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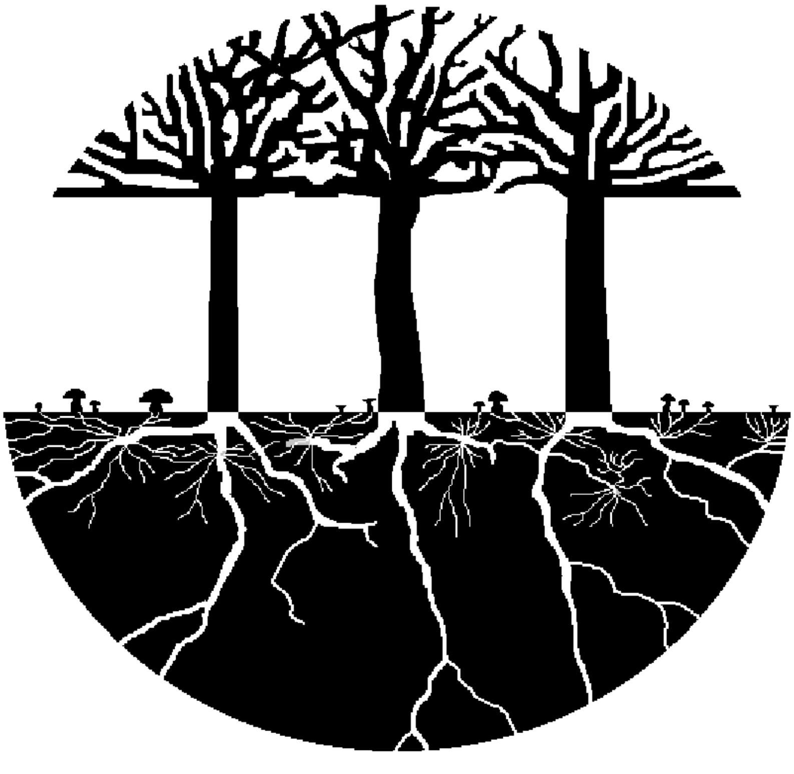

Nils Henriksson

Sveriges Lantbruksuniversitet — Jägmästarprogrammet
Examensarbete i markvetenskap, 30 hp, avancerad nivå A2E — ISSN 1654-1898
Handledare: Torgny Näsholm, SLU, Inst för skogens ekologi och skötsel
Examinator: Tord Magnusson, SLU, Inst för skogens ekologi och skötsel — Umeå 2012

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Uppskattning av N-överföringseffektiviteten hos mykorrhiza genom att mäta N-upptag och transport i Pinus sylvestris

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Nyckelord / *Keywords:*
Nitrogen, xylem sap, water transport, mycorrhiza, transfer efficiency, Pinus sylvestris

ISSN 1654-1898 Umeå 2012

Sveriges Lantbruksuniversitet / *Swedish University of Agricultural Sciences*
Fakulteten för skogsvetenskap / *Faculty of Forest Sciences*
Jägmästarprogrammet / *Master of Science in Forestry*
Examensarbete i markvetenskap / *Master thesis in Soil Science*
EX0177, 30 hp, avancerad nivå/ *advanced level* A2E

Handledare / *Supervisor*: Torgny Näsholm
SLU, Inst för skogens ekologi och skötsel / *SLU, Dept of Forest Ecology and Management*
Examinator / *Examiner:* Tord Magnusson
SLU, Inst för skogens ekologi och skötsel / *SLU, Dept of Forest Ecology and Management*

Contents

Abstract

In a large-scale field experiment, single trees in a Scots pine stand in Northern Sweden were subjected to different amounts of nitrate fertilizer containing the tracer isotope ^{15}N. This was done on two plots, one control plot and one that had been fertilized during the previous 6 seasons, all trees were about 90 years old. Measurements of needle nitrogen (N) content and delta ^{15}N abundance and analyses of N-forms in the xylem sap were conducted. Also, sap flux density was measured with Granier-type sensors, enabling assessment of when added N may have reached the canopy. The hypotheses were 1) that the ectomycorrhizal N transfer efficiency to the plant partner is enhanced by conditions of high soil N availability; and 2) that N transport through the trees' xylem is shifted to compounds with lower C/N ratio when the N availability is increased. The results supported the first hypothesis and gave some support to the second. Sap flux measurements indicated a surprisingly slow water transport in the xylem, suggesting that the added N reached the upper canopy four weeks after fertilization. Xylem sap nitrate concentrations exhibited a peak four weeks after fertilizer addition, corroborating the slow xylem transport rates assessed with the Granier elements.

Key words: nitrogen, xylem sap, water transport, mycorrhiza, transfer efficiency, Pinus sylvestris

Introduction

Background

The growth of trees in boreal forests, such as those of Northern Sweden, is generally extremely N limited (Tamm, 1991). Nitrogen is not a constituent of common minerals and so must be fixed from the atmosphere by those organisms capable of doing so. These are bacteria and so other organisms like plants and fungi, barring those that exist symbiotically with N-fixing bacteria, must receive all their N from decomposed organic matter in the soil or from added N fertilizers or via N deposition. Soil organisms break down organic tissues for carbon, nutrients and energy. Much of the N present in soils is *immobilized* by soil microbes which have incorporated it into tissues that take a very long time to break down. Since their own tissue has a fixed C/N ratio, they require a certain amount of N for every carbon they absorb (Brady & Weil, 2002). If the material these organisms are degrading has a lower C/N ratio than they require, then the N that they do not incorporate is mineralized and becomes available to other organisms (Brady & Weil, 2002). The slow rate of this mineralization is the presumed reason why boreal systems are generally limited by N availability (Näsholm et al., 1998). Plant roots take up N in the forms of nitrate, ammonium and free amino acids (Näsholm et al., 1998; Öhlund & Näsholm, 2001). Recent evidence also suggest complex N in the form of proteins and even intact microorganisms may act as N sources for plants (Paungfoo-Lonhienne et al., 2008; 2010).

Many plants form symbiosis with fungi - mycorrhiza - and a wealth of evidence suggests such symbioses can alleviate plant N-limitation. At the same time it has been shown again and again that plants in boreal forests are generally N-limited. Considering that around 95 percent of fine tree-roots in a boreal forest tend to be infected by mycorrhizal fungi (Taylor et al., 2000), how does this fit together with the traditional view of the obliging mycorrhizal fungi?

