported above (Table 1). An increase in P demand in this forest might have caused a decrease in soluble P availability in soils, leading to enhanced P uptake from organic matter by EMF. This suggests that, in forests undergoing P limitation, P mining by EMF might be important to the decomposition of soil organic matter.

Table 1:
C:N and C:P ratios of the meshbag contents at the beginning of the experiment (initial material) and after 17 months of incubation in the soil humus and mineral layers of the control and N-fertilized plots at the Norway spruce forest. Standard error is in parentheses (n = 3 for the C:N ratios; n=6 for the C:P ratios). Values followed by different lowercase letters indicate significant difference between treatments and the initial material ($p < 0.05$). Modified from Nicolás *et al*. (2017).

	Initial material	**Humus layer**	**Humus layer**	**Mineral layer**	**Mineral layer**
		Control	N-Fertilized	Control	N-Fertilized
C:N	11.9 (0.1) a	14.7 (0.5) b	14.3 (0.3) b	14.8 (0.6) b	14.0 (0.1) b
C:P	53.8 (6.2) a	137.6 (12.7) b	139.2 (13.2) b	159.0 (13.9) b	139.8 (12.1) b

Regarding P uptake from mineral P compounds, such as apatite, low P availability has been reported to increase production of LMWOA by EMF (Van Schöll *et al.,* 2006; Rosling *et al.,* 2009; Smits *et al.,* 2012) and to increase P uptake from this mineral (Wallander, 2000; Casarin *et al.,* 2004; Leake *et al.,* 2008; Smits *et al.,* 2012). Leake *et al.* (2008) grew *P. sylvestris* seedlings inoculated with *Paxillus involutus* and fertilized them either with a full nutrient solution or a solution containing no phosphate. The roots and mycelium were grown in an agar-chamber where either apatite or quartz (no-containing-P mineral) was added to test if apatite could improve the host P nutrition. When no phosphate was provided in the nutrient solution, the P content of the host increased significantly in the presence of apatite. However, when the seedlings were provided with a full nutrient solution, no difference in the seedling P content was observed between apatite and quartz additions. These findings clearly demonstrate the capability of EMF to increase P uptake from mineral sources, such as apatite, when the P availability in soils is low.

Phosphorus uptake and phosphorus status of the mycelium

In axenic cultures, low intracellular P concentrations of the mycelium have been reported to increase P absorption from the growth medium (Cairney & Smith, 1992; Torres-Aquino *et al.,* 2017). Cairney & Smith (1992) studied *Pisolithus tinctorius* uptake of ^{32}P-labelled phosphate after the fungus was exposed to different P concentrations in the growth medium, and they found a negative correlation between the intracellular phosphate concentration and the total amount of ^{32}P taken up. Similarly, Torres-Aquino *et al.* (2017) found that after a period of P starvation, P uptake by *Hebeloma cylindrosporum* was enhanced and high amounts of poly-P granules were produced when the fungus was resupplied with phosphate. The amount of polyphosphate registered in the starved hyphae was as high as the amount registered in the non-starved hyphae. The production of poly-P granules is suggested as a mechanism to reduce free-phosphate concentrations in the cytoplasm, which might activate phosphate transporters in the membrane, thereby increasing P uptake (Cairney & Smith, 1992; Cairney, 2011). Moreover, the production of polyphosphate was not restricted to the amount of phosphate resupplied. Indeed, starved hyphae could produce similar amounts of polyphosphate granules, even when resupplied with a 10-fold lower phosphate concentration than the concentration originally resupplied. These findings suggest that the P status of the hyphae is an important factor that regulates P uptake and storage.

Limitations of axenic experiments for nutrient uptake studies.

Regarding P uptake from poorly soluble P sources, the results from culture experiments are not always consistent with those from experiments in which the fungus is growing in symbiosis with a plant (Rosling, 2009). In a symbiotic system, the host acts both as a sink for nutrients and as a carbon source. It has been proposed that the P taken up in the mycelial front is translocated to the fungus: root interphase in which P is accumulated (Cairney & Smith, 1992; Cairney, 2011). This phosphate efflux maintains low concentrations of intracellular P at the hyphal front, which might enhance P uptake (Cairney & Smith, 1992). Therefore, EMF growing in pure culture systems lack the nutrient sink that allows the reciprocal translocation of C and P. Moreover, in pure culture experiments, glucose and P are generally both provided in the growing medium, which

suppresses the formation of fungal structures adapted to nutrient foraging, uptake, and translocation (Smits *et al.,* 2012).

To overcome some of these problems, in **Paper IV,** I developed an experimental set up designed to resemble a symbiotic system in which the C and P source come from different locations. To accomplish this, I grew *P. involutus* in a divided petri dish system in which carbon and P were provided in the different compartments of the plate (Fig. 13). Using this set up, I examined the capacity of the fungus to take up P from poorly soluble forms. Additionally, to test the effects of P status of the mycelium on P uptake, half of the fungal cultures were previously exposed to a starvation growth phase in which no P was supplied in the growing media (see details in **Paper IV**). Starved and non-starved mycelia were then provided with the following P sources: phosphate, apatite, P-bound goethite, phytic acid or no P addition (as a control). Apatite and P-bound goethite were provided as solid particles on a piece of cellophane paper laying on top of the upper glass bead layer at approximately 1 cm from the dividing wall of the plate (Fig. 14). After 2 weeks of incubation, the part of the mycelium growing on the C compartment was collected to measure total P taken up and transferred from the P compartment. The part of the mycelium growing in contact with the P source was then collected and used for gene expression analysis (data not presented).

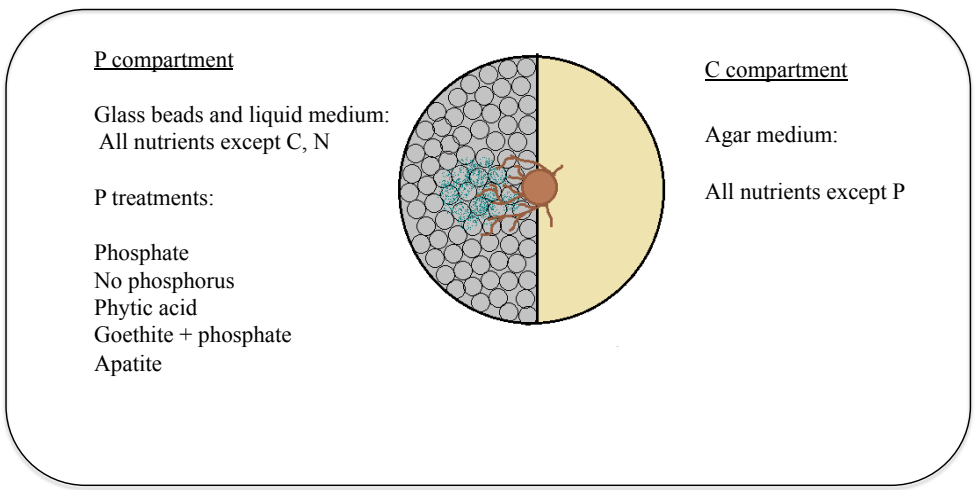

Figure 13: The divided plate set up used in Paper IV explained in detail. The fungus was inoculated at the dividing zone between the two compartments. In the P compartment, liquid growth medium was provided. Different P sources were provided depending on the treatment. On this side of the plate, the fungus grew on three layers of glass beads. In the C compartment, agar and growing medium were added and all of the nutrients but P were provided. Grey circles represent a glass bead system while the beige area represents agar-added growing medium

