Organic and inorganic nitrogen sources for conifer seedlings: abundance, uptake and growth

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Abstract

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Even though the capacity of plants to absorb organic N compounds has been known since the 1940.s it was not until recently that these N sources were recognized as a potentially important source of N for plants. Thus, the classical paradigm that plants only use inorganic N compounds and are forced to play a passive role acquiring N in excess of microbial demand is today questioned. However, despite the accumulating amounts of studies suggesting that plants have the capacity to absorb organic N at comparable rates to inorganic N the quantitative importance of organic-N, as opposed to inorganic N, is still not understood. In this thesis the focus has been to investigate the abundancy, uptake and growth of amino acids in two economically important conifer species Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) Karst.). Moreover, we also tried to examine to what extent amino acids may contribute to the N budget of field grown Scotst pine seedlings. The results show that Scots pine seedlings grown in a dry heath forest have acces to comparable or higher soil solution concentration of free amino acids N compare to inorganic N sources. The seedlings were also able to absorb amino acid N at the same or higher rates as inorganic N sources both when these were supplied at higher concentration in greenhouse grown seedlings and at lower more ecological relevant concentratons in field grown seedlings. Moreover, our growth experiments suggested that amino acids are capable of supporting similar or even higher growth in Scots pine seedlings compared to a commercial fertilizer based on NO_3^- and NH_4^+. The use of arginine as a source of N for growth of Scots pine seedling were also recorded to give a much smaller N loss compared to the commercial fertilizer due to a strong adsorption to the growth substrate. In all the results presented in this thesis strongly suggests that amino acids is an important N source in the N economy of Scots pine and Norway spruce seedlings and that these N sources may be a poteential alternative to inorganic N sources for comersial seedling growth.

Keywords: organic N, amino acids, boreal forest, Scots pine, Norway spruce, uptake, growth.

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Contents

Appendix

List of Papers

The present thesis is based upon the following papers, which will be referred to by their Roman numerals:

I. Öhlund, J. & Näsholm, T. (2001) Growth of conifer seedlings on organic and inorganic nitrogen sources. Tree Physiology 21:1319-1326.

II. Öhlund, J. & Näsholm, T. (2002) Low Nitrogen Losses with a New Source of Nitrogen for Cultivation of Conifer Seedlings. Environmental Science and Technology 36:4854-4859.

III. Öhlund, J. & Näsholm, T. (2004) Regulation of organic and inorganic nitrogen uptake in Scots pine (*Pinus sylvestris* L.) seedlings. Submitted to Tree Physiology.

IV. Öhlund, J. & Näsholm, T. (2004) Mechanisms of amino acid uptake in Scots pine (*Pinus sylvestris* (L).) seedlings. Submitted to Planta.

V. Öhlund, J. & Näsholm, T. (2004) The abundance and utilization of dissolved nitrogen compounds in a *Pinus sylvestris* forest. Submitted to Ecosystems.

Introduction

All living organisms contain nitrogen (N). In fact, N is the fourth most abundant element in our own bodies after hydrogen, oxygen and carbon (Eckert et al. 1996). In humans, as well as in plants N constitute a building block for proteins and DNA molecules (Ganong 1997, Lambers et al. 1998). In plants, N is also an important part of the chlorophyll molecule, which with the help of the sun play a crucial role in converting CO_2 and H_2O into carbohydrate (that we eat) and oxygen (that we breath) in photosynthesis (Salisbury and Ross 1992, Lambers et al. 1998).

Nitrogen - a restless molecule

Nitrogen is a restless molecule; involved in an endless flow trough different pools and molecular forms. The single largest pool of them all is the dinitrogen pool (N_2) which constitute more then three quarters of the air that we breath. Dinitrogen is, however, only accessible for special N fixating prokaryotes such as bacteria which sometimes form a symbiotic relationship with plants and mosses (Marschner 1995, DeLuca et al. 2002). The N fixating prokaryotes reduces N_2 into the plant accessible N form NH_4^+ and in return they receive carbohydrates from the plant. Nitrogen can also move from the atmosphere to the soil through rain in the form of NH_4^+ or NO_3^- (Salisbury and Ross 1992). The NH_4^+ received from the rain arises from industrial burning, volcanic activity or forest fires whereas NO_3^- arises from oxidation of N_2 by oxygen or ozone in the presence of lightning and ultraviolet radiation (Taiz and Zeiger 1998).

Following uptake, plants incorporate N into different N compounds such amino acids and proteins. When the plants finally die or gets eaten the N is returned to the soil. Thus, protein in the dead plant is degraded into smaller organic N compounds mainly by extracellular hydrolytic enzymes that cleaves proteins and peptides to form amino acids (Leake and Read 1989, Marschner 1995). The amino acids are then in part mineralized to NH_4^+, which in turn can be transformed to NO_2^- and NO_3^- by auto- or heterotrophic nitrifying microorganisms (Taiz and Zeiger 1998). Under anaerobic conditions other microorganism may denitrify NO_3^- into N_2, which in turn can be lost to the atmosphere and the N cycle is completed. (Marschner 1995)

Nitrogen and the boreal forest

The boreal forest as well as many other biomes contains vast amounts of N compounds such as amino acids, peptides and proteins (Näsholm et al. 1998, Nordin et al. 2001a, Jones and Kielland 2002, Andersson and Berggren 2003, Paper **V**). Still, growth of plants in this ecosystem is often limited by N (Tamm 1991, Vitousek and Howarth 1991). This contradiction has been explained as a result of the slow mineralization of organic N into NH_4^+ and NO_3^-, the presumed principle N sources of plants (Tamm 1991). However, calculations have shown that plants grown in tundra and taiga ecosystems grow faster than they should be capable of regarding the amounts and rates of production of inorganic N recorded in these soils (Chapin et al. 1988, Ruess et al. 1996). Thus, to sustain such a high growth these plants must have access to other forms of N than the inorganic N forms. So what sources of N

are feeding these plants? The answer to the question may be organic N.

Organic N

The capacity of plants to absorb organic N compounds (foremost amino acid) has been known since the 1940.s (Virtanen and Linkola 1946, Melin and Nilsson 1953) but the significance of organic N in the total N budget of plants has been regarded as small. Even today the recognition of organic N as an important N source is questioned and sentences like: *"even though such amino acids can be absorbed and metabolized by plants, these and other more complex nitrogen compounds contribute little to the plants nitrogen nutrition in a direct way"* can be found in modern textbooks (Salisbury and Ross 1992). Thus, the central dogma has been that plants have to rely on the mineralization of organic N into inorganic N to make up accessible N compounds for the plants (Tamm 1991). Several recent studies have, however, suggested that a number of different plants species grown in the field may absorb amino acids as a direct source of N (Chapin et al. 1993, Kielland 1994, Näsholm et al. 1998,2000, Raab et al. 1999, Persson and Näsholm 2001, Persson et al. 2003). Thus, organic N have become increasingly accepted as a potential important source of N for many different plant species.

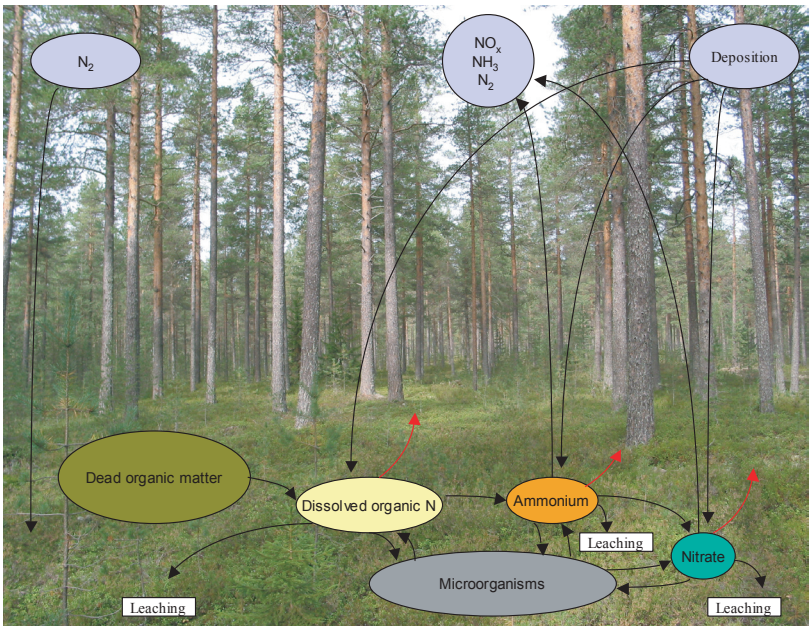

Figure 1. Piture showing the complex nitrogen cycle in the soil of a Pine heath forest. Red arrows show the uptake of different N forms by plants.