Brief history of the discovery of mycorrhizal fungi

Mycorrhizal symbioses play an important role in all major terrestrial biomes (Smith & Read, 2008). They have been suggested as drivers of ecosystem processes in boreal forests (Read et al., 2004) and have interested scientists for more than a century (Jones & Smith, 2004; Trappe & Berch, 1985). Plant diversity in the boreal forest is small in comparison to temperate biomes, and this is especially true when it comes to tree species, which is dominated by the family Pinaceae. An estimated 6000 fungal species are likely to be capable of forming ectomycorrhiza. These are mostly basidiomycetous, but also include ascomycetous fungi (Read et al., 2004). However this includes more than just the species found in boreal forest ecosystems. The number of ectomycorrhizal fungi inhabiting the boreal, Pinaceae-dominated, forests is much smaller. For instance, in their study, Toljander et al., (2006) took soil samples along a 90 meter transect in a boreal forest and estimated the species richness of ectomycorrhizal fungi to be between 110 and 150. They reason that the estimate is probably low. They also stated that the species composition changed depending on soil conditions and vegetation.

The prevailing view concerning the functional characteristics of mycorrhiza has changed over the decades as a result of more studies being conducted and new observations made. For instance, the Hartig net, a feature of ectomycorrhizal infection, was described as early as 1840, by Theodor Hartig. At the time he did not recognize that it was fungal, believing that it was a part of the roots' own physiology (Trappe & Berch, 1985). In 1885, A. B. Frank wrote that certain trees regularly formed a "symbiosis with a fungal mycelium", and further that "this mycelium…takes over the entire nourishing of the tree from the soil". However, many researchers at the time did not believe this and several were convinced that the fungi were pathological or only sporadically occurring structures (Trappe & Berch, 1985). By the late 1930's most workers had become convinced that mycorrhizal seedlings grew faster in moderately nutrient-deficient soils (Harley, 1985), and by the 1990's most researchers in the field accepted as a matter of course that mycorrhizal associations were mutualistic (Jones & Smith, 2004), that is mutually beneficial to both partners. Today it is generally recognized that mycorrhiza can indeed have negative, as well as positive effects on the host plant, depending on the balance between fungal demand for energy and the plant's need for nutrients (Jones & Smith, 2004).

The ectomycorrhizal symbiosis

Ectomycorrhizal fungi are widely recognized as playing a critical part in boreal ecosystems, even to the extent of suggesting them as a driver of ecosystem processes in boreal forests. By occupying the zone within the soil where, in their absence, the roots of their host plants would have produced root hairs they are well placed to influence carbon/nutrient exchanges between pools in the system (Read et al., 2004). The hyphae of ectomycorrhiza penetrate between the root's epidermial and cortical cells, forming a network called the *Hartig net*. The fungus also envelopes the absorbing root tip in a *sheath* or *mantle*. Finally the fungus puts forth what is called the extraradical mycelium, the outwardly expanding system of hyphae which constitutes the interface with the soil and the main nutrient-absorbing area of the fungus.

The fungal mycelia can stretch beyond the depletion zone that can surround the roots and so reach nutrients that may not otherwise have been available to the roots. Also, the fact that the diameter of the hyphae (on average <5 µm, according to Staddon et al., 2003) is so much smaller than that of the fine roots (defined as <1 mm by Persson (1978), and as <2 mm by Vanninen & Mäkelä (1999)) means that the fungus can explore the soil further per unit biomass, making it a more cost-efficient alternative. This also increases the absorbing area per unit biomass. Additionally, Corrêa et al., (2008) showed that ectomycorrhizal colonization can affect the nutrient uptake of the plant by means not dependent on overcoming the spatial limitations of a non-mycorrhizal root system. From this, it seems clear that the plant would benefit from associating with mycorrhizal fungi, but as mentioned above, the net effect on the plant can sometimes be negative for the plant. Predicting when this will be the case is difficult because of conflicting theories concerning the mechanisms governing the balance between fungal energy-demand and plant nutrient-requirement.

Thus it is not always clear exactly what functional role this association occupies under different conditions of nutrient availability. Rather it is often assumed that the symbiosis is mutualistic. But considering that nutrients absorbed by ectomycorrhizal roots must pass through the fungi before reaching the host plant, one might reason that the mycorrhizal association would not be profitable to the host plant until N availability is sufficient for the persistence or growth of the fungal partner. Indeed, there are studies that have shown negative effects on plant growth during conditions of low N availability (Colpaert et al., 1996, Corrêa et al., 2008). Therefore it is not always obvious how to classify mycorrhiza along the mutualism-parasitism spectrum. Egger and Hibbet (2004) suggest that "reciprocal parasitism" may be a more fitting description of the characteristics of mycorrhizal symbioses. Several published papers suggest more varied view of the functions of mycorrhizal associations (Johnson et al., 1997; Jones & Smith, 2004; Egger & Hibbet, 2004). It is important to recognize that mycorrhizae are part of a complex system, and aim for the appropriate scale when studying mycorrhizal function; the larger the scale of interest, the more influence external biotic and abiotic factors can have through indirect interactions (Johnson et al., 1997).

Nitrogen assimilation and transport in within the tree

When N enters the root it can either be in one of the inorganic forms of nitrate or ammonium, or it can be in the form of free amino acids. If it is taken up as nitrate, it will be reduced to ammonium before being attached to a carbon skeleton to synthesize amino acids (Lambers et al., 1998). This is called nitrogen assimilation and carries a large carbon cost.

Once N has been reduced to ammonium it is converted to the amino acid glutamine, after which it undergoes transamination reactions to form other amino acids and is incorporated into larger organic compounds. Thus the actual N-uptake cost is higher for nitrate-N than amino-N, though additional factors shrink this difference in carbon cost (Zerihun et al., 1998).

Transport of N in the xylem may occur as either amino acids or nitrate while ammonium is seldom found in significant concentrations in the sap. The amount of N present as nitrate in the xylem is related to the location in the plant where nitrate reduction takes place, and can differ between species. Nitrate reduction may take place directly in the roots, or the it can be shipped through the xylem to the foliage and be reduced there. According to Smirnoff et al., (1984) the latter is more likely for most plants, except under conditions of limited nitrate supply. There are also suggestions that the capacity for nitrate reduction in the roots may be limited, because all species increase the proportion of nitrate reduced in shoots as the nitrate supply increases (Andrews, 1986). Since severe N limitation is the natural condition in boreal forests, it is probable that most of the absorbed nitrate is reduced in the roots. However Smirnoff et al., (1984) noted a high inducibility of nitrate reducing enzymes in the foliage of species of the genus *Pinus*, remarking that it merited further research.

