Nitrogen Nutrition and Biomass Distribution in Conifers

Aspects of Organic and Inorganic Nitrogen Acquisition

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Abstract

The main objectives of the work presented in this thesis were to increase our understanding of how different chemical forms of nitrogen (N) affect the growth and biomass distribution of conifer seedlings and hence their establishment and performance in field. Growth studies of Scots pine (*Pinus sylvestris* (L.)), Norway spruce (*Picea abies* (L.) Karst.) and the model plant Arabidopsis (*Arabidopsis thaliana*, ecotype Col-0) showed that plants can grow at similar (or higher) rates on organic N sources to those on the inorganic N sources ammonium (NH_4^+) and nitrate (NO_3^-). Cultivation on arginine also improved the field performance of Norway spruce seedlings by increasing their current-year shoot growth, despite smaller initial shoot length. Moreover, plants supplied with organic N distributed a relatively larger proportion of their biomass to root structures than controls with similar total biomass and N contents grown on inorganic N sources. Detailed studies on Arabidopsis revealed that an increase in the root:shoot ratio coincided with high retention of organic N in the roots, implying that the site of assimilation may be of importance for the short-term distribution of biomass. Further, studies on Scots pine seedlings deprived of carbohydrates suggested that the uptake, reduction and assimilation of NO_3^- are highly dependent on recent photoassimilates and that use of organic N may have considerable energetic benefits for plants, especially under conditions that limit carbohydrate supplies.

The results from the studies underlying this thesis highlight the potential role of organic N in the nutrition of conifer seedlings, the links between seedling nutrition, morphology and field performance, and effects of organic N on biomass distribution. They suggest that organic N may serve as an alternative or complement to inorganic N sources in seedling production, and may help attempts to improve seedling establishment in the field.

Keywords: Amino acids, Ammonium, *Arabidopsis thaliana*, Arginine, Carbohydrates, Carbon, Glutamine, Nitrate, *Picea abies*, *Pinus sylvestris*, root:shoot ratio

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Dedication

Mo chuisle mo chroí

Tror du det är så himla lätt då, när man bara tänker på glass?
Om konsten att följa en instruktion

Contents

List of Publications

This thesis is based on the work described in the following papers, which are referred to by the corresponding Roman numerals in the text:

I Cambui C.A., Svennerstam H., Gruffman L., Nordin A., Ganeteg U. and Näsholm T. (2011). Patterns of plant biomass partitioning depend on nitrogen source. *PLoS ONE* 6(4), e19211.

II Gruffman L., Palmroth S. and Näsholm T. Organic nitrogen uptake of Scots pine seedlings is independent of current carbohydrate supply. (submitted manuscript).

III Gruffman L., Jämtgård S. and Näsholm T. Nitrogen status and co-occurrence of organic and inorganic nitrogen sources influence root uptake by Scots pine seedlings. (manuscript).

IV Gruffman L., Ishida T., Nordin A. and Näsholm T. (2012). Cultivation of Norway spruce and Scots pine on organic nitrogen improves seedling morphology and field performance. *Forest Ecology and Management* 276, 118-124.

Papers I & IV are reproduced with kind permission of the publishers.

Linda Gruffman’s contributions to the papers appended to this thesis were as follows:

I Participated in performing the experiments, analysing the data and writing the paper.

II Planned the experiments together with the co-authors, performed the experiments, analysed the data and wrote the paper.

III Planned the experiments together with co-authors, performed the experiments, analysed the data and wrote the paper.

IV Planned and established field trials together with T Näsholm and J Öhlund, analysed the data together with co-authors and wrote the paper.