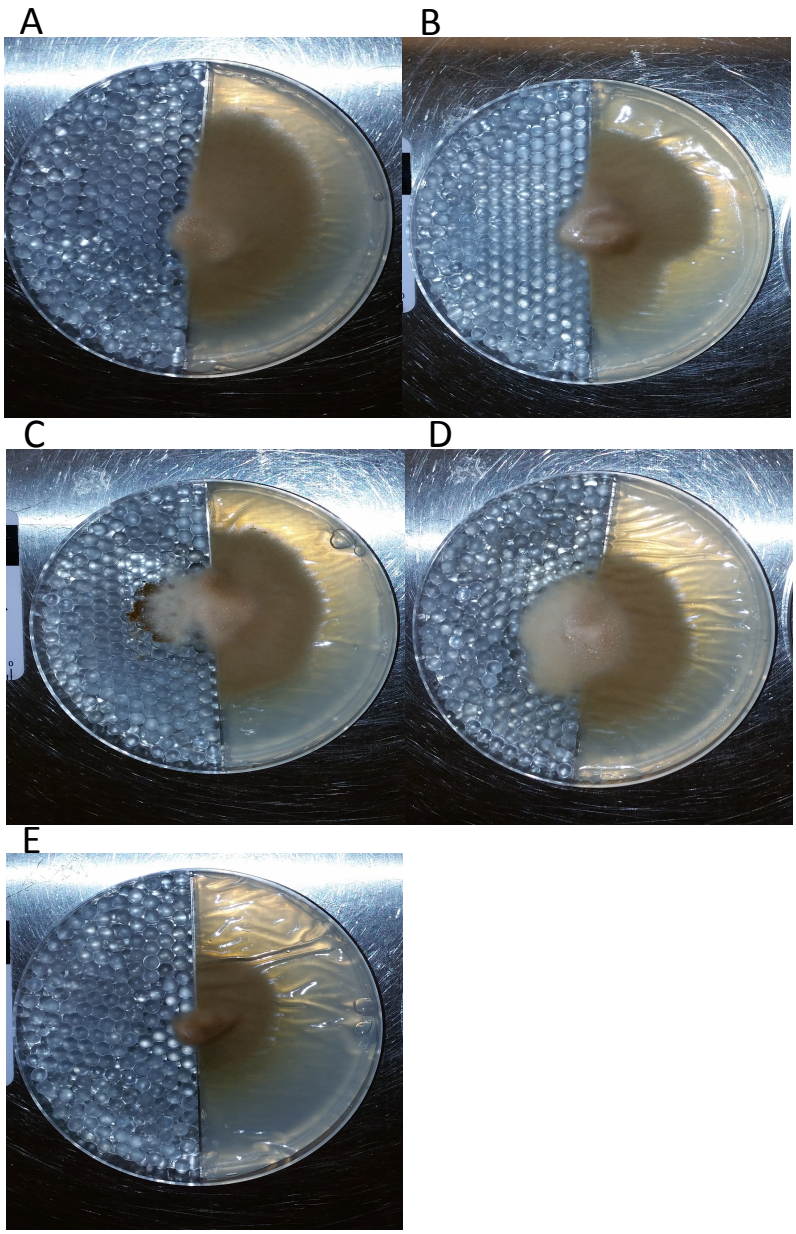

Figure 14: Photographs of *P. involutus* cultures and the different phosphorus source treatments at the end of the experiment: phosphate (A), phytic acid (B), goethite (C), and apatite (D) and no-phosphorus (E)

We found that *P. involutus* was able to access P from all of the sources provided (apatite, phytic acid, and P-bound goethite), although not to the same extent as the easily available phosphate. The fungus was able to translocate P to the part of the mycelium that had access to C. These results support previous evidence that *P. involutus* is able to take P up from both mineral and organic sources. Furthermore, we provide for the first time direct evidence of EMF uptake from P that has been absorbed into a Fe-oxide surface. The capacity of *P. involutus* to release P bound to goethite, which is one of the most abundant Fe mineral in natural soils (Torrent *&* Schwertmann*,* 1992), suggests that this species is of ecological importance for P nutrition.

In **Paper IV**, we also examined whether the capacity of *P. involutus* to reduce Fe^{3+} was related to P uptake and influenced by P source and P starvation. For this purpose, we collected liquid medium from the fungal cultures at the end of the experiment and determined the Fe-reducing capacity.

The Fe-reducing capacity was measured using a Ferrozine assay (Goodell *et al*., 2006). Ferrozine is a compound that reacts with divalent Fe (Fe^{2+}) to produce a colour reaction. The concentration of Fe^{2+} in the solution can be then measured based on the absorbance of the sample using a spectrophotometer. We added Fe^{3+} (in the form of Fe Cl_3) to the liquid medium collected. If *P. involutus*, had produced Fe-reducing metabolites during the experiment, the added Fe^{3+} will be reduced. The Fe-reducing capacity of the medium was estimated as the amount of added Fe^{3+} that was reduced to Fe^{2+} during the assay.

In the P-starved mycelium cultures, we found significant Fe-reducing capacity for all of the P treatments (phytic acid, apatite, goethite and no-P) with exception of the phosphate treatment (Fig. 15). Fe-reducing activity by *P. involutus* could be a mechanism to enhance phosphate uptake from poorly soluble sources. The release of P from apatite and phytic acid in soil is probably very slow and the released phosphate ions may easily be captured by Fe^{3+} present in the soil (Turner, 2008; Rosling*,* 2009; Plassard *et al.,* 2011). However, by producing Fe-reducing compounds, EMF may increase the possibility for assimilation of the newly liberated phosphate ions.

Furthermore, the P source also affected Fe-reduction capacity in which phosphate registered the lowest Fe-reducing capacity and apatite registered the highest. In our experiment, low concentrations of phosphate in the medium solution might have induced Fe-reducing activity as a response to improve phosphate uptake. Phosphate released from the apatite was likely rapidly taken up by the fungus, resulting in low phosphate concentrations in the medium, which may have induced continuing exudation of Fe-reducing metabolites. In **Paper IV**, we report for the first time that Fe-reducing capacity by EMF may be related to P nutrition and dependent on the P source provided. Fe-reduction as a mechanism to improve P

uptake from organic and mineral P sources is a strategy not reported for EMF and should therefore be further investigated.

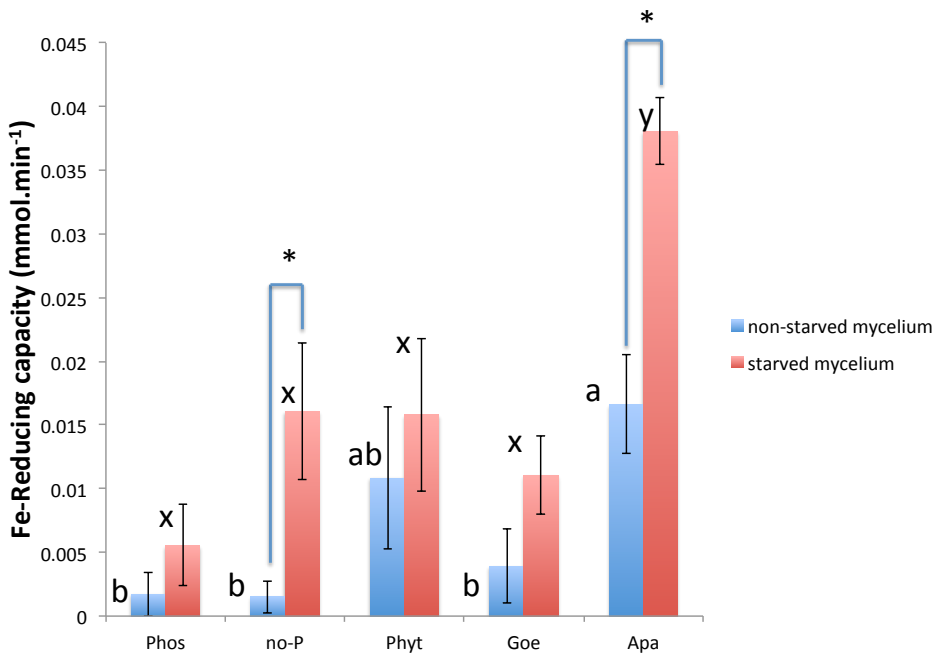

Figure 15: *P. involutus* total Fe-reducing capacity in the growing medium for all P treatments in the starved and non-starved mycelium. The Fe-reducing capacity of the no fungus control was subtracted from all of the treatments. Error bars correspond to two standard errors of the mean (n=4). Phos, no-P, Phyt, Goe, and Apa are abbreviations for the phosphate, no-phosphorus, phytic acid, goethite, and apatite treatments, respectively. Asterisks (*) represent significant differences between the starved and non-starved mycelium. Lower case a and b indicate significant differences between the P sources in the non-starved mycelium according to the Dunn and ANOVA tests. Lower case x and y indicate significant differences between the P sources in the starved mycelium according to Dunn and ANOVA tests.

Contrary to what was expected, we did not see an increase in P uptake in the starved mycelium in comparison with the non-starved mycelium when growing with any of the P sources. However, we reported that the capacity to reduce Fe was increased in the starved mycelium, especially when the fungus was provided with apatite or growing without any P source. Moreover, the total amount of oxalate produced by the fungus was significantly increased in the starved mycelium relative to the non-starved mycelium, but only when no P source was provided (results not shown in **Paper IV**) (Fig. 16). Thus, we show that the P status of the mycelium in *P. involutus* is important to regulation of mechanisms that improve P uptake from poorly soluble sources, even though an effect on total P uptake could not be detected.