Practical applications

The absorption of amino acids by plants may have important ecological as well as economical consequences and raises a number of questions. Our traditional view on the whole N cycle but also our view on N deposition, N fertilization and global warming may have to be challenged. The effect of for example N deposition or forest fertilization may cause shifts in the relative importance of various N forms to plants, shifts that may be of both ecological and practical significance. Additionally, the absorption of amino acids by plants could have economical and practical consequences. The utilization of organic N sources in plants widen the range of different N sources that may be used in commercial plant cultivation. Thus, in the future amino acids as a source of N for cultivation of plants may open new possibilities to compose fertilizer specially adapted for different plants and growing conditions.

Objectives of this thesis

There are two major ecological objectives of the present thesis, Firstly (paper **I-II)** we were trying to reveal to what extent amino acids are able to contribute to the uptake and growth of Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) Karst.) seedlings. In other words is the capacity of these conifers to absorb organic N sufficient to meet the N demand of a growing plant. Secondly, (paper **III-V**) we performed more detailed studies on the uptake pattern and utilization of amino acids in Scots pine seedlings grown both in the field and in the greenhouse. Here the focus was to determine how these organic N sources are absorbed by the seedlings and also to what extent they may contribute to the N budget of field grown plants.

Figure 2. Scots pine seedling, the main studied plant species in the present study.

Nitrogen and plants – an ecological perspective

As mentioned in the introduction section, organic N forms have become increasingly accepted as potential important sources of N for many different plant species. There are, however, numerous difficulties that a plant has to face in order to effectively absorb organic N compounds. This section deals with the ecological factors that affect the uptake of organic as well as inorganic N compounds by plants.

Plant available nitrogen forms

From a plant perspective, the content of N in the soil is actually of limited importance. Thus, a plant growing in soil with high content of N could still suffer from N starvation and a plant growing in soil with low N concentration could be well fed with N. This paradox is explained by the fact that most of the N in the soil is inaccessible for plant uptake. As mentioned in the introduction, soils in cold areas such as the boreal forest and the artic region contain great amounts of different N compounds of high molecular weight. But how many of these N compounds are directly available to the plants? The central dogma has been that plants don't use these N compounds and thus have to rely on the mineralization of organic N compounds into NH_4^+ and NO_3^- (Tamm 1991). However, it has today become increasingly accepted that numerous different plant from Eucalyptus trees (Turnbull et al. 1995) to boreal plants (Näsholm et al. 1998, Persson et al. 2003, Paper **I-V**) and artic species (Schimel and Chapin 1996, Chapin et al. 1993, Kielland 1994, Raab et al. 1999, Lipson et al. 1999a) have the ability to absorb amino acids in comparable rates as inorganic N compounds. Uptake of amino acids has also been recorded in non mycorhizzal plants suggesting that an mycorhizza association is not required for organic N uptake by plants (Schobert and Komor 1987, Raab et al. 1996,1999). Considering the wide range of different amino acids present in most soils, the menu of plant accessible N compounds may be substantial. However, as plants only have been shown to be able to absorb simple organic N compounds such as amino acids and small peptides much of the organic N compounds in soils is still inaccesible for the plants to absorb (Chapin et al. 2002).

Mycorrhizal symbiosis

A large part of the microbial community in the boreal forest consists of mycelia of mycorhizzal fungi (Chalot and Brun 1998). Studies have shown that these mycorhizzal fungi are able to absorb both inorganic as well as organic N sources (Abuarghub and Read 1988, Finlay et al. 1989, Chalot and Brun 1998, Javelle et al. 1999, Wallenda and Read 1999, Hawkins et al. 2000).

Plants have long been known to form symbiotic associations with different fungi (Marschner 1995, Lambers et al. 1998). The ecological importance of these associations has been recognized, especially in N limited systems (Stribley and Read 1980, Vogt et al. 1982, Abuarghub and Read 1988, Finlay et al. 1989, Chalot and Brun 1998, Wallenda and Read 1999). One obvious advantage for plants that form mycorhizzal associations is the extension of the root system through fungal hyphae which increases the absorptive surface area, and thus the ability of plants

to absorb N as well as other nutrients. Ecto and ericoid mycorhizza have also been shown to secrete extracellular hydrolytic enzymes (proteinases) that have a direct role in the decomposition and uptake of organic N by plants (Leake and Read 1989, Smith and Read 1997, Hodge et al. 2001). These hydrolytic enzymes are able to cleave proteins into amino acids that subsequently may be absorbed by their host plant (Abuzindah and Read 1986,1989, Finlay et al. 1992, Lipson et al. 1999a). Thus, plants associated with mycorhizzal fungi may both increase their adsorptive surface area as well as broaden the forms of N that are available to them. Regarding this, it is not surprising that different studies have reported higher growth and uptake rates of N for plants infected with mycorhizzal fungi compared to plants lacking this association (Turnbull et al. 1995, Chalot et al. 2002).

Nitrogen in soil

In boreal and artic soils the degradation of dead organic matter is usually rather slow and will lead to a high proportion of organic N compounds relative to NH_4^+ and NO_3^- that has a more rapid turnover in the soil (Kielland 1995, Näsholm et al. 1998, Nordin et al. 2001a, Jones and Kielland 2002). The main reasons for the slow N mineralization rate and high proportion of organic N compounds are the cold climate and acidic soils found in these regions together with a often recalcitrant litter (Marschner 1995). These conditions are unfavourable for soil microbes that facilitate the breakdown of organic molecules, such as protein and amino acids into inorganic N compounds. Thus, the bulk of organic N compounds found in the boreal soils are usually of a high molecular weight, such as different polymers (proteins, peptides, DNA, RNA, chitin, and lignin) or monomers (nucleic acids and amino sugars) (Lipson and Näsholm 2001). Among the organic N compounds proteins and heterocyclic N compounds (nucleic acids) generally dominate. In a review article on soil organic N, the following proportions of N compounds in different soils were estimated: 40 % proteinaceous material, 35 % heterocyclic N compounds (nucleic acids), 19 % NH_4^+ and 5-6 % amino sugars (Schulten and Schnitzer 1997). However, in contrast to this review Knicker and Lüdemann (1995) recorded that the major form of N in composted *Lolium perenne* and *Triticum sativum* plants were in amide/peptide structures. The proportion of amino acids in the proteinaceous pool could be substantial, even higher than the concentration of inorganic

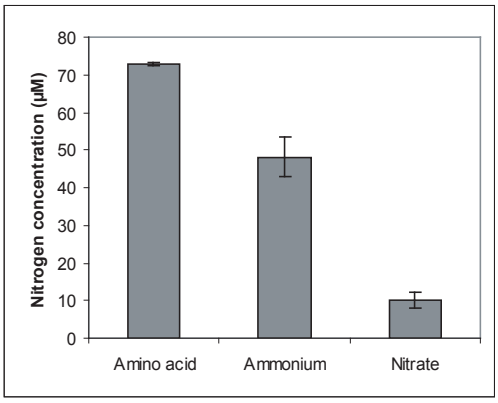

Figure 3. Composition of nitrogen species in the soil solution of a dry heath Pine forest near Umeå, Sweden. Soil samples were collected using lysimeters (see under Soil solution measurements in the Methodological aspects section), error bars represent ± SE, n = 6.

N, and is generally dominated by the acidic amino acids aspartate and glutamate and their amides, together with the neutral amino acids glycine and alanine (Senwo and Tabatabai 1998). In the boreal forest high soil concentrations of arginine, glutamine and aspargine are also commonly found (Nordin et al. 2001a). As these amino acids have been shown to serve as N storage compounds in plants (Näsholm and Ericsson 1990, Näsholm et al. 1997, Nordin et al. 2001b) their high concentrations in the soil could be due to the leakage from dead plant tissue . There are also reports suggesting high levels of arginine and serine in tundra soil extracts and pore water (Kielland 1995). However, only a small amount of the amino acid N pool recorded in the soil is dissolved in the soil solution and referred to as free amino acids, while the bulk of amino acid as well as inorganic N compounds is tightly bound to colloids or minerals and thus less accessible for the plants (Lipson and Näsholm 2001).

Nitrogen turnover

The high proportion of organic N compounds relative to NH_4^+ and NO_3^- in artic and boreal soils together with comparable uptake affinities for both organic and inorganic N compounds recorded in plants, are strong indications of a substantial plant uptake and utilization of organic N sources in these ecosystem (Chapin et al. 1993, Kielland 1995, Schimel and Chapin 1996). However, the use of soil N concentration to assess the utilization of organic N may be hazardous. The amino acids pool in soils is highly dynamic, turning over several times a day, (Kielland 1995, Jones 1999, Jones and Kielland 2002). Therefore, a high concentration of a certain N compound in the soil may not necessarily suggest a high plant usage of this N compound, but may instead suggest the reverse. Using the same logic, a low concentration of a certain N compound may not necessarily mean a low plant usage of that N compound, but may instead suggests a high turnover and hence a high usage of that particular N compound.