In woody plants in general, and conifers in particular, amino acids dominate the xylem N transport. According to the findings of Nordin et al., (2001), amino N is present in the wood of *Pinus sylvestris* mostly as glutamine. They also reported that arginine made up an important constituent of the N in the xylem sap, especially in fertilized trees. It has also been shown for *P. sylvestris* and *Picea abies* that excess uptake of N leads to accumulation of N in the form of arginine (Näsholm and Ericson 1990; Stoermer et al., 1997) in the needles but the extent to which this needle-N accumulation results in increased rates of arginine transport in the xylem sap, or if it s caused by de novo synthesis in needles is not known. Plants may use different compounds for N translocation from roots to shoots as a result of altered C/N balance (Corruzzi & Last, 2000) Thus, one may speculate that trees exposed to high rates of N fertilizers may increasingly use e.g. arginine for N translocation as this amino acid has a C/N ratio of 1.5, compared to 2.5 for glutamine. Conifers, in general, exhibit a root-based N assimilation. This suggests that any absorbed nitrate may be reduced in the roots and nitrate in xylem sap may not be detectable.

The response to nitrate availability of shifting reduction to the roots or leaves is a useful tool for measuring the nitrate availability, for instance after fertilization. The concentration of glutamine in the xylem sap may also be measured to give a general picture of how much inorganic N is being assimilated.

Aims and Hypothesis

The goal of this study is to investigate the extent to which absorbed N is retained in the fungi (*mycorrhizal transfer efficiency*) in a boreal forest at long-term and short-term conditions of low and high N availability. By measuring sap flow, total N and ^{15}N ratio of foliage and analyzing the organic and inorganic composition of N-compounds in the xylem sap, estimations will be made of the amount of applied N-fertilizer that reaches the canopy, and how long this transport takes.
The working hypotheses of this study are 1) that the ectomycorrhizal N transfer efficiency to the plant partner is enhanced under conditions of high soil N availability; and 2) that N transport through the trees' xylem is shifted to compounds with lower C/N ratio when the N availability is increased.

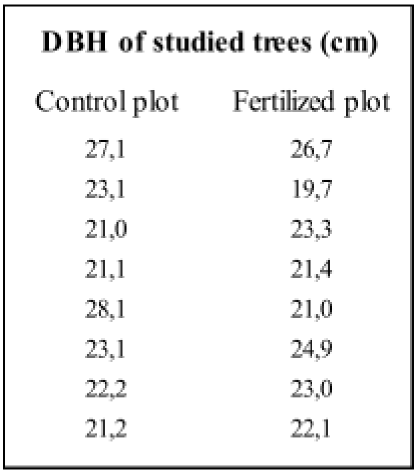
Methods

Study Area

The study area is a Scots pine forest in Northern Sweden, located close to the town of Vindeln (lat 64.2, 19.7). The stand is even-aged and ca 90 years old, growing on deep sandy sediment. Two plots, separated by a couple of kilometers, were used; one previously untreated stand, and one that had been fertilized for the previous 6 years at a rate of 100 kg N ha^{-1} y^{-1}. The ground vegetation of the previously untreated plot consisted of lichens and ericaceous dwarf shrubs, mostly *Vaccinium vitis-ideae* and *Calluna vulgaris*; on the previously fertilized plot the ground was covered mainly with *V.myrtillus* and *V. vitis-ideae.*

Experiment layout

In this tracer study, 16 trees were chosen; 8 growing on the fertilized plot and 8 growing on a control plot. Most trees had a diameter at breast height of 20-25 cm, and were around 20 meters tall. On each plot, 4 of the trees received 90 liters of water and the other 4 received 90 liters of 10 mM $Ca(NO_3)_2$ solution. All trees also received 33.75 mg of the tracer isotope ^{15}N, in the form of a 25 µM solution of $Na^{15}NO_3$. The application was executed on the morning of the 17th of August, 2011, and the treated area was 9 m^2 surrounding the base of the tree. The treatment consisting of only water and the labeled isotope is designated *0+label*; and the treatment including calcium nitrate is referred to as *N+label.*

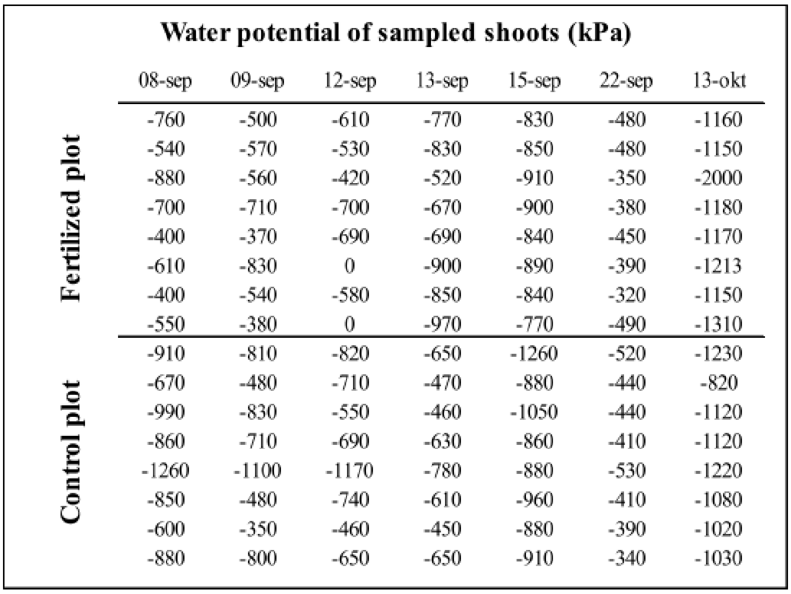


DBH of studied trees (cm)

Control plot	Fertilized plot
27,1	26,7
23,1	19,7
21,0	23,3
21,1	21,4
28,1	21,0
23,1	24,9
22,2	23,0
21,2	22,1

Sampling

Using pole-scissors, leading shoots were repeatedly collected from the upper 50 percent of the canopy of all 16 trees on 9 separate occasions during weeks 2, 3, 4, 5, and 8 after treatment. The shoots were sealed in air-tight bags and taken to the lab, where they were placed in a pressure chamber to extract samples of the xylem fluid. A pressure of 300 kPa above the equilibrium pressure (eq. ranged between approximately -400 kPa and -1200 kPa) in the shoot was applied. The escaping water was collected in Eppendorf tubes and frozen. This means that the absolute pressure applied was not always the same from shoot to shoot, but it was constant in relation to the water potential of the tree at the time of sampling.