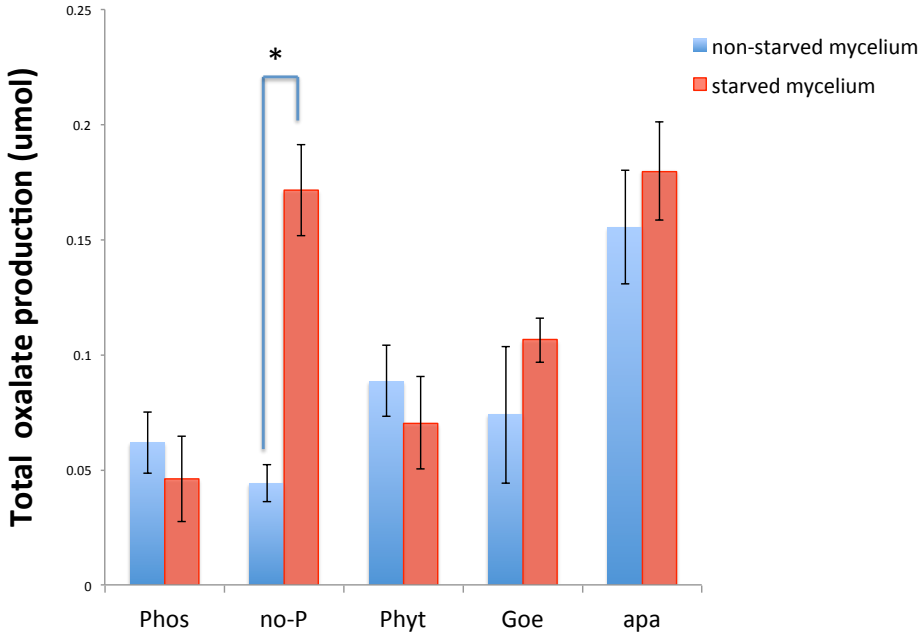

Figure 16: *P. involutus* oxalate production for all of the P treatments in starved and non-starved mycelium. Error bars correspond to two standard errors of the mean (n=4). Phos, no-P, Phyt, Goe, and Apa are abbreviations of the phosphate, no-phosphorus, phytic acid, goethite, and apatite treatments, respectively. Asterisks (*) represent significant differences between the starved and non-starved mycelium according to ANOVA tests.

As shown here, *P. involutus* has the capacity to release P from different poorly soluble sources (including P bound to Fe oxides surfaces) and to produce Fe-reductants that facilitate uptake of the newly released P. This might confer this species with an adaptive advantage over species in the EMF community in soils, especially under P limiting conditions. In a forest with high P demand, *P. involutus* could be rewarded with a higher C allocation from the host in return for P taken up from minerals and organic compounds. This supports the results reported by Lilleskov *et al.* (2012a), who found that this species is favoured under high P demand conditions, probably because of its high P uptake capacity. *Boletus badius* is another species that has been reported to increase in abundance in response to P limitation (**Paper II**) and suggested to be efficient for P uptake in forest soils (Kottke *et al.,* 1998). In a pilot study, we isolated *B. badius* from the P-limited spruce forest described in previous sections. Preliminary analyses showed that *B. badius* exhibited significant levels of Fe-reducing capacity that were enhanced by P starvation. These findings corroborate the idea that Fe reductive dissolution could be a mechanism to avoid Fe-P binding, and that this strategy is being used in natural soils by EMF.

In conclusion, in this chapter, I show why EMF association can be important for P nutrition in high latitude forests. EMF can access P from different P pools via different strategies, and this is enhanced by low P availabilities. This can be crucial in forests facing P limitation. Other EMF species responsive to P limitation in high latitude forests should be studied to test the potential mechanisms by which species deal with nutrient limitation and how EMF communities adapt to shifts in nutrient regime.

Conclusions and future perspectives

In this thesis I highlight the importance of P over various aspects of EMF at a northern conifer forest receiving high N deposition levels. I show that P regulates EMF biomass production and EMF communities' assembly. Also, P limitation favors the abundance of EMF species better adapted to P uptake from non-easily available P sources, which are abundant in natural soils.

N has been considered the most important nutrient limiting plant growth and regulating EMF growth and EMF communities' assemblage in high latitude forests. However, since N deposition can lead to a shift in nutrient limitation increasing P demand, P can become an important element for high latitude forest nutrition. The shift from N to P limitation has been widely discussed for these forests (Vitousek *et al*, 2010; Peñuelas *et al.,* 2013; Jonard *et al.,* 2015), yet studies showing a positive effect of P fertilization on tree growth are scarce in temperate forests and boreal forests. In **Paper II,** I provide strong evidence about the effect of P fertilization on tree growth and foliar P concentration and I show that regions receiving high N deposition levels can transition from N to P limitation. The forest analyzed in our studies has more elevated N deposition levels in comparison to other northern conifer forests, though these levels are low in comparison temperate regions in central Europe. Even so, under these conditions a strong effect of P was detected. Other P fertilization experiments are needed especially in temperate forests to evaluate the response of EMF to the transition from N to P limitation as done in this study.

Under high soil N availability the host trees reduce belowground C allocation, which results in reduced EMF biomass production. In this thesis I show that an increase in P availability also decreases EMF total production in forest where N deposition has resulted in P limitation. P-rich sources like apatite have a stronger positive effect on the total EMF production but only under P limiting conditions. This corroborates that in this forest the P status of the trees regulates EMF production and suggest that P regulates belowground C allocation to the fungal symbionts. Interestingly, EMF foraging on N rich sources (urea) was also decreased by an increase in P fertility, which may indicates that a general reduction of belowground C allocation results in a reduction for foraging to other nutrient sources.

Here I have suggested that the effect of P on EMF biomass production is a result of a decreased belowground C partitioning by the host. However, I have measured only EMF biomass of mycelium colonizing the meshbags and I have not estimated the total belowground C allocation since I have no data on root production, respiration and EMF root colonization. Therefore, it is difficult to state which nutrient N or P is more important for belowground C partitioning. N could be more important regulating fine root production while P can be more important regulating EMF extramatrical production as suggested by Ekblad *et al.* (2016) and Blanes *et al.* (2012). More studies on root respiration, fine root production and EMF root colonization and are needed to identify how N and P nutrient limitation affects belowground C allocation to different components of the rhizosphere and soils. Additionally, EMF total production seems to be a more robust tool to assess extramatrical mycelium responses to nutrient limitation than measurements of the standing biomass of one given incubation time in meshbags or the standing biomass from soils.

P had a stronger effect on EMF community's composition than N. Moreover, P strongly regulates EMF community's structure especially in presence of apatite in P limited plots. This clearly indicates that EMF community composition is related to the trees nutrient necessities and species adapted for P foraging and uptake are favored by a higher C allocated by the host when P demand increases.

Besides the overall main effect on the EMF community structure, P limitation strongly favored single species like *Boletus badius* (especially responsive to apatite in meshbags under P limiting conditions) that have been shown to be efficient to take up and store P from soils. The increase in abundance of *B. badius* indicates a potential role of this species in tree P nutrition, similar to what was suggested for other species responsive to an increase of P demand in N saturated forests like *P. involutus*.

It is necessary to do more studies on the ecology and the physiology of individual species that respond to an increase of N deposition and so P demand, such as *B. badius* and *P. involutus,* to determine the importance of these species for forest P nutrition. Studies that test the EMF capacity to take up P from organic and mineral compounds, the rate of P uptake in comparison with other species, the ability to subsist with minutes amounts of P, or the ability to transfer P to the host according to the host needs, are necessary to evaluate how EMF will adapt to increase P demand in high latitude forests. Here, I have isolated *B. badius* from the forest soils and I aimed to test P uptake from different sources by this potential P efficient species in axenic cultures and symbiosis experiments.

I have shown that *P. involutus* has the capacity to take up P from poorly soluble sources like phytic acid, apatite and P bound to secondary minerals like goethite that are abundant in many soils. Furthermore, I observed that *P. involutus* induces

Fe-reduction when provided with these sources especially when it grows with apatite. This Fe-reducing capacity could be a strategy by the fungus to enhance phosphate uptake probably by preventing binding between the phosphate release by the mineral and Fe^{+3} present in the soils. A follow up study could be to analyze which reducing metabolites are involved in the Fe-reduction activity registered for *P. involutus*. Involutin has been detected as a secondary metabolite involved in N mining form organic compounds. I observed that *B. badius* also presents Fe-reducing activity in response to P starvation. It would be important to isolate the compounds and metabolites (as done by Shah *et al.* (2015)) produced during P starvation to test the metabolites involved in P uptake from these two boletal species.

The P status of the hyphae of *P. involutus* increases Fe-reducing capacity when the fungus is provided with apatite or when the fungus was growing without any P source. The total oxalate production followed a similar pattern as the Fe reducing capacity. This suggests that the P status of the hyphae regulates some strategies to weather poorly soluble P sources. The majority of studies about physiological responses of EMF to low P availabilities have focused on low external phosphate concentration as a proxy for P limitation. It is important to study how the P status of the mycelium affects P uptake and regulate strategies to increase P uptake. Understanding how the P status of the hyphae influence on P uptake will allow us to understand how P limitation in forests affect P nutrition from the perspective of the fungus own needs.

The similar pattern between oxalate production and Fe-reducing capacity might suggest a synergetic effect of these compounds during mineral weathering. A similar synergistic effect has been suggested for Fe binding molecules (siderophores) and oxalate during organic matter degradation (Clarholm *et al.,* 2015) and iron oxides dissolution (Reichard *et al*., 2005). The high Fe-reducing capacity and oxalate production when apatite is provided as a P source requires further studies. A trascriptome study on the mycelium in contact with the P sources provided in the experiment from **Paper IV** is being developed to analyze the genes involved in apatite weathering and to test if apatite provided to starved mycelia leads to an up regulation of genes involved in Fe-reduction compounds and oxalate production.