The turnover of N in the soil includes three major steps. First, larger organic N compounds in the litter are degraded into smaller organic N compounds by the action of hydrolytic enzymes (e.g proteinases) (Leake and Read 1989). These smaller organic N compounds are then degraded into amino acids by microbes such as bacteria and fungi. Alternativly proteins may also be directly degraded into amino acids by the action of endopeptidases. The amino acids produced may then either be taken up directly by the plant or further mineralized to NH_4^+. Ammonium may finally be oxidized, via NO_2^- to become NO_3^- (nitrification).

Thus, N in soil is involved in an endless flow through different pools and molecular forms. The challenge for the future is to monitor these flows and determine the production and utilization of different N sources by plants and microorganisms. As discussed above, the mineralization of amino acids into NH_4^+ and NO_3^- has traditionally been considered to be the rate-limiting step for the production of plant available N sources (Tamm 1991). However, as suggested by Jones and Kielland (2002) the transformation of proteins into amino acid and not the transformation of amino acids into inorganic N, may instead be the rate-limiting step for the N availability of plant grown in artic or boreal ecosystems.

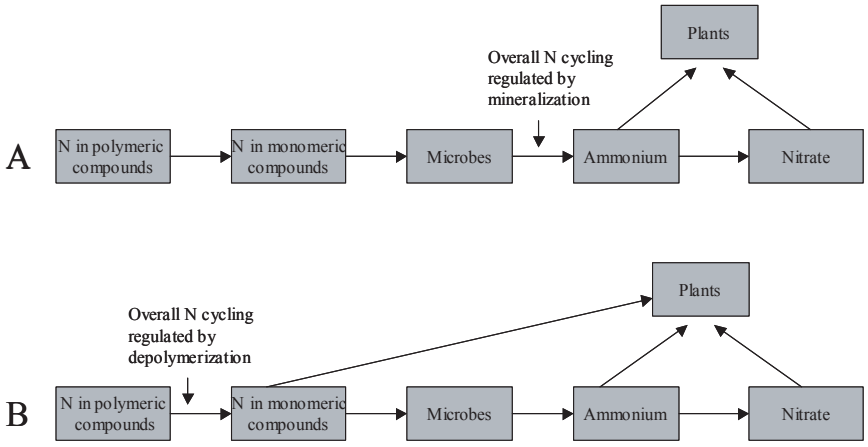

Figure 4. Figure showing **A**) the classical model of N cycling and N uptake in plants where overall N cycling is regulated by mineralization, and **B**) an alternative model where overall N cycling is regulated by depolymerization of soil organic matter into monomers (i.e amino acids).

Delivery of N to roots

There are two main routes which nutrients are transported to the root or mycorhizzal surface of the plant: by diffusion or by mass flow (Marschner 1995). The diffusion of N compounds is driven by the concentration gradient from the root surface and outward into the soil and is generally considered to be the single most important way of nutrient transport in areas with low nutrient concentrations (Lambers et al. 1998). On the other hand, mass flow of N in soil water normally driven by the transpiration from shoots, is believed to be most important in soils with high nutrient concentrations (Lambers et al. 1998). In some areas, mass flow could also be driven by gravitation, causing water movements in the soil. One such example is hillsides where large amounts of water are transported down the hill and on its way carries substantial amounts of N (Chapin et al. 1988).

Diffusion is driven by the concentration gradient from the root surface and outwards into the soil and hence the diffusion coefficient of the N compound is an important determinant of the transport rate and thus the utilization of different N compounds in plants (Lambers et al. 1998, Taiz and Zeiger 1998). As organic N compounds have a higher molecular weight than inorganic N compounds, their relative movement in the soil may be slower. However, other factors such as adsorption or fixation of N compounds in soil material may also have a large effect on the diffusion rate of N compounds. For example, cations such as NH_4^+ may easily be adsorbed to negatively charged surfaces, containing e.g. carboxyl groups ($R{\cdot}COO^-$) and thus be more immobile in the soil compared to anions such as NO_3^- (Marschner 1995).

As the importance of organic N in the N budget of plants until recently have been regarded as small, relativly few studies regarding their diffusion coefficients in diffrent soils have been performed (Jones 1999). In paper **II** it was suggested that the positively charged amino acid arginine was even more immobile in peat compared to NH_4^+, which is usually regarded as a rather immobile cation. Besides diffusion and mass flow there are also a third way in which a plant can access N; through root interception (Lambers et al. 1998). This way is generally regarded as less significant compared to diffusion and mass flow and involves the actual growth of the roots to new areas in the soil (Marschner 1995). Some plants and mosses also have the capacity to form symbiotic associations with special prokaryotes such as blue-green bacteria and cyanobacteria that possess the ability to absorb N_2 and transform it into accessible NH_4^+ in exchange for carbohydrates from the plant (Marschner 1995, DeLuca et al. 2002).

Table 1. Nitrogen supply through root interception, mass flow and diffusion in a sedge tundra ecosystem. All data in kg/ha. Modified from Shaver and Chapin 1991.

	Amount taken up (kg N/ha)	Root interception	Mass flow	Diffussion
Nitrogen	**22**	**-**	**0.1**	**21.9**

Competition for N

The chances for a plant to absorb N is strongly limited by both the molecular form and amount of N that is available for plant uptake, as well as the actual movement and delivery of different N compounds to the root surface of the plant. These limiting factors are, however, not the only difficulties plants faces in their scavenging for N. In a N limited ecosystem such as the boreal forest, plants are also forced to compete for N both with other plants but foremost with microbes such as bacteria and fungi (Kaye and Hart 1997). Traditionally, microbes have been considered superior in this competition and forced plants to play a passive role acquiring N in excess of microbial demand (Marschner 1995, Owen and Jones 2001, Bardgett et al. 2003). Another assumption has been that plants only utilize inorganic N and thus have to rely on microbes for the mineralization of organic N compounds (Harmsen and van Schreven 1955). Hence, plants have been considered to be weak competitors for all forms of N and particularly for organic N compounds. However, the findings that several different plant species including conifers can short-circuit the N mineralization step by direct absorption of simple organic N compounds in field situations suggests that plants actually are able to successfully compete with microorganisms for N (Chapin et al. 1993, Schimel and Chapin 1996, Kielland 1994, Näsholm et al. 1998, Persson et al. 2003). The challenging question is instead to determine the competitive *ability* of plants vs. microbes for different

N sources. Several attempts to increase our understanding of the competitive relationship between plants and microbes have been made (Schimel and Chapin 1996, Hodge et al.1998, Lipson and Monson 1998, Lipson et al. 1999b, Bardgett et al. 2003). Some of these studies have concluded that microbes are superior to plants in the competition of N in general and organic N in particular (Hodge et al.1998, Owen and Jones 2001, Bardgett et al. 2003), while other argue that plants are well capable of competing with microbes (Schimel and Chapin 1996, Lipson and Monson 1998, Lipson et al. 1999b). The general opinion, however, is still that microbes are superior to plants in the competition for N due to their rapid growth rate, high substrate affinities, high surface-to-volume ratio and thus are the first to absorb any new N compound in the soil. This conclusion is probably true on a face to face competition between plants and microbes, but not necessarily the case on a whole soil scale. As soils are highly heterogenic they contain microsites with different chemical conditions such as % N, C:N ratio, lignin content, extractable inorganic N, pH etc. (Hodge et al. 1999a,b, Hodge et al. 2000a, Hodge 2003, Vance and Chapin 2001). These differences will cause an intense diffusion of available N compounds between different microsites and thus offer opportunities for plants to absorb these N sources simply by being the first to encounter an available N source. Considering the wide range of different available N sources and the high turnover of N in most soils, such opportunities are probably quite common (Jones and Kielland 2002, McFarland et al. 2002). When the extended period of time that a plant retains N compared to microbes is also taken into account, plants would be expected to increase their competitive ability in the long run. Hence, a plant would have more opportunities to absorb the same N compound compared to a microbe that only would get a few chances during its short lifespan (Kaye and Hart 1997, Hodge et al. 2000b). Besides the spatial and temporal factors described above, local and spatial differences in temperature may also influence the competitive relationship between plants and microbes. In a study by Vinolas et al. (2001a) it was recorded that the microbial mineralization rate became slower at lower soil temperature. If assuming that the amino acid N uptake rate of plants remain constant at lower soil temperature it may be suggested that the competitive pressure from microbes over N would decrease when the soil temperature drops and thus provide opportunities for an increased amino acid N uptake in plants. This suggestion is, however, questioned in a study on the alpine sedge *Kobresia myosuroides* where no effect on the competition between plants and microbes were recorded following climatic disturbances (Lipson and Monson 1998). Another factor that may increase the chances of a plant to compete with microbes for N is the formation of mycorrhiza (Stribley and Read 1980, Chalot and Brun 1998, Lipson et al. 1999a, Hodge 2003). As previously mentioned plants associations with mycorhizzal fungi increases the absorptive surface which in turn increases the nutrient uptake of the plant. Ecto and ericoid mycorhizzal fungi have also been shown to secrete extracellular hydrolytic enzymes that have a direct role in the decomposition of organic N (Abuarghub and Read 1988, Smith and Read 1997). However, the formation of mycorhizza makes it difficult to separate symbiotic microbes from microbes competing with plants. Thus, studies of N competition between plants and microbes in the field tend to include symbiotic mycorhizzal fungi in the group of microbes that compete with plants. As N taken up by symbiotic mycorhizzal fungi later may be transported and taken up by the plant, such studies may underestimate plant N acquisition (Finlay et

al. 1989, Lipson et al. 1999a).