Water potential of sampled shoots (kPa)

	08-sep	09-sep	12-sep	13-sep	15-sep	22-sep	13-okt
Fertilized plot	-760	-500	-610	-770	-830	-480	-1160
	-540	-570	-530	-830	-850	-480	-1150
	-880	-560	-420	-520	-910	-350	-2000
	-700	-710	-700	-670	-900	-380	-1180
	-400	-370	-690	-690	-840	-450	-1170
	-610	-830	0	-900	-890	-390	-1213
	-400	-540	-580	-850	-840	-320	-1150
	-550	-380	0	-970	-770	-490	-1310
Control plot	-910	-810	-820	-650	-1260	-520	-1230
	-670	-480	-710	-470	-880	-440	-820
	-990	-830	-550	-460	-1050	-440	-1120
	-860	-710	-690	-630	-860	-410	-1120
	-1260	-1100	-1170	-780	-880	-530	-1220
	-850	-480	-740	-610	-960	-410	-1080
	-600	-350	-460	-450	-880	-390	-1020
	-880	-800	-650	-650	-910	-340	-1030

Subsequently current-year needles were collected from the shoots and dried at 60 degrees Centigrade and ground. Sap flux density was measured on all 16 trees with Granier-type sensors (Granier, 1987). The sensors were installed in the outer 20 mm of the sapwood on the north side of the trees to minimize the effects of solar radiation on the sensors.

Analyses

Through isotope ratio mass spectrometry, the needles were analyzed to investigate whether they had received any of the ^{15}N. Analysis of the needle tissue also included the total N content, as percent of dry weight. Chromatographic analysis of the extracted xylem fluid was performed in order to examine the amino acid composition. The xylem fluid was also analyzed for NO_3-content employing a spectrophotometric method described in Miranda et al., (2001), as modified by Hood-Nowotny et al., (2010).

Calculations

The diameter under bark and sapwood thickness of the studied trees were measured and used to calculate sapwood area at breast height. Five trees from each plot were felled and their needle mass was calculated from sample branches and sapwood area below the living crown was measured. These were then averaged to get an estimated needle mass and sapwood area below the crown of the trees used in the current study.

A *crown base/breast height* ratio of sapwood area was calculated (A_{cb}:A_{bh}). Finally, since the flow rate in the xylem diminishes closer to the heartwood and the sensors only measured sap flow in the outer xylem, the data was modified using data from a parallel study (Zhang et al., unpublished data). With the sap flow and the sapwood area at breast height known it was possible to calculate the amount of water (kg) that had passed the measuring point since the time of treatment. The speed at which water reached the crown was calculated by dividing the flow rate at breast height by the ratio A_{cb}:A_{bh}.

Though the xylem-sap concentrations of all amino acids were analyzed, only arginine and glutamine were used and presented. This was because glutamine nearly always stood for 80 – 96 percent of the total concentration of amino-N. Arginine was included because previous studies suggest that excess N taken up may be stock-piled in the form of arginine (Nordin et al., 2001).

Results

1. *Water, nitrate and amino-N transport in the xylem*

Approximately four weeks after treatment (26-27 days), increased concentrations of nitrate were found in the xylem sap of all trees (Figure **1**). Calculations of sap-flow predicted that water taken up at the time of treatment would during this period of time have travelled around 13 meters (Figure **1**). This was roughly the height of sampling in the trees' foliage.

On the fertilized plot, this nitrate pulse was followed by an increase in the concentration of arginine in the xylem sap (Table **1**; Figure **2**).

Based on these results, samples taken before the time when the nitrate pulse appeared (September 12th) are henceforth treated as not having been influenced by treatment. This time point will from now on be referred to as the *treatment arrival*, or *N arrival in the foliage* (Figure **1**).

The total amount of amino-N in the xylem sap before treatment arrival was on average approximately twice as high in trees growing on the fertilized plot as it was in trees from the control plot, and about three times higher after the treatment arrival (Table **1**). On the fertilized plot about 94 % of this N was on average represented by glutamine before the pulse, and it decreased to about 92 % after the nitrate pulse in both 0+label and N+label trees. At the same time the proportion of amino-N present as arginine increased from about 1,7 % to 4,5 % in the 0+label trees and from about 1,5 to 2,7 in the N+label trees (Table 1). The same pattern was visible in the N+label trees on the control plot (a 123 % increase in arginine-N), but it did not occur in the 0+label trees on the control plot. In general there was less variation over time of the amino-N composition of trees on the control plot (Figure **2**).