References

Adeleke, R. A., Cloete, T. E., Bertrand, A., & Khasa, D. P. (2012). Iron ore weathering potentials of ectomycorrhizal plants. *Mycorrhiza*, *22*(7), 535-544.

Agerer, R. (2001). Exploration types of ectomycorrhizae. *Mycorrhiza*, *11*(2), 107-114.

Akselsson, C., Westling, O., Alveteg, M., Thelin, G., Fransson, A. M., & Hellsten, S. (2008). The influence of N load and harvest intensity on the risk of P limitation in Swedish forest soils. *Science of the total environment*, *404*(2-3), 284-289.

Akselsson, C., Belyazid, S., Hellsten, S., Klarqvist, M., Pihl-Karlsson, G., Karlsson, P. E., & Lundin, L. (2010). Assessing the risk of N leaching from forest soils across a steep N deposition gradient in Sweden. *Environmental Pollution*, *158*(12), 3588-3595.

Allison, S. D., Czimczik, C. I., & Treseder, K. K. (2008). Microbial activity and soil respiration under nitrogen addition in Alaskan boreal forest. *Global Change Biology*, *14*(5), 1156-1168.

Almeida, J. P., Rosenstock, N. P., Forsmark, B., Bergh, J., & Wallander, H. (2018). Ectomycorrhizal community composition and function in a spruce forest transitioning between nitrogen and phosphorus limitation. Fungal Ecology. In press: doi.org/10.1016/j.funeco.2018.05.008

Alvarez, M., Gieseke, A., Godoy, R., & Härtel, S. (2006). Surface-bound phosphatase activity in ectomycorrhizal fungi: a comparative study between a colorimetric and a microscope-based method. *Biology and fertility of soils*, *42*(6), 561.

Antibus, R. K., Sinsabaugh, R. L., & Linkins, A. E. (1992). Phosphatase activities and phosphorus uptake from inositol phosphate by ectomycorrhizal fungi. *Canadian Journal of Botany*, *70*(4), 794-801.

Bae, K., Fahey, T. J., Yanai, R. D., & Fisk, M. (2015). Soil nitrogen availability affects belowground carbon allocation and soil respiration in northern hardwood forests of New Hampshire. *Ecosystems*, *18*(7), 1179-1191.

Bahr, A., Ellström, M., Akselsson, C., Ekblad, A., Mikusinska, A., & Wallander, H. (2013). Growth of ectomycorrhizal fungal mycelium along a Norway spruce forest nitrogen deposition gradient and its effect on nitrogen leakage. *Soil Biology and Biochemistry*, *59*, 38-48.

Bahr, A., Ellström, M., Bergh, J., & Wallander, H. (2015). Nitrogen leaching and ectomycorrhizal nitrogen retention capacity in a Norway spruce forest fertilized with nitrogen and phosphorus. *Plant and Soil*, *390*(1-2), 323-335.

Baldwin, D. S., & Mitchell, A. M. (2000). The effects of drying and re-flooding on the sediment and soil nutrient dynamics of lowland river–floodplain systems: a synthesis. *Regulated Rivers: Research & Management: An International Journal Devoted to River Research and Management*, *16*(5), 457-467.

Bartlett, E. M., & Lewis, D. H. (1973). Surface phosphatase activity of mycorrhizal roots of beech. *Soil Biology and Biochemistry*, *5*(2), 249-257.

Baum, C., & Makeschin, F. (2000). Effects of nitrogen and phosphorus fertilization on mycorrhizal formation of two poplar clones (*Populus trichocarpa* and *P. tremula* x *tremuloides*). *Journal of Plant Nutrition and Soil Science*, *163*(5), 491-497.

Becquer, A., Trap, J., Irshad, U., Ali, M. A., & Claude, P. (2014). From soil to plant, the journey of P through trophic relationships and ectomycorrhizal association. *Frontiers in Plant Science*, *5*, 548.

Belyazid, U.J. & Belyazid, S. (2012). Phosphorus cycling in boreal and temperate forest ecosystems. *Belyazid Consulting and Communication AB, Malmö, Sweden*.

Belyazid, U.J. & Belyazid, S. (2013). Nitrogen in boreal forest ecosystems - The influence of climate change, nitrogen fertilization, whole-tree harvest and stump harvest on the nitrogen cycle. *Belyazid Consulting and Communication AB, Malmö, Sweden*.

Bending, G. D., & Read, D. J. (1995). The structure and function of the vegetative mycelium of ectomycorrhizal plants. V. The foraging behaviour of ectomycorrhizal mycelium and the translocation of nutrients from exploited organic matter. *New Phytologist*, *130*(40), 1-409.

Bergh, J., Nilsson, U., Allen, H. L., Johansson, U., & Fahlvik, N. (2014). Long-term responses of Scots pine and Norway spruce stands in Sweden to repeated fertilization and thinning. *Forest Ecology and Management*, *320*, 118-128.

Berner, C., Johansson, T., & Wallander, H. (2012). Long-term effect of apatite on ectomycorrhizal growth and community structure. *Mycorrhiza*, *22*(8), 615-621.

Bindschedler, S., Cailleau, G., & Verrecchia, E. (2016). Role of fungi in the biomineralization of calcite. *Minerals*, *6*(2), 41.

Binkley, D., & Högberg, P. (2016). Tamm review: revisiting the influence of nitrogen deposition on Swedish forests. *Forest Ecology and Management*, *368*, 222-239.

Blanes, M. C., Emmett, B. A., Viñegla, B., & Carreira, J. A. (2012). Alleviation of P limitation makes tree roots competitive for N against microbes in a N-saturated conifer forest: A test through P fertilization and 15N labelling. *Soil Biology and Biochemistry*, *48*, 51-59.

Bödeker, I. (2012). Functional ecology of ectomycorrhizal fungi. PhD thesis. Swedish University of Agricultural sciences. Uppsala

Bonfante, P., & Genre, A. (2010). Mechanisms underlying beneficial plant–fungus interactions in mycorrhizal symbiosis. *Nature Communications*, *1*, 48.

Braun, S., Thomas, V. F., Quiring, R., & Flückiger, W. (2010). Does nitrogen deposition increase forest production? The role of phosphorus. *Environmental Pollution*, *158*(6), 2043-2052.

Cairney, J. W. (2011). Ectomycorrhizal fungi: the symbiotic route to the root for phosphorus in forest soils. *Plant and Soil*, *344*(1-2), 51-71.

Cairney, J. W. G., & Smith, S. E. (1992). Influence of intracellular phosphorus concentration on phosphate absorption by the ectomycorrhizal basidiomycete *Pisolithus tinctorius*. *Mycological Research*, *96*(8), 673-676.

Cannon, J. P., Allen, E. B., Allen, M. F., Dudley, L. M., & Jurinak, J. J. (1995). The effects of oxalates produced by Salsola tragus on the phosphorus nutrition of Stipa pulchra. *Oecologia*, *102*(3), 265-272.

Casarin, V., Plassard, C., Hinsinger, P., & Arvieu, J. C. (2004). Quantification of ectomycorrhizal fungal effects on the bioavailability and mobilization of soil P in the rhizosphere of *Pinus pinaster*. *New Phytologist*, *163*(1), 177-185.

Clarholm, M., Skyllberg, U., & Rosling, A. (2015). Organic acid induced release of nutrients from metal-stabilized soil organic matter–the unbutton model. Soil Biology and Biochemistry, 84, 168-176.

Celi, L., Barberis, E., & Marsan, F. A. (2000). Sorption of phosphate on goethite at high concentrations. *Soil Science*, *165*(8), 657-664.

Chacon, N., Silver, W. L., Dubinsky, E. A., & Cusack, D. F. (2006). Iron reduction and soil phosphorus solubilization in humid tropical forests soils: the roles of labile carbon pools and an electron shuttle compound. *Biogeochemistry*, *78*(1), 67-84.

Chapin, F. S., Walker, L. R., Fastie, C. L., & Sharman, L. C. (1994). Mechanisms of primary succession following deglaciation at Glacier Bay, Alaska. *Ecological Monographs*, *64*(2), 149-175.

Cromack Jr, K., Sollins, P., Graustein, W. C., Speidel, K., Todd, A. W., Spycher, G., et al. (1979). Calcium oxalate accumulation and soil weathering in mats of the hypogeous fungus *Hysterangium crassum*. *Soil Biology and Biochemistry*, *11*(5), 463-468.

Cosgrove, D.J. (1967) Metabolism of organic phosphates in soil. In: *Soil Biochemistry* (A.C. McLauren and G.H. Peterson, editors). Marcel Dekker, New York, USA.