The competitive relationship between plants and microbes for different types of N compounds has been suggested to show great variation. (Jones and Hodge 1999, Lipson et al. 1999b). As microorganisms generally are considered to be carbon limited the competition pressure from microorganisms over organic N source (containing both C and N), may be more intense compared to what is the case for inorganic N sources. However, differences in competition pressure for various organic N sources have also been observed between plants and microbes (Kielland 1994, Lipson et al. 1999b). In a study by Lipson et al. (1999b) it was suggested that microbes were able to grow 3.25 times better on glutamate compared to glycine and it was argued that this difference could be explained by that glycine constitutes a poor carbon source for microbes compared to other amino acids. Thus, plants may have been specialised to absorb amino acids that are under less intense microbial competition. There are also studies suggesting a temporal separation between microbes and plants in their uptake of N (Lipson et al. 1999c). Thus, the competitive pressure between microbes and plants could be less pronounced at certain times of the year/day and thus offer increased chances for the plants to absorb N.

Even though microbes generally are considered the nr 1 N competitor for plants, the competition over N among plants themselves could also be substantial. Plants competition over N occurs not only between other plant species but also between different individuals within the same species. There are, however, studies that suggest that plants may have developed niches in order to ease this plant to plant competition and allow co-existence in N limited environments. In a study by McKane et al. (2002) it was suggested that different species of artic tundra plants were differentiated in timing, depth and chemical form of N uptake, and that species dominance was strongly correlated with uptake of the most available N forms.

Taken together, competition over N is a complex issue and the outcome of this competition may depend on numerous factors. A deeper knowledge of soil processes and plant-microbe interactions is necessary to understand the importance of both organic and inorganic N in different plant species. As short time studies only offer a snap shot of the processes occurring in the soil, their use to determine these complex processes is limited. New techniques that make it possible to monitor the flow and concentrations of different N compounds during longer time periods are necessary to further increase our understanding of the utilization of different N compounds in plants.

Plant uptake of nitrogen

Pathways of nitrogen transport

Nitrogen compounds are transported to the root mainly by diffusion and mass flow. Well at the surface of the root cells, these N compounds enter a network of pores, so called interfibrillar and intermicellar spaces, in the root cell wall (Marschner 1995). These pores contain carboxyl groups ($R{\cdot}COO^-$) that act as cation exchangers (Clarkson and Lüttge 1980). Thus, cations such as Ca^{2+} may bind to these sites before being transported over the plasma membrane (Sentenac and Grignon 1981).

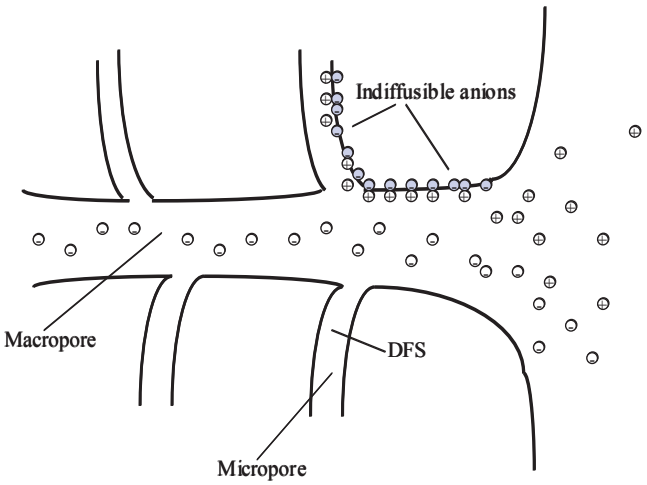

Figure 5. Cation absorption in the Donnan free space (DFS) of the plant cell wall. Cations are absorbed by negatively charged carboxyl groups ($R{\bullet}COO^-$) that act as cation exchangers in the cell wall of plants.

In paper **III** and **IV** we present data that suggest that such an electrostatic binding also occur for the positively charged amino acid arginine. The binding of cations in the cell wall increases the concentration and vicinity of these molecules to active uptake sites in the plasma membrane (Marschner 1995). Thus, the binding of cations in the apoplast of the cell wall may have a positive effect on the uptake rate of such molecules (Marschner 1995). When a N compound finally enters the plasma membrane of the root cell, there are two different ways in which it could be transported across the membrane and reaches the cytosol of the cell: by passive diffusion or by active transport. However, since the main function of the plasma membrane is to form a barrier between the cytosol and the apoplasm, it only allows passive diffusion of gases, such as O_2 and CO_2, small polar molecules such as H_2O and small non polar molecules (Garret and Grisham 1999). Thus, larger molecules

like amino acids and ions like NH_4^+ and NO_3^- cannot passively pass the plasma membrane at appreciable rates, but have to rely on active transport in order to enter the cytoplasm of the cell. There are three different transporters that are involved in the active transport of solutes over the plasma membrane: **A**) *proton pumps or ATPase* **B**) *ion channels* **C**) *carriers*. The main function of the proton pumps is to transport protons from the cytoplasm to the apoplasm, leading to an electrical potential gradient as well as a proton gradient between the cytolsol and the apoplast (Lambers et al. 1998). This extrusion of protons from the cytoplast provides the driving force for the transport of solutes through channels and carriers. Ion channels allow a simultaneous transport of several molecules along their concentration or electrical potential gradient, and are thus able to transport solutes at high rates (Taiz and Zeiger 1998). Movements of solutes through carriers on the other hand, involves the interaction of the solute and the protein binding site on the carrier witch triggers a conformation change on the carrier protein. This conformation change allows the transport of the solute through the carrier. Thus, in contrast to ion-channels, carriers are regarded to be rather substrate specific and only able to transport one solute at a time. There are three main types of carriers: *uniporters*, *symporters* and *antiporters* (Taiz and Zeiger 1998). Uniporters are carriers that speed up the transport of for example cations down their electrochemical gradient; symporters allow inward transport of two different solutes simultaneously over the plasma membrane, and antiporters facilitate a simultaneous inward and outward transport of solutes over the plasma membrane.

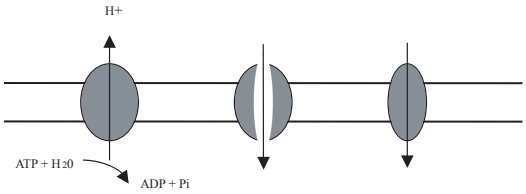

Figure 6. The three types of transporters in plants: proton pumps or ATPase (left), channels (middle) and carriers (right).

Inorganic nitrogen transport

The uptake kinetics of NH_4^+ is divided into two distinct systems: a low-affinity transport system (LATS) and a high affinity transport system (HATS) (Kronzucker et al. 1996, Glass et al. 2001). The LATS of NH_4^+ is generally thought to act through channels since no saturation of the uptake rate has been recorded even at high NH_4^+ concentrations (Kronzucker et al. 1996). In contrast to LATS, the uptake through HATS has been suggested to follow Michaelis-Menten kinetics, which indicates that uptake through this system is mediated by carriers (Lüdewig et al. 2002). Several genes encoding for these high affinity transporters have also been identified in *Arabidopsis thaliana* (Gazzarrini et al. 1999).

Nitrate and other anions normally use symporters or proton-anion co-transport to cross the plasma membrane (Crawford and Glass 1998). In contrast to NH_4^+, the uptake of NO_3^- is so far categorized into three kinetically different phases: a constitutive high-affinity system (CHATS) an inducible high-affinity system (IHATS) and a linear low-affinity transport system (LATS), (Kronzucker et al. 1995, Crawford and Glass 1998). The first uptake system, referred to as the CHATS, is a high affinity transport system working in plants unexposed to NO_3^-. It is characterized by a slightly higher affinity and lower uptake rate compared to IHATS (Kronzucker et al. 1995). The second uptake system referred to as the IHATS is working in higher concentrations compared to CHATS and is characterised by its induction after exposure to NO_3^- (Min et al. 2000). The last uptake system, referred to as LATS, is a linear and non-saturable uptake system and is working at high [NO_3] (Kronzucker et al. 1995). As for NH_4^+, several genes encoding for both high and low affinities transporters for NO_3^- have been identified in plants (Glass et al. 2001). Nitrate transporters are also active in the cytosol of cells, transporeting NO_3^- over the vacuole membrane.