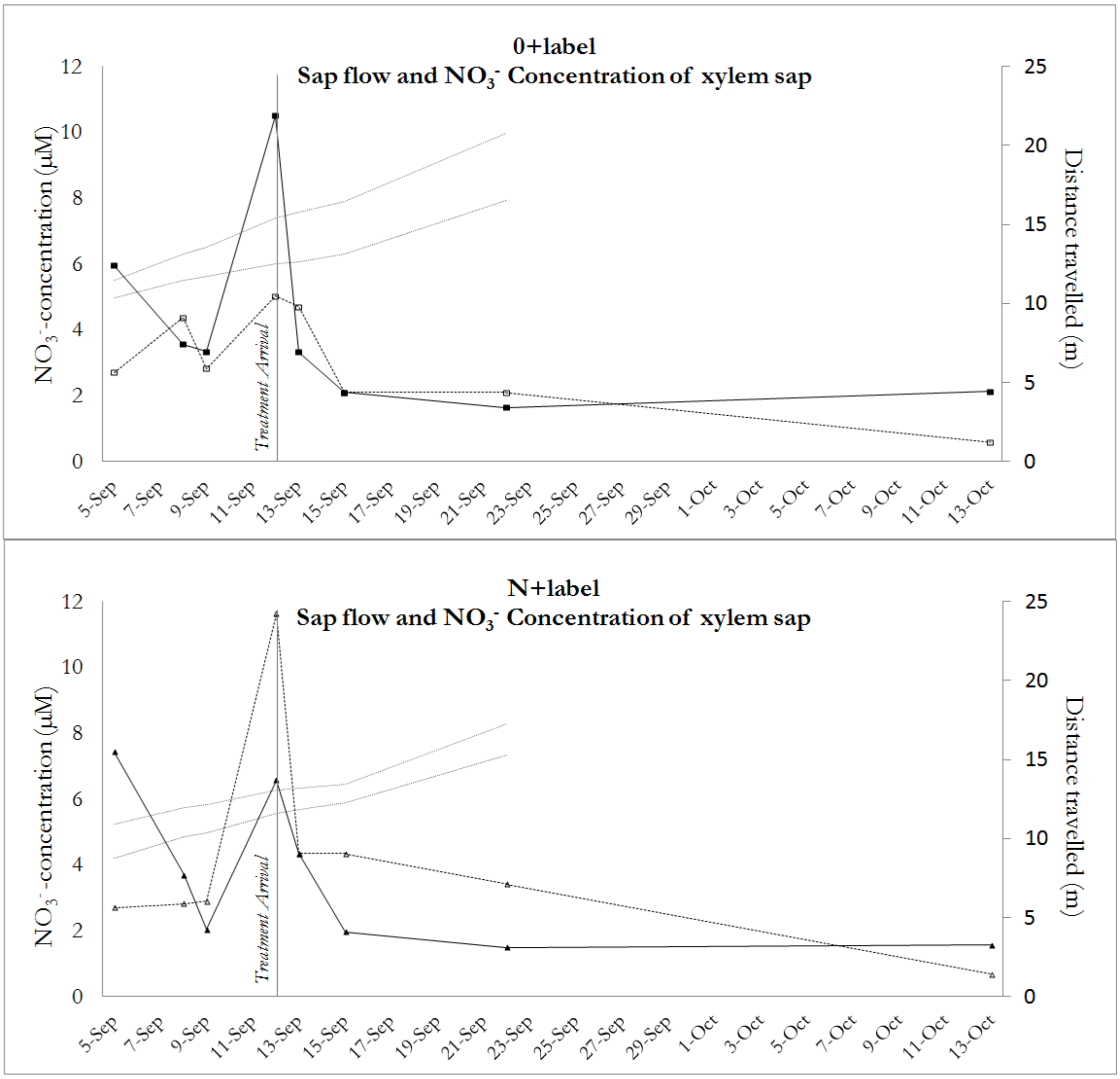
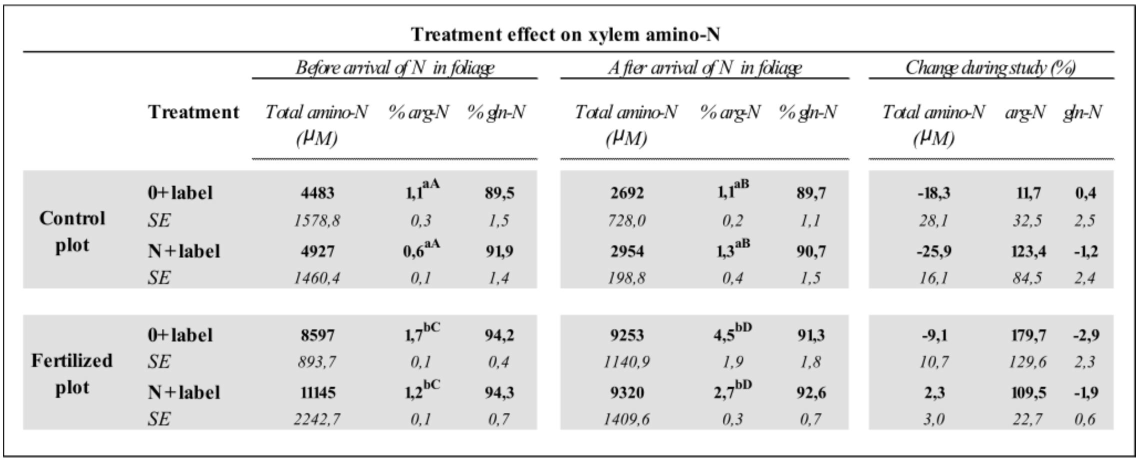


Figure 1 The average NO_3^- concentration over time in the xylem of sampled current-year shoots in the upper half of the canopy. The top chart gives the values for 0+label trees and the bottom chart gives the values for N+label trees. In both charts the dashed lines represent trees on the control plot, and solid lines represent the fertilized plot. The dotted lines show the average calculated distance (in meters, secondary axis) travelled by water through the xylem. The vertical line marks the time point where the nitrate peak appears, referred to as the *treatment arrival*.

Table 1. The amino-N composition of the xylem sap before and after the appearance of the nitrate pulse. Values are treatment-averages for both the control plot and the fertilized plot. The total amino-N concentration (µM) and the percentage of this N to be found in arginine and glutamine are presented. The change in these parameters are presented in percent increase or decrease compared to values measured before the nitrate pulse appeared. Below each average is given the corresponding standard error. An ANOVA test revealed a significant difference in % arginine between the control and fertilized plots, and also between % arginine before and after N arrival within each plot ($p \leq 0.05$). Different lower-case letters indicate significant differences between plots while different upper-case letters indicate significant differences between time-points. There was no significant difference between the treatments.



Treatment effect on xylem amino-N

		Before arrival of N in foliage			*After arrival of N in foliage*			*Change during study (%)*		
	Treatment	*Total amino-N (µM)*	*% arg-N*	*% gln-N*	*Total amino-N (µM)*	*% arg-N*	*% gln-N*	*Total amino-N (µM)*	*arg-N*	*gln-N*
Control plot	**0+label**	**4483**	**1,1^{aA}**	**89,5**	**2692**	**1,1^{aB}**	**89,7**	**-18,3**	**11,7**	**0,4**
	SE	*1578,8*	*0,3*	*1,5*	*728,0*	*0,2*	*1,1*	*28,1*	*32,5*	*2,5*
	N+label	**4927**	**0,6^{aA}**	**91,9**	**2954**	**1,3^{aB}**	**90,7**	**-25,9**	**123,4**	**-1,2**
	SE	*1460,4*	*0,1*	*1,4*	*198,8*	*0,4*	*1,5*	*16,1*	*84,5*	*2,4*
Fertilized plot	**0+label**	**8597**	**1,7^{bC}**	**94,2**	**9253**	**4,5^{bD}**	**91,3**	**-9,1**	**179,7**	**-2,9**
	SE	*893,7*	*0,1*	*0,4*	*1140,9*	*1,9*	*1,8*	*10,7*	*129,6*	*2,3*
	N+label	**11145**	**1,2^{bC}**	**94,3**	**9320**	**2,7^{bD}**	**92,6**	**2,3**	**109,5**	**-1,9**
	SE	*2242,7*	*0,1*	*0,7*	*1409,6*	*0,3*	*0,7*	*3,0*	*22,7*	*0,6*