Davidson, E. A. (2008). Biogeochemistry: fixing forests. *Nature Geoscience*, *1*(7), 421.

Du, E., & Fang, J. (2014). Weak growth response to nitrogen deposition in an old-growth boreal forest. *Ecosphere*, *5*(9), 1-9.

Ekblad, A., Mikusinska, A., Ågren, G. I., Menichetti, L., Wallander, H., Vilgalys, R., et al. (2016). Production and turnover of ectomycorrhizal extramatrical mycelial biomass and necromass under elevated CO2 and nitrogen fertilization. *New Phytologist*, *211*(3), 874-885.

Elser, J. J., Bracken, M. E., Cleland, E. E., Gruner, D. S., Harpole, W. S., Hillebrand, H., et al. (2007). Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology letters*, *10*(12), 1135-1142.

Erisman, J. W., Dammers, E., Damme, M. V., Soudzilovskaia, N., & Schaap, M. (2015). Trends in EU nitrogen deposition and impacts on ecosystems. *EM: Air and Waste Management Association's Magazine for Environmental Managers, September, 65, 31-35*.

Falkowski, P., Scholes, R. J., Boyle, E. E. A., Canadell, J., Canfield, D., Elser, J., et al. (2000). The global carbon cycle: a test of our knowledge of earth as a system. *Science*, *290*(5490), 291-296.

Finlay, R. D. (2008). Ecological aspects of mycorrhizal symbiosis: with special emphasis on the functional diversity of interactions involving the extraradical mycelium. *Journal of Experimental Botany*, *59*(5), 1115-1126.

Fink, J. R., Inda, A. V., Tiecher, T., & Barrón, V. (2016). Iron oxides and organic matter on soil phosphorus availability. *Ciência e Agrotecnologia*, *40*(4), 369-379.

Finzi, A. C. (2009). Decades of atmospheric deposition have not resulted in widespread phosphorus limitation or saturation of tree demand for nitrogen in southern New England. *Biogeochemistry*, *92*(3), 217-229.

Gadd, G. M. (1999). Fungal production of citric and oxalic acid: importance in metal speciation, physiology and biogeochemical processes. In *Advances in Microbial Physiology* (Vol. 41, pp. 47-92). Academic Press.

Gadd, G. M. (2006). *Fungi in Biogeochemical Cycles* (Vol. 24). Cambridge University Press.

Galloway, J. N., Townsend, A. R., Erisman, J. W., Bekunda, M., Cai, Z., Freney, J. R., et al. (2008). Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science*, *320*(5878), 889-892.

Gerke, J. (2015). Phytate (inositol hexakisphosphate) in soil and phosphate acquisition from inositol phosphates by higher plants. a review. *Plants*, *4*(2), 253-266.

Gill, A. L., & Finzi, A. C. (2016). Belowground carbon flux links biogeochemical cycles and resource-use efficiency at the global scale. *Ecology Letters*, *19*(12), 1419-1428.

Goodell, B., Daniel, G., Jellison, J., & Qian, Y. (2006). Iron-reducing capacity of low-molecular-weight compounds produced in wood by fungi. *Holzforschung*, *60*(6), 630-636.

Graf, E., Empson, K. L., & Eaton, J. W. (1987). Phytic acid. A natural antioxidant. *Journal of Biological Chemistry*, *262*(24), 11647-11650.

Gundersen, P., Callesen, I., & De Vries, W. (1998). Nitrate leaching in forest ecosystems is related to forest floor C/N ratios. In *Nitrogen, the Confer-Ns* (pp. 403-407).

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. *Mycological Research*, *112*(6), 681-688.

Hedwall, P. O., Nordin, A., Strengbom, J., Brunet, J., & Olsson, B. (2013). Does background nitrogen deposition affect the response of boreal vegetation to fertilization?. *Oecologia*, *173*(2), 615-624.

Hendricks, J. J., Mitchell, R. J., Kuehn, K. A., & Pecot, S. D. (2016). Ectomycorrhizal fungal mycelia turnover in a longleaf pine forest. *New Phytologist*, *209*(4), 1693-1704.

Hobbie, E. A., & Agerer, R. (2010). Nitrogen isotopes in ectomycorrhizal sporocarps correspond to belowground exploration types. *Plant and Soil*, *327*(1-2), 71-83.

Houlton, B. Z., Wang, Y. P., Vitousek, P. M., & Field, C. B. (2008). A unifying framework for dinitrogen fixation in the terrestrial biosphere. *Nature*, *454*(7202), 327.

Högberg, M. N., Högberg, P., & Myrold, D. D. (2007). Is microbial community composition in boreal forest soils determined by pH, C-to-N ratio, the trees, or all three?. *Oecologia*, *150*(4), 590-601.

Högberg, M. N., Briones, M. J., Keel, S. G., Metcalfe, D. B., Campbell, C., Midwood, A. J., et al. (2010). Quantification of effects of season and nitrogen supply on tree below-ground carbon transfer to ectomycorrhizal fungi and other soil organisms in a boreal pine forest. *New Phytologist*, *187*(2), 485-493.

Hyland, C., Ketterings, Q., Dewing, D., Stockin, K., Czymmek, K., Albrecht, G., & Geohring, L. (2005). Phosphorus basics—The phosphorus cycle. *Agronomy Fact Sheet Series*, *12*, 1-2.

Hui, D., Deng, Q., Tian, H., & Luo, Y. (2017). Climate change and carbon sequestration in forest ecosystems. Handbook of Climate Change Mitigation and Adaptation, 555-594.

Irshad, U., Brauman, A., Villenave, C., & Plassard, C. (2012). Phosphorus acquisition from phytate depends on efficient bacterial grazing, irrespective of the mycorrhizal status of *Pinus pinaster*. *Plant and Soil*, *358*(1-2), 155-168.

Janssens, I. A., Dieleman, W., Luyssaert, S., Subke, J. A., Reichstein, M., Ceulemans, R., et al. (2010). Reduction of forest soil respiration in response to nitrogen deposition. *Nature Geoscience*, *3*(5), 315.

Jonard, M., Fürst, A., Verstraeten, A., Thimonier, A., Timmermann, V., Potočić, N., et al. (2015). Tree mineral nutrition is deteriorating in Europe. *Global change Biology*, *21*(1), 418-430.

Keith, H., Raison, R. J., & Jacobsen, K. L. (1997). Allocation of carbon in a mature eucalypt forest and some effects of soil phosphorus availability. *Plant and Soil*, *196*(1), 81-99.

Kjøller, R., Nilsson, L. O., Hansen, K., Schmidt, I. K., Vesterdal, L., & Gundersen, P. (2012). Dramatic changes in ectomycorrhizal community composition, root tip abundance and mycelial production along a stand-scale nitrogen deposition gradient. *New Phytologist*, *194*(1), 278-286.

Kottke, I., Qian, X. M., Pritsch, K., Haug, I., & Oberwinkler, F. (1998). *Xerocomus badius–Picea abies*, an ectomycorrhiza of high activity and element storage capacity in acidic soil. *Mycorrhiza*, *7*(5), 267-275.

Kuylenstierna, J. C. I., Hicks, W. K., Cinderby, S., & Cambridge, H. (1998). Critical loads for nitrogen deposition and their exceedance at European scale. In *Nitrogen, the Confer-Ns* (pp. 591-598).

Landeweert, R., Hoffland, E., Finlay, R. D., Kuyper, T. W., & van Breemen, N. (2001). Linking plants to rocks: ectomycorrhizal fungi mobilize nutrients from minerals. *Trends in Ecology & Evolution*, *16*(5), 248-254.

Lapeyrie, F., Ranger, J., & Vairelles, D. (1991). Phosphate-solubilizing activity of ectomycorrhizal fungi in vitro. *Canadian Journal of Botany*, *69*(2), 342-346.

Leake, J. R., Duran, A. L., Hardy, K. E., Johnson, I., Beerling, D. J., Banwart, S. A., & Smits, M. M. (2008). Biological weathering in soil: the role of symbiotic root-associated fungi biosensing minerals and directing photosynthate-energy into grain-scale mineral weathering. *Mineralogical Magazine*, *72*(1), 85-89.

LeBauer, D. S., & Treseder, K. K. (2008). Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology*, *89*(2), 371-379.

Li, Y., Niu, S., & Yu, G. (2016). Aggravated phosphorus limitation on biomass production under increasing nitrogen loading: a meta-analysis. *Global Change Biology*, *22*(2), 934-943.

Lilleskov, E. A., Fahey, T. J., Horton, T. R., & Lovett, G. M. (2002a). Belowground ectomycorrhizal fungal community change over a nitrogen deposition gradient in Alaska. *Ecology*, *83*(1), 104-115.

Lilleskov, E. A., Hobbie, E. A., & Fahey, T. J. (2002b). Ectomycorrhizal fungal taxa differing in response to nitrogen deposition also differ in pure culture organic nitrogen use and natural abundance of nitrogen isotopes. *New Phytologist*, *154*(1), 219-231.