In addition to the influx of NO_3^- and NH_4^+ through different uptake systems, studies have shown that a simultaneous efflux of both NO_3^- and NH_4^+ occurs in plants (Crawford and Glass 1998, Britto et al. 2001). Today it is known that the efflux of NO_3^- and NH_4^+ occurs passively over the plasma membrane and increases with external concentrations, but the proteins involved in the efflux pathway are still unknown (Crawford and Glass 1998, Britto et al. 2001).

Taken together, the uptake systems of both NO_3^- and NH_4^+ in plants are well studied and numerous genes that encode for these transporters have been identified (Gazzarrini et al. 1999, Rawat et al. 1999, Glass et al. 2001). Moreover, several studies have suggested that plants are able to regulate the expression of these genes in order to match the N demand of the plant (Rawat et al. 1999, Glass et al. 2001, Vidmar et al. 2000, Nazoa et al. 2003). These studies have shown that a decrease in plant N status leads to an up-regulation of the high-affinity NH_4^+ and NO_3^- transporters, while an increase in plant N status leads to a down-regulation of the same transporters. In this way, plants are able to adjust their uptake of N in response to their current need (Glass et al. 2001). Both NH_4^+ itself, and the downstream metabolite glutamine have been suggested as controlling agents for the regulation of transcription of NH_4^+ and NO_3^- high-affinity transporters (Feng et al. 1994, Rawat et al. 1999, Glass et al. 2001, Nazoa et al. 2003). These findings were supported by paper **III** where a high N supply resulted in a down-regulation of the NH_4^+-N uptake rate in Scots pine seedlings. Simultaneously, an increase in the endogenous NH_4^+ and a glutamine concentration were recorded suggesting that not only NH_4^+ itself but also the amino acid glutamine could regulate the uptake of N (Gessler et al. 1998, Rawat et al. 1999).

Organic nitrogen transport

As amino acids constitute the building blocks of peptides and proteins they are objects of an intense transport between different organs of the plant through the xylem and phloem. Additionally, a continuous transport of amino acids between

different organelles in the cell occurs. Thus, the direct uptake of amino acids from the apoplasm is only one of many functions that amino acid transporters may perform. The transport of amino acids over the plasma membrane is considered to be facilitated by proton-coupled symporters (Reinhold and Kaplan 1984, Buch 1993, Tanner and Caspari 1996, Heremans et al. 1997, Neelam et al. 1999). Thus, the inward flux of amino acids over the plasma membrane is performed by symport carriers that bind both the proton and the substrate (Reinhold and Kaplan 1984). Studies have suggested the presence of a number of different amino acid symporters in plants (Fischer et al. 1995, Tanner and Caspari 1996). Some of these symporters have been regarded as rather substrate specific for a certain group of amino acids (Li and Buch 1991) but most studies argue that single amino acid symporters may have a broader substrate specificity, capable of transporting both basic neutral and acidic amino acids (Fischer et al. 1995, Neelam et al. 1999).
In contrast to the well-studied uptake regulation of inorganic N uptake, studies on the regulation of amino acids uptake are rare (Persson and Näsholm 2002, 2003). In paper **III** we found that Scots pine seedlings down-regulated the uptake of arginine, but not of glycine, in response to an increase in endogenous N status. Such different responses of uptake of various N sources to increased endogenous N of plants may cause shifts in the relative importance of various N forms to plants.

Nitrogen transport through mycorhizza

As previously mentioned, the role of mycorhizzal fungi for the uptake of both organic and inorganic N compounds in plants has been widely recognised and well studied (Stribley and Read 1980, Vogt et al. 1982, Abuarghub and Read 1988, Chalot et al. 1991, Finlay et al. 1989, Chalot and Brun 1998, Wallenda and Read 1999). It has been suggested in several papers that ectomycorrhizal fungi associated with plants are able to transfer N compounds from the soil solution to their host plant, and thereby play a crucial role in the N nutrition of plants (Finlay et al. 1989,1992, Lipson et al. 1999a). Despite the apparent importance of mycorhizza for the N nutrition of plants, data on the actual mechanism of mycorhizzal N uptake is limited. It is, however, believed that transport of both amino acids and inorganic N compounds over the plasma membrane of mycorhizza fungi are pH dependent and thus mediated by an active transport mechanism (Chalot et al. 1996, Javelle et al. 1999, 2001). There are studies suggesting that amino acids are transported over the mycorhizza cell membrane through proton symport mechanism similar to that observed in plants (Chalot et al. 1996,2002, Nehls et al. 1999).

All together, much is today known about how amino acids are transported in plants and mycorhizzal fungi. However, from an ecological point of view, the capacity of plants to transport amino acids says little about how these N compounds are utilized in the field. It is even possible that the function of amino acid transport from the apoplast to the cytosol is to retain amino acids lost from the cell (Shobert and Komor 1987). It should also be mentioned that many studies on amino acid carriers have been performed on unicellular organisms such as yeast (*Saccharomyces cerevisiae*; Grabeel and Grenson 1970, Grenson 1992, Fischer et al. 1998, Marvier et al. 1998) or the model plant *Arabidopsis thaliana* (Fischer et al. 1995, Heremans et al. 1997, Okumoto et al. 2002). Thus, the presence of amino acid carriers in for

example conifers is still unknown.

Methodological aspects

Studies of uptake processes of plant roots or mycorhizzal fungi have suggest that affinities and rates of uptake may be in the same order of magnitude for both inorganic and organic N compounds (Chapin et al. 1993, Kielland 1994, Schimel and Chapin 1996, Raab et al. 1996, Näsholm et al. 1998, Schmidt and Stewart 1999, Persson and Näsholm 2002, Paper **I-IV**). Many of these studies have, however, been performed under controlled laboratory settings, labelling roots in solutes containing different forms of organic as well as inorganic N compounds. This approach excludes factors like microbial competition and diffusion rates of different N compounds in the soil that may strongly affect the rate at which a certain N compound is delivered and taken up by the plant. Another difficulty with these kinds of laboratory studies is that they often exclude mycorhizza. By washing the roots before labelling, most of the mycorhizzal fungi will be lost. As mycorhizzal fungi are regarded as an important factor for plant N uptake, the loss of mycorhizza may strongly affect the uptake of different N forms. Thus, these studies only provide data about plants *capacity* to absorb different organic N compounds, but cannot quantify the importance of these N sources in the total N economy of field grown plants. Moreover, as suggested by Schobert and Komor (1987) the capacity to transport amino acids from the apoplast to the cytosol could have other functions than retaining amino acids from the soil solution, for example a way for the plant to recover amino acids lost from the phloem. Several studies have also reported on poor growth on organic N sources in non mycorhizza plants (Finlay et al. 1992, Turnbull et al. 1995). Still, these plants were able to take up these organic N sources. The fact that plants, unable to grow on organic N still are able to absorb these N sources further support the conclusion that the capacity to absorb amino acids and the utilization and growth on them are two completely different things. From an ecological point of view, the interesting questions is to determine if the plant capacity to absorb amino acids is realized in the field, and if so to what extent they contributes to the N economy of these plants.

A critical issue when studying amino acid uptake in plants is to determine the actual form of N utilized by the plants. Usually amino acids, dually labelled with ^{13}C and ^{15}N at all C and N positions (universally labelled) have been used (Näsholm et al.1998, Persson et al. 2003, Paper **I-IV**). The use of universally labelled amino acids provides an important advantage over single label tracers (only labelled at the N positions). In contrast to single label tracers the use of dual label tracers enable us to trace intact uptake of amino acids in the plant by comparing the amounts of ^{13}C to ^{15}N recorded in the seedlings with the ratio of ^{13}C to ^{15}N recorded in the tracer. This relationship gives a minimum estimation of the fraction of intact amino acid uptake by the plants (Näsholm et al. 1998, Näsholm and Persson 2001). However, there is one large problem using labelled compounds to measure uptake in plants - the loss of label due to respiration and excretion. This problem is especially serious for carbon, which is rapidly lost through respiration (Näsholm and Persson 2001). This will lead to a rapid loss of carbon from the plant which makes it impossible to

separate intact from mineralized uptake of amino acid N. The solution to the problem has been to perform short time uptake studies to minimize the loss of carbon. Generally, a sudden doze of labelled amino acids has been supplied to the field, and after 1-2 hours plants have been harvested and analyzed for their ^{15}N and ^{13}C content. A sudden supply of amino acids may locally occur in the field, for example when a cell lyses and its cytosol is released into the soil or when plant tissues high in N storage compounds (arginine) are decayed (Nordin et al. 2001b, Jones 1999). However, when considering the amounts and concentrations of amino acids generally used in these kind of studies, it seems unrealistic that such a high amino acid burst would naturally occur in the field. A high and sudden supply of amino acids might alter the competitive relationship between plants and microbes and thus give a false picture of plants organic N uptake capacity. When also regarding the temporal differences in the competition between plants and microbes (see above), a few hour long uptake study cannot provide conclusive evidence of the role of organic N in plants total N budget.