N forms in xylem sap

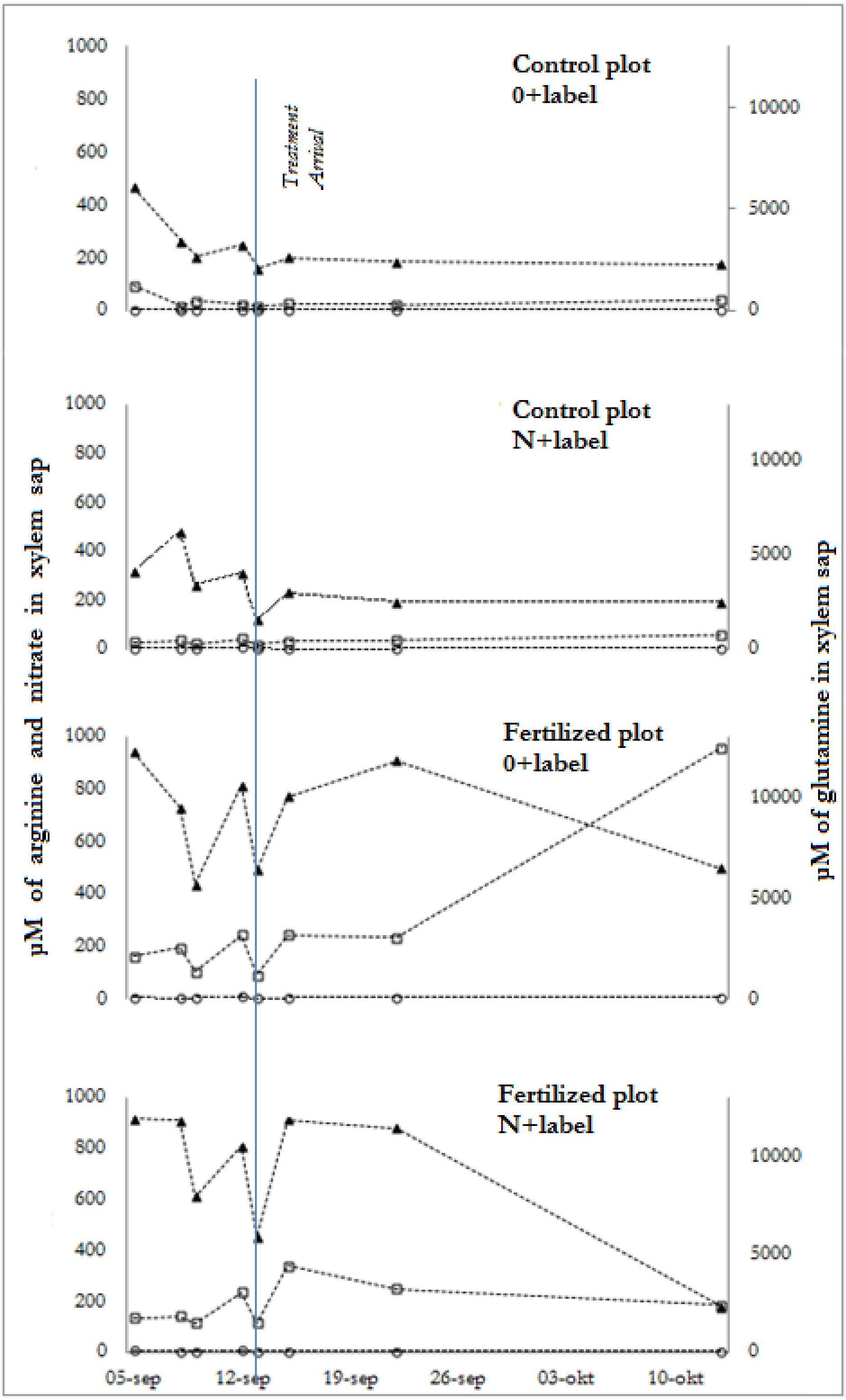
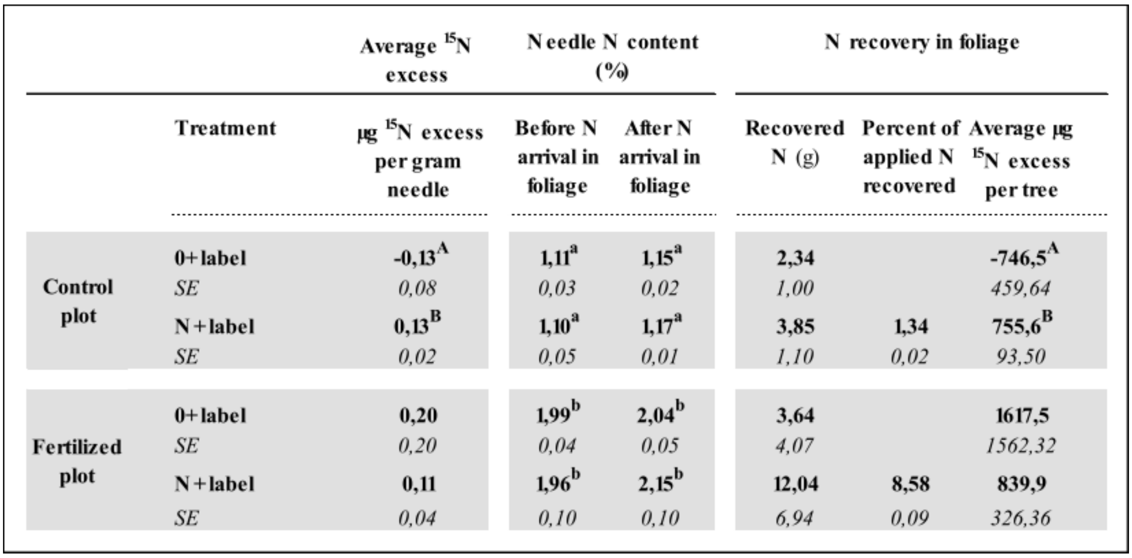


Figure 2 The four charts show the concentrations of N compounds in the forms of arginine (open squares) and glutamine (filled triangles) and nitrate (open circles) in the xylem sap at each sampling event throughout the study. In all charts, the glutamine concentration corresponds to the right-hand y-axis. The vertical line marks the treatment arrival.

2. *Foliage N content and ^{15}N-labeling*

The calculated amount of N fertilizer that was recovered in the trees' foliage was on average more than six times higher on the fertilized plot, but there was a large variation between individual trees (Table 2). On the control plot, the absorbed proportion was lower but more even between trees. The total N content of needles (% dry weight) was higher on the fertilized plot than on the control plot (Table 2). This was true irrespective of what treatment the trees received during the current study. The same pattern was found for the increase of ^{15}N content in the needles (Table **2**).

Table 2 The average needle N content of 0+label and N+label trees in percent of dry weight before and after treatment arrival. Excess ^{15}N values are shown first in µg per g needle, and also in µg per tree to give an idea of the total amount taken up by the trees. An ANOVA test shows that ^{15}N excess is significantly higher after N arrival in N+label trees on the control plot, and also the needle N content between plots ($p \leq 0.05$). Different lower case letters designate a significant difference between plots, and different upper case letters indicate a significant difference between treatments.