Lilleskov, E. A., Hobbie, E. A., & Horton, T. R. (2011). Conservation of ectomycorrhizal fungi: exploring the linkages between functional and taxonomic responses to anthropogenic N deposition. *Fungal Ecology*, *4*(2), 174-183.

Linder, S. (1995). Foliar analysis for detecting and correcting nutrient imbalances in Norway spruce. *Ecological: Bulletins (Copenhagen), 44,* 178-190.

Lott, J. N., Greenwood, J. S., & Batten, G. D. (1995). Mechanisms and regulation of mineral nutrient storage during seed development. *Seed Development and Germination. Marcel Dekker, New York*, 215-235.

Lovett, G. M., Arthur, M. A., Weathers, K. C., Fitzhugh, R. D., & Templer, P. H. (2013). Nitrogen addition increases carbon storage in soils, but not in trees, in an eastern US deciduous forest. *Ecosystems*, *16*(6), 980-1001.

Mafla, P. M. (2018). Molecular diversity and host specificity in ectomycorrhizal communities in the tropical forest of Yasuní National Park, Ecuador. Master thesis, Lund University.

Mainwaring, D. B., Maguire, D. A., & Perakis, S. S. (2014). Three-year growth response of young Douglas-fir to nitrogen, calcium, phosphorus, and blended fertilizers in Oregon and Washington. *Forest Ecology and Management*, *327*, 178-188.

Makarov, M., Haumaier, L., & Zech, W. (2002). The nature and origins of diester phosphates in soils: a 31 P-NMR study. *Biology and Fertility of Soils*, *35*(2), 136-146.

Malajczuk, N., & Cromack Jr, K. (1982). Accumulation of calcium oxalate in the mantle of ectomycorrhizal roots of *Pinus radiata* and *Eucalyptus marginata*. *New Phytologist*, *92*(4), 527-531.

Marschner, H., & Dell, B. (1994). Nutrient uptake in mycorrhizal symbiosis. *Plant and Soil*, *159*(1), 89-102.

Martin, M., Celi, L., & Barberis, E. (2002). Extractability and plant availability of phosphate from P-goethite complexes. *Communications in Soil Science and Plant Analysis*, *33*(1-2), 143-153.

Mayor, J. R., Mack, M. C., & Schuur, E. A. (2015). Decoupled stoichiometric, isotopic, and fungal responses of an ectomycorrhizal black spruce forest to nitrogen and phosphorus additions. *Soil Biology and Biochemistry*, *88*, 247-256.

McGroddy, M. E., Daufresne, T., & Hedin, L. O. (2004). Scaling of C: N: P stoichiometry in forests worldwide: Implications of terrestrial redfield-type ratios. *Ecology*, *85*(9), 2390-2401.

Mittal, A., Singh, G., Goyal, V., Yadav, A., Aneja, K. R., Gautam, S. K., & Aggarwal, N. K. (2011). Isolation and biochemical characterization of acido-thermophilic extracellular phytase producing bacterial strain for potential application in poultry feed. *Jundishapur Journal of Microbiology*, *4*(4), 273-282.

Näsholm, T., Kielland, K., & Ganeteg, U. (2009). Uptake of organic nitrogen by plants. *New Phytologist*, *182*(1), 31-48.

Nehls, U., & Plassard, C. (2018). Nitrogen and phosphate metabolism in ectomycorrhizas. *New Phytologist*, *220*(4), 1047-1055.

Nicolás, C., Almeida, J. P., Ellström, M., Bahr, A., Bone, S. E., Rosenstock, N. P., Bargar, J.R., Tunlid, A., Persson, P., & Wallander, H. (2017). Chemical changes in organic matter after fungal colonization in a nitrogen fertilized and unfertilized Norway spruce forest. *Plant and Soil, 419*(1-2)*,* 113-126.

Nilsson, L. O., & Wallander, H. (2003). Production of external mycelium by ectomycorrhizal fungi in a Norway spruce forest was reduced in response to nitrogen fertilization. *New Phytologist*, *158*(2), 409-416.

Norisada, M., Motoshige, T., Kojima, K., & Tange, T. (2006). Effects of phosphate supply and elevated CO2 on root acid phosphatase activity in *Pinus densiflora* seedlings. *Journal of Plant Nutrition and Soil Science*, *169*(2), 274-279.

Nworie, O. E., Qin, J., & Lin, C. (2017). Differential effects of low-molecular-weight organic acids on the mobilization of soil-borne arsenic and trace metals. *Toxics*, *5*(3), 18.

Op De Beeck, M., Troein, C., Peterson, C., Persson, P., & Tunlid, A. (2018). Fenton reaction facilitates organic nitrogen acquisition by an ectomycorrhizal fungus. *New Phytologist*, *218*(1), 335-343.

Osman, K. T. (2012). *Soils: principles, properties and management*. Springer Science & Business Media. Dordrecht, The Netherlands.

Pampolina, N. M., Dell, B., & Malajczuk, N. (2002). Dynamics of ectomycorrhizal fungi in an Eucalyptus globulus plantation: effect of phosphorus fertilization. *Forest Ecology and Management*, *158*(1-3), 291-304.

Pardo, L. H., Fenn, M. E., Goodale, C. L., Geiser, L. H., Driscoll, C. T., Allen, E. B., et al. (2011). Effects of nitrogen deposition and empirical nitrogen critical loads for ecoregions of the United States. *Ecological Applications*, *21*(8), 3049-3082.

Peñuelas, J., Poulter, B., Sardans, J., Ciais, P., Van Der Velde, M., Bopp, L., et al. (2013). Human-induced nitrogen–phosphorus imbalances alter natural and managed ecosystems across the globe. *Nature Communications*, *4*, 2934.

Pihl Karlsson, G., Akselsson, C., Hellsten, S., & Karlsson, P. E. (2017). Krondroppsnätet i södra Sverige: Övervakning av luftföroreningar och dess effekter i skogsmiljön. Resultat till och med september 2016.

Plassard, C., Louche, J., Ali, M. A., Duchemin, M., Legname, E., & Cloutier-Hurteau, B. (2011). Diversity in phosphorus mobilisation and uptake in ectomycorrhizal fungi. *Annals of Forest Science*, *68*(1), 33-43.

Radwan, M. A., Shumway, J. S., DeBell, D. S., & Kraft, J. M. (1991). Variance in response of pole-size trees and seedlings of Douglas-fir and western hemlock to

nitrogen and phosphorus fertilizers. *Canadian Journal of Forest Research*, *21*(10), 1431-1438.

Rastetter, E. B., Vitousek, P. M., Field, C., Shaver, G. R., & Herbert, D. (2001). Resource optimization and symbiotic nitrogen fixation. *Ecosystems*, *4*(4), 369-388.

Read, D. J., & Perez-Moreno, J. (2003). Mycorrhizas and nutrient cycling in ecosystems–a journey towards relevance?. *New Phytologist*, *157*(3), 475-492.

Read, D. J., Leake, J. R., & Perez-Moreno, J. (2004). Mycorrhizal fungi as drivers of ecosystem processes in heathland and boreal forest biomes. *Canadian Journal of Botany*, *82*(8), 1243-1263.

Reichard, P. U., Kraemer, S. M., Frazier, S. W., & Kretzschmar, R. (2005). Goethite dissolution in the presence of phytosiderophores: rates, mechanisms, and the synergistic effect of oxalate. Plant and Soil, 276(1-2), 115-132.

Richardson, A.E. (1994). Soil microorganisms and phosphorus availability. In: Soil Biota Management in Sustainable Farming Systems (C.E. Pankhurst, B.M. Doulse, V.V.S.R. Gupta and P.R. Grace editors). CSIRO, Australia.

Rosenstock, N. P., Berner, C., Smits, M. M., Krám, P., & Wallander, H. (2016). The role of phosphorus, magnesium and potassium availability in soil fungal exploration of mineral nutrient sources in Norway spruce forests. *New Phytologist*, *211*(2), 542-553.

Rosling, A., Lindahl, B. D., & Finlay, R. D. (2004). Carbon allocation to ectomycorrhizal roots and mycelium colonising different mineral substrates. New Phytologist, 162(3), 795-802.

Rosling, A., Suttle, K. B., Johansson, E., Van Hees, P. A., & Banfield, J. F. (2007). Phosphorous availability influences the dissolution of apatite by soil fungi. *Geobiology*, *5*(3), 265-280.

Rosling, A., & Rosenstock, N. (2008). Ectomycorrhizal fungi in mineral soil. *Mineralogical Magazine*, *72*(1), 127-130.

Rosling, A. (2009). Trees, mycorrhiza and minerals–field relevance of in vitro experiments. *Geomicrobiology Journal*, *26*(6), 389-401.

Sanz-Penella, J. M., & Haros, M. (2014). Whole grain and phytate-degrading human bifidobacteria. In *Wheat and rice in disease prevention and health* (pp. 17-31).