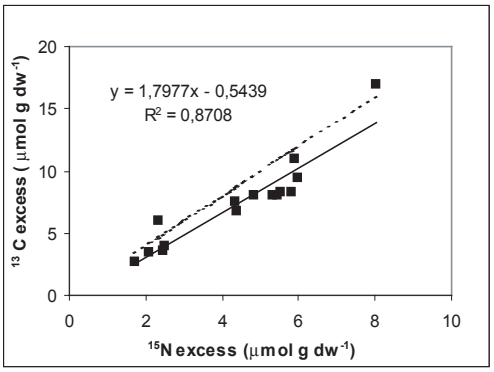

Figure 7. Relationship between excess ^{13}C and excess ^{15}N in Scots pine roots labelled with U-[$^{13}C_2$], [^{15}N]-glycine. Each symbol represents one analysis of roots from one seedling. Broken lines indicate the slopes corresponding to 100% of amino-N absorbed as intact amino acid (glycine slope = 2.0). Solid lines indicate the regressions relating to U-[$^{13}C_2$], [^{15}N]-glycine treated seedling roots. Thus, the slope of 1.8 suggests that at least 90 % of N taken up from glycine was taken up as intact glycine molecules in this experiment.

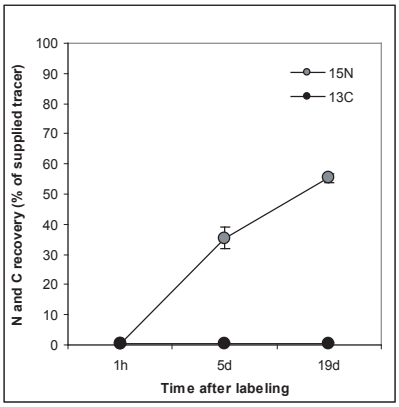

Figure 8. Relative ^{13}C and ^{15}N recovery (% of supplied tracer) in Scots pine seedlings grown in pots and injected with U-[$^{13}C_6$], [$^{15}N_4$]-arginine. The figure shows the relative recovery of ^{13}C and ^{15}N 1h, 5days or 19 days after arginine injection, error bars represent ± SE, n = 6.

Used techniques

In paper **I** and **II**, potted Scots pine seedlings grown on both inorganic and organic N sources were labelled using U-[$^{13}C_6$], [$^{15}N_4$]-arginine and U-[$^{13}C_2$], [^{15}N]-glycine. The uptake of amino acids was then analyzed using both gas chromatography-mass spectrometry (GC-MS) and isotope ratio mass spectrometry (IRMS) (Paper **I**). The GC-MS techniques make it possible to verify the presence of universally labelled amino acids in seedling and also to monitor the metabolic fate of the amino acids while the IRMS provides a high precision determination of the isotopic ratios between ^{13}C and ^{15}N recorded in the seedlings and thus a possibility to determine the minimum level of intact uptake of the amino acids. The analysis of ^{13}C and ^{15}N enrichments in plants supplied with universally labelled amino acids by IRMS can, however, be problematic. As plants contains much higher levels of C compared to N the endogenous dilution effect of ^{13}C is much larger than the endogenous dilution effect of ^{15}N (Näsholm and Persson 2001). This will result in a more difficult reading of the ^{13}C enrichments in the plant compared to ^{15}N enrichment witch may have sever effect on the conclusions about the levels of intact amino acid uptake recorded in plants.

In paper **III** and **V**, high performance liquid chromatography (HPLC) was used to determine the amino acid content in fertilized seedling roots (Paper **III**) and to determine the uptake of a number of different N compounds depleted from an incubation solution (Paper **V**). The advantages in using HPLC techniques are the possibilities to simultaneously follow the uptake of a number of different N compounds in the same sample. In paper **IV**, ^{14}C labelled amino acids were used. The use of radiotracers offers high sensitivity, which allows the use of low label concentration without loosing precision. However, the use of ^{14}C labelled amino acids makes it impossible to be absolutely certain that the amino acids are taken up as intact molecules, since minor carbon fractions from the ^{14}C labelled amino acid also could be taken up by the seedlings.

Soil solution measurements

Characterization of soluble N compounds in soil have generally been performed using different extraction techniques such as water extraction (Kielland 1995, Nordin et al. 2001a), salt extraction (Jones and Kielland 2002) or centrifugal extraction (Näsholm et al. 1998) of sieved soil. The effects of excavating and sieving soil before extraction as well as the effects of the extraction *per se* on the composition of soluble N compounds may, however, be severe. Studies on amino acid turnover in soil suggest a mean amino acid half-lives of a few hours (Jones 1999, Vinolas et al. 2001b, Jones and Kielland 2002). Moreover, individual amino acids have also been suggested to display large differences in their respective turnover rates (Lipson et al. 1999b, Jones and Hodge 1999). Thus, measuring amino acid concentrations in soil extracts may not reflect the true amounts and compositions of these substances due to the rapid and variable decomposition of amino acids during handling and extraction of soil samples. In paper **V** we used an alternative method for sampling of soil solution based on the use of small lysimeters (Raab et al. 1996,1999, Andersson and Berggren 2003). The lysimeter, driven by an applied vacuum, has small enough pores to minimize the problems with microbial

decomposition of organic compounds in the sampled solution (Andersson 2003). This technique may therefore provide an alternative to destructive soil sampling methods and better reflect the composition of the soil solution.

In paper **V** we also tested a microdialysis technique in order to sample the soil solution. This technique is commonly used in clinical studies and involves the use of minute dialysis membranes fitted to small probes through which a perfusate can be pumped (Yoshitake et al. 2003, Böttcher et al. 2003, Sommer and Larsen 2003). The advantage of using microdialysis technique to sample soil water is thus, that it may sample an external solution through diffusional induced movements instead of applied vacuum as when using the lysimeter technique. As a significant part of nutrients are transported to roots through diffusional induced movements, the microdialysis technique may give important complementary information to the lysimeter technique.

If assuming that N delivery to the plant root or mycorhizzal fungi mainly is driven by mass flow of nutrient in the soil and diffusional transport of nutrient (Marschner 1995, Lambers et al. 1998) the combination of the lysimeter and microdialysis techniques may thus, better reflect the composition and concentrations of N compounds that plant roots or mycorhizzal fungi encounter after all competition as well as fluxes of N has taken place.

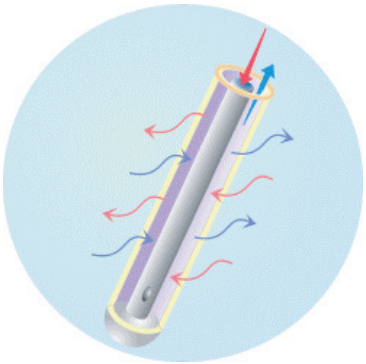

Figure 9. Picture showing the function of the microdialysis probe (CMA/ microdialysis, Stockholm, Sweden). Solute is pumped through the inner tube of the probe and returned through the outer tube ending in a microfraction collector. Exchange of molecules is conducted by simple diffusion when the solute passes the permeable microdialysis membrane (membrane cut-off at 20 000 Daltons).