		Average ^{15}N excess	Needle N content (%)		N recovery in foliage		
	Treatment	µg ^{15}N excess per gram needle	Before N arrival in foliage	After N arrival in foliage	Recovered N (g)	Percent of applied N recovered	Average µg ^{15}N excess per tree
Control plot	**0+label**	**-0,13^{A}**	**1,11^{a}**	**1,15^{a}**	**2,34**		**-746,5^{A}**
	SE	*0,08*	*0,03*	*0,02*	*1,00*		*459,64*
	N+label	**0,13^{B}**	**1,10^{a}**	**1,17^{a}**	**3,85**	**1,34**	**755,6^{B}**
	SE	*0,02*	*0,05*	*0,01*	*1,10*	*0,02*	*93,50*
Fertilized plot	**0+label**	**0,20**	**1,99^{b}**	**2,04^{b}**	**3,64**		**1617,5**
	SE	*0,20*	*0,04*	*0,05*	*4,07*		*1562,32*
	N+label	**0,11**	**1,96^{b}**	**2,15^{b}**	**12,04**	**8,58**	**839,9**
	SE	*0,04*	*0,10*	*0,10*	*6,94*	*0,09*	*326,36*

Discussion

The hypotheses of this study were: 1) that the ectomycorrhizal N transfer efficiency to the tree is enhanced by heightened soil N availability; and 2) that N transport through the trees' xylem is shifted to compounds with lower C/N ratio when the N availability is increased. The results of the total N and ^{15}N analyses of needles do not allow the hypothesis concerning mycorrhizal transfer efficiency to be dismissed. Taken together with results from a parallel study (discussed below) the results support hypothesis 1. The results from the xylem sap analyses showed changes in amino-N composition of the xylem sap as a result of heightened N availability, showing that a change is indeed induced by heightened N availability. This lends some support to hypothesis 2.
Finally, the results indicated that the water transport in the xylem is significantly slower than expected.

Water transport
The time at which the nitrate pulse was detected in the xylem sap was the same irrespective of plot or treatment, with the exception that three of the four 0+label trees on the control plot received it one day after the rest of the trees. This indicates an unexpectedly slow transport rate of N, taking

approximately four weeks to reach the upper canopy (approximately 13 meters). Assuming passive transport, the fact that the timing of the nitrate pulse coincided with the calculated time it should have taken the water to reach the approximate sampling height suggested that the nitrate in the pulse was taken up at the time of treatment. Based on this information, the date at which the nitrate pulse was detected was treated as the point in time when the applied water (and applied N) reached the part of the crown where sampling took place.

Xylem amino-N composition and N recovery in the foliage

After receiving the treatment, the trees altered the amino-N composition in their xylem in favor of arginine, more than doubling the proportion of amino-N transported in this form. That the same shift was visible in the N+label trees on the control plot (an 123 % increase in arginine-N), but did not occur in the 0+label trees, indicated that it was not an effect of long-term high N availability. Rather the increase in arginine-N was induced by the experiment's single sudden event of increased soil N availability. These findings thus support the second hypothesis of this study – *that N transport through the trees' xylem is shifted to compounds with lower C/N ratio when the N availability is increased.*
The calculated amount of N fertilizer that found its way into the trees' foliage was on average six times higher on the fertilized plot (0 % - 20 % recovery of applied N), but there was large variation between individual trees. On the control plot, the absorbed proportion was lower (0.75% - 4.5% recovery) but more even between trees (Table **2**). The recovery of applied N in the trees on both plots was within the expected range, as reported by Melin et al., (1983). On average the increase in needle N content was higher on the fertilized plot than on the control plot, and higher for N+label trees than for 0+label trees, irrespective of which plot they grew on.
So far the results presented here merely show how much N was taken up during the studied period, but the tracer isotope data helps shed some light on how much of this N was actually from the N applied in the beginning of the experiment. However, the excess values of ^{15}N were very small, the highest being 0.74 µg per gram needle (Table 2) corresponding to a shift in ∂^{15}N of about 0.8. On the control plot, the values of 0+label trees were very close to zero µg/g, while the N+label trees had somewhat higher values. On the fertilized plot the difference between treatments was smaller and reversed. However this was affected by one 0+label tree which had a very high excess value (0.74 µg per gram needle), and if this value had been excluded then the excess value of ^{15}N would be higher for N+label trees on the fertilized plot as well. This suggests that the uptake of the applied N was higher on the fertilized plot than on the control plot, and higher in N+label trees than 0+label trees. Especially on the control plot, the ^{15}N uptake pattern, while somewhat diffuse, agreed with what was shown for total needle N-content, pointing toward the conclusion that more of the applied N was taken up by the N+label trees than by the 0+label trees.

Ectomycorrhizal transfer efficiency

In a parallel study, utilizing the same labeling experiment, ectomycorrhizal sporocarps were collected around the treated trees on the control plot (Hasselquist, *unpublished data*), and analyzed for ^{15}N abundance. The fertilized plot was more or less devoid of sporocarps. When compared to the natural abundance of ^{15}N in each species (Taylor, et al., 1997; Hasselquist, *unpublished data*), the collected fruit bodies were found to have increased their ^{15}N abundance during the study. Furthermore, the sporocarps were found to have increased their ^{15}N abundance more if they grew on a 0+label plot (by approximately 5.9 δ-units' increase) than under a N+label tree (approximately 0.75 δ-units' increase). Together with the lower ^{15}N recovery in the trees on the control plot compared to the fertilized plot, this suggests that under N-limitation, ectomycorrhizal fungi retain much of the N they absorb, rather than transfer it to the trees.

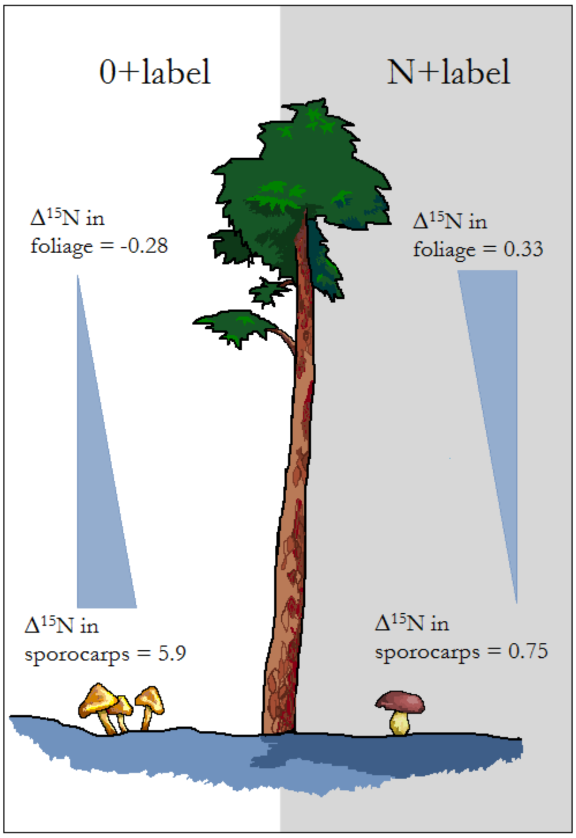


Figure 3 Schematic representation of the relationship between average $\Delta^{15}N$ of ectomycorrhizal sporocarps and foliage on the control plot.