Scheinost, A. C. (2005). Metal oxides. In *Encyclopedia of Soils in the Environment*, 428–438.

Schulten, H. R., & Schnitzer, M. (1997). The chemistry of soil organic nitrogen: a review. *Biology and Fertility of Soils*, *26*(1), 1-15.

Schulte-Uebbing, L., & de Vries, W. (2018). Global-scale impacts of nitrogen deposition on tree carbon sequestration in tropical, temperate, and boreal forests: A meta-analysis. *Global Change Biology*, *24*(2), e416-e431.

Shah, F., Schwenk, D., Nicolás, C., Persson, P., Hoffmeister, D., & Tunlid, A. (2015). Involutin is a Fe3+ reductant Secreted by the Ectomycorrhizal Fungus *Paxillus involutus* during Fenton-based Decomposition of Organic Matter. *Applied and Environmental Microbiology*, AEM-02312.

Simard, S., Asay, A., Beiler, K., Bingham, M., Deslippe, J., He, X., et al. (2015). Resource transfer between plants through ectomycorrhizal fungal networks. In *Mycorrhizal Networks* (pp. 133-176). Springer, Dordrecht.

Smith, S. E., & Read, D. J. (2010). *Mycorrhizal symbiosis*. Cambridge, UK: Academic press.

Smith, S. E., Jakobsen, I., Grønlund, M., & Smith, F. A. (2011). Roles of arbuscular mycorrhizas in plant phosphorus (P) nutrition: interactions between pathways of P uptake in arbuscular mycorrhizal (AM) roots have important implications for understanding and manipulating plant P acquisition. *Plant Physiology*, pp-111.

Smits, M. M., Bonneville, S., Benning, L. G., Banwart, S. A., & Leake, J. R. (2012). Plant-driven weathering of apatite–the role of an ectomycorrhizal fungus. *Geobiology*, *10*(5), 445-456.

Sohrt, J., Lang, F., & Weiler, M. (2017). Quantifying components of the phosphorus cycle in temperate forests. *Wiley Interdisciplinary Reviews: Water*, *4*(6), e1243.

Sponseller, R. A., Gundale, M. J., Futter, M., Ring, E., Nordin, A., Näsholm, T., & Laudon, H. (2016). Nitrogen dynamics in managed boreal forests: Recent advances and future research directions. *Ambio*, *45*(2), 175-187.

Sterner, R. W., & Elser, J. J. (2002). *Ecological stoichiometry: the biology of elements from molecules to the biosphere*. Princeton University Press.

Stevens, P. A., Harrison, A. F., Jones, H. E., Williams, T. G., & Hughes, S. (1993). Nitrate leaching from a Sitka spruce plantation and the effect of fertilization with phosphorus and potassium. *Forest Ecology and Management*, *58*(3-4), 233-247.

Strullu-Derrien, C., Selosse, M. A., Kenrick, P., & Martin, F. M. (2018). The origin and evolution of mycorrhizal symbioses: from palaeomycology to phylogenomics. *New Phytologist*, 220(4), 1012-1030.

Suz, L. M., Barsoum, N., Benham, S., Dietrich, H. P., Fetzer, K. D., Fischer, R., et al. (2014). Environmental drivers of ectomycorrhizal communities in Europe's temperate oak forests. *Molecular Ecology*, *23*(22), 5628-5644

Talbot, J. M., & Treseder, K. K. (2010). Controls over mycorrhizal uptake of organic nitrogen. *Pedobiologia*, *53*(3), 169-179.

Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N. S., Wijesundera, R., et al. (2014). Global diversity and geography of soil fungi. *Science*, *346*(6213), 1256688.

Terry, N., & Ulrich, A. (1973). Effects of phosphorus deficiency on the photosynthesis and respiration of leaves of sugar beet. *Plant Physiology*, *51*(1), 43-47.

Thelin, G., Rosengren-Brinck, U., Nihlgård, B., & Barkman, A. (1998). Trends in needle and soil chemistry of Norway spruce and Scots pine stands in South Sweden 1985–1994. *Environmental Pollution*, *99*(2), 149-158.

Torrent, J., & Schwertmann, U. (1992). Fast and slow phosphate sorption by goethite-rich natural minerals. In *Clays Clay Minerals*, 40(1), 14-21.

Torres-Aquino, M., Becquer, A., Le Guernevé, C., Louche, J., Amenc, L. K., Staunton, S., et al. (2017). The host plant *Pinus pinaster* exerts specific effects on phosphate efflux and polyphosphate metabolism of the ectomycorrhizal fungus *Hebeloma*

cylindrosporum: a radiotracer, cytological staining and ^{31}P NMR spectroscopy study. *Plant, Cell & Environment*, *40*(2), 190-202.

Treseder, K. K. (2004). A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO2 in field studies. *New Phytologist*, *164*(2), 347-355.

Turner, B. L. (2008). Resource partitioning for soil phosphorus: a hypothesis. *Journal of Ecology*, *96*(4), 698-702.

Van Hees, P. A., Rosling, A., Essén, S., Godbold, D. L., Jones, D. L., & Finlay, R. D. (2006). Oxalate and ferricrocin exudation by the extramatrical mycelium of an ectomycorrhizal fungus in symbiosis with *Pinus sylvestris*. *New Phytologist*, *169*(2), 367-378.

Van Hees, P. A. W., Lundström, U. S., & Giesler, R. (2000). Low molecular weight organic acids and their Al-complexes in soil solution—composition, distribution and seasonal variation in three podzolized soils. *Geoderma*, *94*(2-4), 173-200

Van Schöll, L., Hoffland, E., & Van Breemen, N. (2006). Organic anion exudation by ectomycorrhizal fungi and *Pinus sylvestris* in response to nutrient deficiencies. *New Phytologist*, *170*(1), 153-163.

Van Tichelen, K. K., & Colpaert, J. V. (2000). Kinetics of phosphate absorption by mycorrhizal and non-mycorrhizal Scots pine seedlings. *Physiologia Plantarum*, *110*(1), 96-103.

Veresoglou, S. D., Peñuelas, J., Fischer, R., Rautio, P., Sardans, J., Merilä, P., et al. (2014). Exploring continental-scale stand health–N: P ratio relationships for European forests. *New Phytologist*, *202*(2), 422-430.

Vincent, A. G., Vestergren, J., Gröbner, G., Persson, P., Schleucher, J., & Giesler, R. (2013). Soil organic phosphorus transformations in a boreal forest chronosequence. *Plant and Soil*, *367*(1-2), 149-162.

Vitousek, P. M., Porder, S., Houlton, B. Z., & Chadwick, O. A. (2010). Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen–phosphorus interactions. *Ecological Applications*, *20*(1), 5-15.

von Liebig, J. F. (1855). *Die grundsätze der agricultur-chemie mit rücksicht auf die in england angestellten untersuchungen* (in England: The relations of chemistry to agriculture and the agricultural experiments of Mr. J.B. Lawes). 1st and 2nd ed Friedrich Vieweg und Sohn Publ. Co., Braunschweig, Germany.

Wallander, H., & Nylund, J. E. (1992). Effects of excess nitrogen and phosphorus starvation on the extramatrical mycelium of ectomycorrhizas of Pinus sylvestris L. *New Phytologist*, *120*(4), 495-503.

Wallander, H., Arnebrant, K., Östrand, F., & Kårén, O. (1997). Uptake of 15 N-labelled alanine, ammonium and nitrate in *Pinus sylvestris* L. ectomycorrhiza growing in forest soil treated with nitrogen, sulphur or lime. *Plant and Soil*, *195*(2), 329-338.

Wallander, H. (2000). Uptake of P from apatite by *Pinus sylvestris* seedlings colonised by different ectomycorrhizal fungi. *Plant and Soil*, *218*(1-2), 249-256.

Wallander, H., Nilsson, L. O., Hagerberg, D., & Bååth, E. (2001). Estimation of the biomass and seasonal growth of external mycelium of ectomycorrhizal fungi in the field. *New Phytologist*, *151*(3), 753-760.

Wallander, H., Ekblad, A., & Bergh, J. (2011). Growth and carbon sequestration by ectomycorrhizal fungi in intensively fertilized Norway spruce forests. *Forest Ecology and Management*, *262*(6), 999-1007.

Wallander, H., Ekblad, A., Godbold, D. L., Johnson, D., Bahr, A., Baldrian, P., et al. (2013). Evaluation of methods to estimate production, biomass and turnover of ectomycorrhizal mycelium in forests soils–A review. *Soil Biology and Biochemistry*, *57*, 1034-1047.

Wardle, D. A., Walker, L. R., & Bardgett, R. D. (2004). Ecosystem properties and forest decline in contrasting long-term chronosequences. *Science*, *305*(5683), 509-513.

Wardle, D. A., Jonsson, M., Mayor, J. R., & Metcalfe, D. B. (2016). Above-ground and below-ground responses to long-term nutrient addition across a retrogressive chronosequence. *Journal of Ecology*, *104*(2), 545-560.