Growth on organic nitrogen

The capacity of plants to short circuit N mineralization and absorb organic N compounds as a direct source of N has challenged us to question the classical paradigm that plants only use inorganic N compounds and are forced to play a passive role, acquiring N in excess of microbial demand (Marschner 1995). From an ecological point of view it is, however, important to verify the function and importance of this capacity. Maybe plants absorption of organic N only is a way

for the plant to retain organic N compounds lost from the phloem and thus play a minor role in their N budget (Shobert and Komor 1987). Alternatively, absorption of organic N could be an evolutionary adaptation for plants grown in areas with slow mineralization rates and high organic N content and thus have an important role in the N budget of plants. Assuming that organic N compounds do have an important role in the N budget of plants, to what extent are they able to contribute to the growth of the plant? In other words, is it possible to grow plants on organic N compounds as the dominant or sole N source or is the plants capacity to absorb and metabolize organic N sources not sufficient to meet the N demand of the plant? In order to answer this question we supplied the basic amino acid arginine and the neutral amino acid glycine as the sole N sources to Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L) Karst.) seedlings (paper I). Arginine was chosen for it basic characteristics and its high N content (four N atoms). As arginine also have been shown to be used as an N storage compound in plants further made this amino interesting to examine as a sole N source for Pine seedlings (Näsholm and Ericsson 1990, Nordin et al. 2001b). Glycine was chosen for its neutral characteristics and its high uptake rates recorded in different uptake studies (Chapin et al. 1993, Raab et al. 1996, Schmidt and Stewart 1999). To be able to compare the growth on the amino acids with growth on inorganic N we also grew seedlings on different mixtures of NH_4^+ and NO_3^-. We found that seedling growth supported by the two amino acids was comparable to that of the nutrient solutions based on NH_4^+ and NO_3^-, whereas NH_4^+ as sole or dominant N source resulted in base cation deficiency at the 3 or 10 mM treatment (Engels and Marschner 1993). In boreal forest soil solutions, however, the concentration of NH_4^+ as well as other N sources normally is well below 1 mM and thus no problem with cation deficiency may be expected. Seedlings supplied with the amino acids also had a balanced nutrient composition without any deficiency symptoms in any of the N concentrations tested. Thus, these data suggest that the function of amino acid N absorption in both Scots pine and Norway spruce seedlings is as a source of N and that amino acids play an important role in the N budget of these conifers. However, as the seedlings were grown under normal, non-sterile conditions we could not be sure that the seedlings actually were growing on intact amino acids as these could have been mineralized prior to uptake. To approach this question, seedlings from both species were labelled with universally labelled arginine and glycine for 2 hour. By plotting excess ^{13}C *vs*. excess ^{15}N recorded in the seedlings we arrived at figures indicating that all, or a large fraction of the absorbed N from the two amino acids in fact was due to uptake of intact arginine and glycine (se under the methodological section). Moreover, GC-MS analysis of root extracts verified the presence of universally labelled arginine and glycine in seedling roots or mycorrhiza. Thus, it seems like the seedlings actually were absorbing N from intact amino acids. However, if the seedlings acquired N during a long period of time between two fertilization events, part of the supplied amino acid N could have been mineralized prior to uptake. There were, however, observations that indicated that the seedlings actually absorbed the bulk of amino acid N as intact molecules. The most important evidence for this was the pH recorded in the soil solutions. The data showed that seedlings supplied with a NH_4^+ dominated fertilizer had a significantly lower pH in their soil solution compared to amino acid grown seedlings. As the uptake of NH_4^+ is an acidifying process, whilst uptake of amino acids leads to alkalization of the soil solution, (Rygiewicz

et al. 1984, Rollwagen and Zaoski 1988, Buch 1993, Ficher et al. 1998) the higher pH found in the soil solution of the amino acid grown seedlings suggests that the bulk of amino acids were taken up in their intact form. Thus, these results strongly suggest that the function of organic N uptake for Scots pine and Norway spruce is as a source of N for growth, and that organic N may play an important role in the N budget of these conifers.

Nevertheless, there are studies that have reported relatively poor growth on non-mycorhizza plants supplied with organic N, thus questioning the role of organic N as a primary source of N for plants (Turnbull et al. 1995). As plants have been suggested to be strongly adapted to the current N conditions in the environment, these findings may not be surprising (Nordin et al. 2001a, McKane et al. 2002). Consequently, plants grown in areas with low organic N concentrations could be expected to have a less developed capacity to grow on organic N sources compared to plants grown in areas with high organic N concentrations.

Practical applications

Loss of N during various forms of plant cultivation is today a well recognized problem with severe environmental effects (Hershey and Paul 1982, Boon and Niers 1983, Rathier and Frink 1989, Alexander 1993, Fare et al. 1994, Andersen and Hansen 2000). This problem also includes conifer nurseries that supply large quantities of N on a rather small surface area (Juntunen et al. 2001). Even though the total amount of N loss in conifer nurseries is low compared to for example agricultural N losses, the N loss from conifer nurseries could still have severe effects on the local environment. Studies have shown that the loss of N in conifer nurseries could be up to 85 % of the added amounts of N (Hannertz and Rosenberg 2001). In paper **II** we focused on the causes for this low N recovery in a conifer nursery at Gideå, Sweden, and tested an alternative N source, namely the amino acid arginine, for commercial growth of Scots pine seedlings. Arginine was chosen for its cation characteristics and for the high growth it supported in paper **I**. We recorded that the losses of N from the commercial fertilizer were mainly due to a high loss of NO_3^-. This was suggested to be caused by the high mobility of NO_3^- in the growth substrate, in combinations with a slow NO_3^- uptake rate in the seedlings. As several studies have shown that conifers generally have a low uptake rate for NO_3^- compared to NH_4^+, these results were not surprising (Rygiewicz and Bledsoe 1986, Marschner et al. 1991, Kronzucker et al. 1997, Donaldsson Knoepp et al. 1993, Kamminga-van Wijk and Prins 1993, Gessler et al. 1998). More suprisingly we recorded that seedlings supplied with arginine grew better and had a higher N concentration compared to seedlings grown on a commercial fertilizer based on NO_3^- and NH_4^+. Mineral nutrient analysis of seedling needles revealed that the arginine fed seedlings had a balanced nutrition in agreement with previous mineral nutrient analyses on Scots pine (Ingestad and Kähr 1985). Isotopic data also revealed that the arginine treatment gave a significantly higher N recovery (80 %) compared to the commercial fertilizer treatment (50 %). Thus, it appeared as arginine was strongly adsorbed by the growth substrate and then slowly taken up by the seedlings.

All together, this study suggests that the use of arginine as a source of N may give a desirable combination of high growth and low N losses when supplied to seedlings grown in conifer nurseries. Thus, arginine as a source of N for cultivation of plants may be an interesting alternative to commercial fertilizers based on NH_4^+ and NO_3^-. In the future, the use of amino acids as a source of N for cultivation of plants may open new possibilities to compose fertilizer specially adapted for different plants and growing conditions.

Figure 10. Commercial growth of Scots pine seedlings at one of Holmen ABs conifer nurseries, Gideå, Sweden. On the left side seedlings were grown on the amino acid arginine as sole N source, and on the right side seedlings were grown on a commercial fertilizer based on NH_4^+ and NO_3^-.

Uptake of organic N

In paper **I** and **II** it was shown that arginine and glycine were comparable to inorganic N as N sources for growth of Scots pine and Norway spruce seedlings. Thus, the primary function of amino acid uptake in these seedlings seemed to be as a source of N for growth. However, from both a physiological and ecological point of view several questions remain to be answered. First *how are plants absorbing these organic N sources?* Are plants capable of regulating their uptake of organic N? Is the uptake pattern of organic N sources different from that of inorganic N sources? Second and more important *what is the significance of these organic N sources in the N budget of field grown plants?* Is the importance of organic N sources minor in plants total N budget? Or are organic N sources feeding plants with substantial amounts of N?

Several different amino acid symporters have been identified in plants as well as in mycorhizzal fungi (Fischer et al. 1998, Nehls et al. 1999). These transporters have yet not been identified in conifers but the homology among the so far studied plants may suggest that these transporters could be ubiquitous among plants. However, in order to further increase our understand of the role of amino acids in field situations we have to increase our knowledge about plants uptake of these organic N sources on a larger scale. In paper **III** we investigated if Scots pine seedlings both grown in the greenhouse and in the field were able to regulate their uptake of arginine and glycine in response to changes in N supply in a similar way as they are able to regulate their uptake of inorganic N sources (Rawat et al. 1999, Vidmar et al. 2000, Glass et al. 2001, Nasoa et al. 2003). We recorded that seedlings down-regulated their uptake rate of both arginine-N and NH_4^+-N in response to increasing N supply while no regulation was observed for glycine-N. The different responses to increased endogenous N recorded in arginine and glycine labelled seedlings could suggest that an N supply such as N deposition or forest fertilization may cause shifts in the relative importance of various N forms to plants, shifts that may be of both ecological and practical significance. In this study we also recorded a high passive/adsorptive uptake of arginine-N in seedlings and speculated that this was due to a strong binding of arginine to the negative charges of the root apoplast. This speculation was later confirmed in paper **IV** where we were able to follow the uptake of arginine in more detail, using a sensitive radiotracer technique. In this paper we compared the uptake pattern of arginine, glycine, glutamate and methylamine (an NH_4^+ analogue) and found that large quantities arginine was strongly adsorbed to the surface of plant roots. We also recorded that the adsorbed arginine eventually was absorbed into root cells. Thus it may be possible to consider this adsorption of arginine as the first step of the uptake of this amino acid. A high and strong adsorption of arginine to roots or mycorrhiza