In summary, less of the applied N was recovered in the foliage of 0+label trees than in N+label trees, while the $\Delta^{15}N$ of the collected sporocarps hint that more N was immobilized in the mycorrhizal fungi attached to 0+label trees. This supports the first hypothesis of this study - *that the ectomycorrhizal N transfer efficiency to the plant partner is enhanced by conditions of high soil N availability*. Too few sporocarps were found on the fertilized plot to make the same inference there, but the N uptake pattern of the trees was the same as on the control plot. This at least gives no reason to discard the hypothesis concerning mycorrhizal transfer efficiency.

There are several previous studies dealing with the question of how to classify the functional role of mycorrhizal associations (Johnson et al., 1997; Egger & Hibbet, 2004; Corrêa et al., 2008). The scientific community has gone from conflicting opinions of the functional role of mycorrhiza, to viewing it as almost exclusively beneficial to the plant, to recognizing that the picture is more complicated than this. In their study, Corrêa et al., (2008) suggest that mycelial growth of the mycorrhizal fungi could be an important N sink to the plant, in the sense that it increases the N requirement of the fungi, lowering transfer efficiency. Further, their results indicate that the plant continues to provide carbon to the fungus even as the N supplied by it becomes increasingly less. Thus the tree increases its carbon allocation to the roots in response to the increased N *availability in the soil* rather than to increased N *gain by the plant*. It is thus recognized that mycorrhiza can indeed have negative, as well as positive effects on the host plant, depending on the balance between the fungal demand for energy and the plant's need for nutrients.

The most common way of approaching this question is by a cost-benefit analysis, in which the benefit is represented by heightened capacity for uptake of soil nutrients, and the cost is in terms of photosynthetic carbon allocated to mycorrhizal roots. In such analyses, plant fitness is often used as a measure of cost/benefit, but plant fitness can be a complicated concept in natural systems since it is defined at an individual level (Johnson et al., 1997), and does not take into account interactions between species and between individuals within a species. In their paper, Johnson et al., (1997) stress the importance of acknowledging the potential of interactions at population, community and ecosystem levels to mediate plant fitness and suggest that the great challenge when assessing plant responses to mycorrhizal formation is to focus on a scale that is appropriate to the question of interest. It is also important to remember that carbon allocation to the fungi is only a cost to the plant if it could otherwise have been used to increase its fitness, conversely an increased gain of soil nutrients through the fungal pathway can only be considered a benefit if the nutrient is limiting to plant growth or development. In the current study, carbon transport to the roots was not measured and therefore no inference could be made concerning the carbon cost of mycorrhiza at different levels of N-availability. In other words, since only the "benefit" was measured, it was not possible to perform a cost/benefit analysis.

An alternative to the concept of benefit when studying the effects of mycorrhizal association is the term *mycorrhizal profit*, introduced by Corrêa et al., (2008), in order to have a more quantitative measure of the effects. Profit in this sense is a direct measure of the increase in value generated by employing a limited resource in one use as opposed to another, such as investing in mycorrhizal roots compared to non-mycorrhizal roots. This more quantitatively defined concept allows the profit of investing in mycorrhizal roots to be "calculated as the ratio between any given parameter of an individual mycorrhizal plant (m) relative to a mean value for nonmycorrhizal plants (NM) grown in the same experimental conditions: m/NM" (Corrêa et al., 2008). However this type of study would be difficult to perform in the field, since it is necessary to maintain nonmycorrhizal control trees.

The functional role of a mycorrhizal symbiosis is thus dependent upon any processes that affect the balance between the carbon sink-strength of the mycorrhizal fungi and the nutrient gain of the tree through the fungal pathway. Thus factors that may nudge the outcome of the symbiosis toward mutualism or parasitism include soil nutrient availability, photosynthetic efficiency of the host plant, mycorrhizal transfer efficiency, light conditions and fungal and plant genotypes (Johnson et al., 1997; Egger & Hibbet, 2004; Corrêa et al., 2008).

Early recommendations to foresters in Sweden suggested that the growth response of fertilized trees may not be linearly related to the amount of N applied, but rather having a breaking point where the response suddenly becomes greater. Based on the results of this study, and those of Corrêa et al., (2008), this could perhaps be explained by a change in mycorrhizal transfer efficiency. This might be tested by replicating the current experiment, but with more treatments, increasing by installments the rations of applied N, to find the point at which the fungal transfer efficiency increases. It would also be desirable to include a larger ration of ^{15}N tracer isotope in order to ensure more reliable results.

Conclusions

The results of this experiment indicate that for N-limited boreal forests it seems that adding a larger amount of N may increase the proportion that reaches the trees, and suggests, as an explanation of this, that mycorrhizal transfer efficiency is enhanced by increased soil N availability. It may thus be more accurate to think of mycorrhiza as being less beneficial under conditions of low nutrient availability, the benefit to the plant host becoming more obvious when the soil nutrient supply reaches levels above the immediate requirements of the fungal partner. However, the layout of the current experiment did not provide a specific "critical N availability" where this increased transfer efficiency occurred.

Further results also show that the speed at which water is transported through the xylem is unexpectedly slow (taking approximately 4 weeks to reach the canopy, about 13 meters). This has implications for planning fertilization measures, as well as being interesting from a scientific perspective.

Finally, the analysis of the N-composition of xylem sap showed that the total amount of amino-N was three times higher on the fertilized plot than on the control plot, but was not different between treatments. However, the proportion of amino-N present as arginine did increase slightly, and glutamine decreased by roughly the same amount, with added N on both plots.

Acknowledgements

Elin Norlin, Erich Inselsbacher, Niles Hasselquist, Ram Oren, Sari Palmroth, Yanqun Zhang, and supervisor Torgny Näsholm who all provided an inexperienced student with assistance.

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