Zavišić, A., Yang, N., Marhan, S., Kandeler, E., & Polle, A. (2018). Forest Soil Phosphorus Resources and Fertilization Affect Ectomycorrhizal Community Composition, Beech P Uptake Efficiency, and Photosynthesis. Frontiers in Plant Science, 9.

Zhang, F. S., Ma, J., & Cao, Y. P. (1997). Phosphorus deficiency enhances root exudation of low-molecular weight organic acids and utilization of sparingly soluble inorganic phosphates by radish (*Raghanus sativus* L.) and rape (*Brassica napus* L.) plants. *Plant and Soil*, *196*(2), 261-264.

Zhang, S., Wang, R., Zhang, L., Li, S., Feng, G., & Ding, X. (2014). Iron-reducing bacteria can enhance the activation and turnover of the Fe (III)-fixed phosphorus for mycorrhizal plants. *Journal of Plant Nutrition and Soil Science*, *177*(2), 208-215.

Zhou, M., Yan, G., Xing, Y., Chen, F., Zhang, X., Wang, J., et al. (2019). Nitrogen deposition and decreased precipitation does not change total nitrogen uptake in a temperate forest. *Science of the Total Environment*, *651*, 32-41.

Popular science summary

Mycorrhizal associations

Mycorrhizal fungi are organisms that live in association with plant roots and provide plants with water and nutrients in exchange for the sugars obtained during photosynthesis. In forested ecosystems mycorrhizal fungi are crucial for tree nutrition and trees rely heavily on these associations to take up key elements like nitrogen and phosphorus. As result, big amounts of the atmospheric CO_2 converted in sugars by trees are delivered to the soils to support the fungal partners.

In boreal and temperate forests, nitrogen is present in the soils in forms that are generally not available for tree consumption. Due to the cold conditions in these ecosystems, the cycling of nutrients in the soils is slow and nitrogen accumulates in complex organic molecules (slowly degrading dead plant and animal tissues) with no nutritious value for the trees. However, mycorrhizal fungi have developed different strategies to access the nitrogen locked in such molecules. For that reason, a great amount of carbon is sent to the fungal partners to enhance nitrogen uptake that is in high demand. Indeed, temperate and boreal forests soils are one of the most important reservoirs for carbon in the world.

Mycorrhizal associations, nitrogen deposition and phosphorus demand

Nitrogen pollution caused by human industrial activities deposits from the atmosphere onto forest soils, increases the availability of nitrogen and reduces the need of the trees to maintain the fungal partners. As a consequence, less carbon is delivered to the soils and roots causing a decrease in the growth of mycorrhizal fungi, which might impact ecosystem functioning. A reduction in mycorrhizal biomass can lead to less nitrogen uptake, which will be leached through the soils.

Initially, excess nitrogen favors tree growth, but eventually the trees stop growing because a new nutrient is now in high demand and is required to keep sustaining growth. This nutrient is phosphorus.

Like nitrogen, phosphorus in soils can be present in form of organic molecules. Besides, phosphorus is also found in soil minerals and ores. Mycorrhizal fungi have the ability to release phosphorus from organic molecules and also from ores and minerals. Therefore, when phosphorus tree demand increases in forests,

mycorrhizal fungi growth is expected to be stimulated to enhance phosphorus foraging and uptake from the soils.

In this thesis I measured ectomycorrhizal growth in a forest where nitrogen deposition has resulted in high phosphorus demand. Mycorrhizal growth was measured using ingrowth meshbags, which are bags made of a 25 micrometers mesh containing sand. The size of the mesh prevents the growth of the plants fine roots but allows the growth of the fungal filaments (series of attached cells that extend into the soil). This method allows the study of mycorrhizal fungi colonizing the inside of the bags in the search for nutrients. Moreover, I added nutrients rich in nitrogen and phosphorus inside the meshbags to see how nutrient foraging is affected in forests with high P demand. The meshbags were then placed underground in the forest where they were colonized internally by mycorrhizal fungi.

I found that when phosphorus demand increases in the forest, there is also an increase in mycorrhizal growth suggesting that trees rely again on these fungal associations and send carbon belowground to support mycorrhizal fungi and improve phosphorus nutrition. The increase in mycorrhizal growth was significantly enhanced when the meshbags contained either nitrogen or phosphorus rich nutrients. This suggests that higher mycorrhizal growth due to P demand influences nutrient foraging for phosphorus but also for nitrogen. Thus, the increase in mycorrhizal growth can help the ecosystems to retain more nitrogen and prevent leaching. Moreover, enhanced carbon deliver to the soils can increase carbon sequestration, which is important to mitigate CO_2 emissions.

Phosphorus demand and mycorrhizal communities

Hundreds of different mycorrhizal species inhabits the forest soils and more than tens of different species are associated with the root system of the same individual tree. These species have different abilities to take up phosphorus from soils and to endure the conditions where the trees allocate less carbon due to high nutrient availability.

In this thesis I also studied how the communities of mycorrhizal fungi species are affected when phosphorus is in high demand. I extracted DNA from soils and meshbags collected in the forest described above. Based on the DNA sequences abundance, I estimated mycorrhizal fungi species abundance and the structure of the mycorrhizal fungal communities. I found that phosphorus demand had a strong effect on mycorrhizal communities and stimulated mycorrhizal species more efficient to release phosphorus from organic molecules and soil minerals.

Boletus badius is one of the species that increased in abundance during phosphorus demand conditions. Previous studies showed that *Boletus badius* has the ability to take up and storage high amounts of phosphorus from soils.

Moreover, it thrives next to phosphorus rich mineral sources especially in high phosphorus demand conditions suggesting that it has the ability to release phosphorus from minerals.

In a laboratory experiment I showed that *Paxillus involutus* (another species previously reported to thrive under P demand conditions) had the ability to utilize phosphorus from different compounds that form part of the soil phosphorus pool. Furthermore, I demonstrated the elegant chemical mechanisms that this species has to extract phosphorus from soil minerals and ores. It can produce molecules called organic acids that have strong affinity for mineral surfaces and when released they bind the mineral and displace phosphorus, which can be taken up by the fungus. Moreover, *Paxillus involutus* has the ability to produce molecules that donate electrons to the iron present in certain minerals bound to phosphorus. When iron gains this electrons it gets reduced and phosphorus is released and becomes available for plant and fungi consumption.

In conclusion, mycorrhizal growth in forests is a dynamic process regulated by nitrogen and phosphorus availability. These dynamic processes are very important for carbon delivery and storage in the soils. Mycorrhizal community composition assemblage is also dependent on the nutrient status of the trees, and mycorrhizal species adapted to take up phosphorus from different sources can be important to help trees endure phosphorus deficiency.

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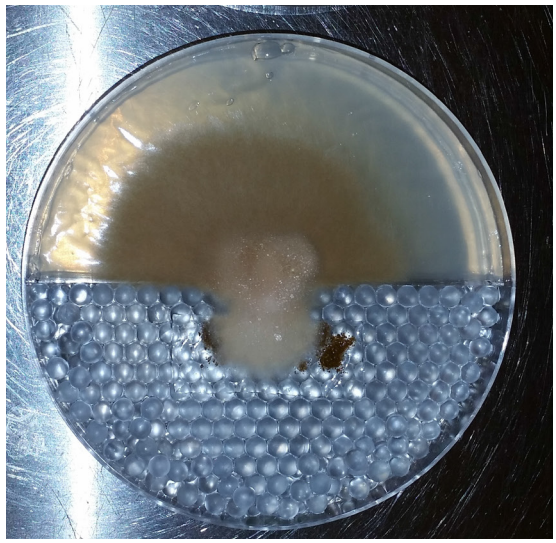

List of papers

I. Nicolás, C., Almeida, J. P., Ellström, M., Bahr, A., Bone, S. E., Rosenstock, N. P., Bargar, J.R., Tunlid, A., Persson, P., & Wallander, H. (2017). Chemical changes in organic matter after fungal colonization in a nitrogen fertilized and unfertilized Norway spruce forest. *Plant and Soil*, *419*(1-2), 113-126.

II. Almeida, J. P., Rosenstock, N. P., Forsmark, B., Bergh, J., & Wallander, H. (2018). Ectomycorrhizal community composition and function in a spruce forest transitioning between nitrogen and phosphorus limitation. *Fungal Ecology*. In press: doi.org/10.1016/j.funeco.2018.05.008

III. Almeida, J.P., Ekblad, A., Rosenstock, N., & Wallander, H. Turnover and production of ectomycorrhizal mycelia in an unfertilized and phosphorus fertilized Norway spruce forest (manuscript).

IV. Almeida, J.P, Tunlid, A., Persson, P., Johansson, T., & Wallander, H. Phosphorus uptake from poorly soluble phosphorus sources by *Paxillus involutus* in relation to iron reducing capacity and phosphorus starvation. (Manuscript).

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