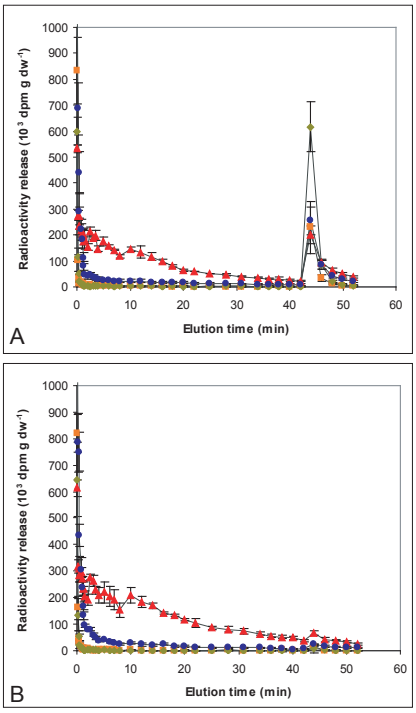

Figure 11. Plots showing radioactivity in eluates (10^3 dpm g dw^{-1}) of seedlings labelled with: L-glutamine UL-^{14}C (orange squares), L-glycine-2-^{14}C (green squares), L-arginine-UL-^{14}C (red triangles), methylamine-^{14}C hydrochloride (blue circles). Figure **A** represents untreated seedlings and figure **B** seedlings pre-treated with the protonphore CCCP. The time periods for the 38 successive elutions were: 5 s (2×), 10 s (2×), 15 s (6×), 30 s (4×), 1 min (4×), 2 min (15×), 3 min (5×). After the last two-minute elutions the seedling roots were submerged in liquid nitrogen for 5 seconds to break root cell membrane. The frozen seedlings were then thawed for 3 minutes and the elution procedure was continued, Error bars represent ± SE, n = 3.

fungi could be an important property for the plants ability to compete and take up this N source in the field. For example the binding of arginine and other cations in plant cell walls increases the concentration and vicinity of these molecules to active uptake sites in the plasma membrane which may increase the uptake rate of these molecules (Marschner 1995). In paper **IV** we also recorded that the uptake of amino acids differ from that of the inorganic N sources in that efflux is negligible. Thus, studies comparing uptake of inorganic and organic N forms by plants would tend to overemphasize uptake of inorganic N because they fail to take the differences in efflux into account.

The importance of organic N

In paper **I** and **II** it was shown that arginine and glycine were comparable to inorganic N as N sources for growth of Scots pine and Norway spruce seedlings and in paper **III** and **IV** amino acid uptake was studied in more detail. However, these papers say little about the actual utilization and importance of amino acids for plants grown in the field. As previously mentioned the respiration of C when supplying dual label amino acids to plants makes it hard to perform long time incubation studies and still be able to separate intact amino acid uptake from mineralized amino acid uptake. In paper **V** we, therefore, used another approach in order to asses the utilization of organic N sources in Scots pine seedlings grown in the field. By the use of both lysimeter and microdialysis technique we sampled the soil solution of a dry heath Scots pine forest for several days and calculated the mean concentration of N compounds recorded in the samples. Thus, this N concentration reflected the mean soil solution concentration of N compounds that the root or mycorhizzal fungi were sensing during the sampling period after all fluxes as well as competition of N had taken place. This N compound concentration was then used as a template for uptake studies in the laboratory using Scots pine seedlings collected at the sampling site. Thus, Pine seedlings were incubated in a mixture containing the same composition of N compounds as well as pH as recorded in the soil solution in the field. We used unlabeled N compounds and followed the decline in the solution for more that four

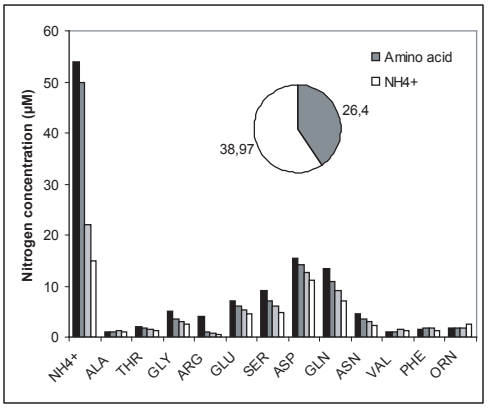

Figure 12. Decline (bars) and total uptake (circular bar) of amino acid-N and NH_4^+-N during a 260 min long incubation of ten pine seedlings in an incubation solution containing the same composition of amino acid-N and NH_4^+-N as was recorded in lysimeter soil water samples in field. The seedlings were collected at the lysimeter soil sampling site and the pH of the incubation solution was the same as recorded in the lysimeter samples (i.e. 4.5).

hours using HPLC measurements. The incubation showed that the plants absorb all amino acids except alanine, valine, phenylalanine and ornithine. Taken together, the amino acids contributed with more than 40 % of the total N uptake in the seedlings during the incubation while the remaining 60 % were derived from the uptake of NH_4^+. It is however, important to realise that these results only reflect the utilization of diffrent N compunds in Scots pine seedlings at one single occation and at the present experimental setup and not nessisarily a general pattern of N utilazation in the field. Nevertheless, the results correlates well to previous studies on field collected roots of artic plants where up to 82 % of the total N uptake was suggested to be derived from the uptake of amino acids (Chapin et al. 1993, Kielland 1994). High importance of amino acid N in the total N budget has also been reported in grass (*Puccinellia phryganodes*) as well as in modelling experiment (Jones and Darrah 1994, Eckersten and Beier 1998, Henry and Jefferies 2002).

As described in the methodological section the use of lysimeter and microdialysis technique for characterization of soluble N compounds in soil has several advantages compared to classical extraction techniques. As the lysimeters sample the soil solution through applied vacuum and the microdialysis membrane sample the soil solution through diffusional induced movements these two techniques may together provide relevant information about the N composition in the soil solution with a minimum disturbances of the system. If assuming that diffusion and mass flow are the major processes in which N is transported to plant roots or mycorhizzal fungi and that the soil solution is an important carrier of N from the soil to the surfaces of roots or mycorhizzal fungi (Leadley et al. 1997), the high amino acid uptake rate recorded in this study thus suggested that amino acid N may be of great importance in the N budget of Scots pine seedlings grown in these ecosystems.

Conclusion and future challenges

Taken together the results presented in this thesis suggest that:

Amino acids constitute a significant N form in boreal forest soils. Our data show that Scots pine seedlings growing in dry heath forests have access to comparable or higher soil solution concentrations of free amino acid N compared to inorganic N sources.

Uptake rates of amino acids in the seedlings were comparable or higher then than inorganic N sources, both at higher N compound concentration in greenhouse grown seedlings, and at lower more ecological realistic concentrations in field grown seedlings.

Field grown Scots pine seedlings are able to absorb a wide range of diffrent amino acids supplied at field relevant concentrations.

Scots pine seedlings down-regulate their uptake of NH_4^+-N and arginine-N in response to increased endogenous N concentrations or N supply, while no down-

regulation of the uptake rate of glycine-N was recorded.

Our radiotracer studies suggest that uptake of amino acids in Scots pine seedlings differ from that of the inorganic N in that efflux is negligible. If this is correct studies comparing uptake of inorganic and organic N forms would tend to overeestimate the uptake of inorganic N because they fail to take the differences in efflux into account.

A high adsorption of arginine in the AFS was recorded before taken up by root cells. Sutch an cationic amino acids adsorption may be an efficient scavenging mechanism and represent a method through which plants may compete for these nutrients in the field.

The amino acid arginine and glycine supported growth in both Scots pine and Norway spruce to the same extent as a commercial fertilizer based on NO_3^- and NH_4^+.

Amino acids for commercial growth of conifers may provide alternatives to inorganic N sources and decrease N losses due to a strong absorption in the growth substrate.

Thus, the results presented in this thesis support previus findings suggesting that organic N compund constitute a potential important source of N for plants. However, several questions still remains unanswered. As discussed above the *capacity* of plants to absorb organic N sources has been known for a long time and is well recognised in numerous studies. But even though an accumulating number of studies have suggested that plants are able to absorb organic N sources in field situations the quantitative importance of organic-N, as opposed to inorganic N for the N nutrition of these plants is still not understood. This may largely be explained by the complexity in performing realistic uptake studies in the field. In order to understand the significance of organic N for plants it is important to be able to follow the uptake of these N sources during longer periods of time. It is also important to increase our knowledge about the fluxes and turnover of organic N sources in different soils. We also have to increase our knowledge about the physiological properties of these "new" N sources, which may influence their mobility and transport in soils. The high heterogeneity in the abundance of these N sources (microsites) must also be furthere studied to help us understand the availability of these organic N sources in different ecosystems. If we are able to understand the underling factors that governs the uptake and utilazation of amino acids in plant we may in the future be able to determine the significant of these N compounds in diffrent ecosystems.

Thus, much works remains to be done in order to fully understand the role of organic N sources in plants total N budget. Development of new techniques and approaches may make it possible to challenge this question and reveal the importance of organic N uptake in plants.

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