1 Introduction

1.1 Background

The objectives of the studies this thesis is based upon were to deepen our knowledge of the nitrogen (N) nutrition of conifers and the effects of organic and inorganic N sources on their biomass distribution and growth. Nitrogen is one of the key elements of all living organisms, including plants, as it is a building block of numerous vital macromolecules such as amino acids, proteins and DNA. Amongst many other physiological processes of plants, N is also involved in capturing light energy since it is an essential component of chlorophyll and the proteins in protein/chlorophyll complexes. Together with hydrogen and oxygen, carbon (C) and N are the most abundant elements in plants. Furthermore, N and C cycling within the plant are highly interconnected; C gained from photosynthesis in leaves or needles provides energy and structural building blocks for root uptake and assimilation of nitrogenous compounds, while a significant proportion of N gained through these C investments is used in synthesis of the light energy-capturing and C-fixing molecules and structures.

As an essential element of both structural and functional components of plants, the availability of N is decisive for controlling plant productivity. However, N availability in most agricultural and forest ecosystems is limiting for plant production (Vitousek *et al.*, 1997; Tamm, 1991; Vitousek & Howarth, 1991). Thus, ever since the introduction of the Haber-Bosch process, the industrial fixation of N, through the conversion of unreactive atmospheric dinitrogen (N_2) into plant available inorganic N used for fertilizers, has increased exponentially. The use of N fertilizers has enabled an enormous increase in crop production, thereby sustaining the increase of the human population. However, the use of industrial fertilizers has also accelerated the global N cycle, with accompanying accelerations in the global C cycle and other elemental cycles (Rockström *et al.*, 2009; Gruber & Galloway, 2008; Vitousek *et al.*, 1997).

Although the vast majority of fertilizer used today is applied in agriculture, the demands on forest production are likely to increase further and thereby the use of N fertilizers in this sector as well. A profound difference between crop and forest production is that forestry is expected to supply both products and services i.e. not only produce timber and fiber but also play important roles in C sequestration, maintenance of water quality and biodiversity, and the provision of suitable habitats for wildlife and recreation (Burger, 2009). This places additional pressure on N management in forest ecosystems.

In contrast to agricultural soils, boreal forest soils are dominated by organic N (Inselsbacher & Näsholm, 2012). Plants have the capacity to take up and use organic N (Näsholm *et al.*, 2009; Näsholm *et al.*, 1998), but the extent to which organic N serves as a significant N source is currently unclear. Research on organic N nutrition is still at an early stage compared to inorganic N nutrition, although the ability to take up organic N was recognized more than a century ago (Hutchinson & Miller, 1911). Increasing our understanding of the relative importance of organic and inorganic N forms for seedling production and forestry is critical for improving fertilizer use and possibly mitigating negative consequences of using fertilizers. Several fundamental issues need to be clarified. Firstly, it will be important to elucidate how different N sources affect seedling growth, quality and dry matter distribution among plant parts, and to get insights to the underlying mechanisms responsible. Secondly, it will be important to determine if organic N can serve as a complement to, or substitute for, inorganic N sources in seedling production.

1.2 Soil Nitrogen

Nitrogen is present in the soil in diverse compounds, ranging from the simple inorganic forms NH_4^+ and NO_3^-, to organic forms such as amino acids, peptides and proteins. The physical and chemical properties of these compounds, such as their size and charge, strongly affect their mobility and plant availability in the soil. Both inorganic and organic N forms may be absorbed to soil particles or present as solutes in the soil solution. However, in early successional stages, natural ecosystems depend on the conversion of atmospheric N_2 to plant available N forms to meet the N requirements of early colonizing species. These inputs are mainly provided through biological N fixation by free-living bacteria and symbiotic associations between N-fixing bacteria and plants, together with lightning-induced wash-out of atmospheric N (cf. Raven *et al.*, 1998; Vitousek *et al.*, 1997). As succession progresses, N continuously accumulates in the ecosystem, which in northern boreal forests to a considerable degree may be accounted for by the association between cyanobacteria (*Nostoc* sp.) and feather mosses (*Pleurozium schreberi* and *Hylocomium splendens*) (Zackrisson *et al.*, 2009; Zackrisson *et al.*, 2004; DeLuca *et al.*, 2002). In addition, anthropogenic N inputs in the form of forest fertilization and atmospheric N deposition also contribute to total N inputs. In conventional forest fertilization practices in Sweden, 150 kg N ha^{-1} is added about 15 years before final harvest, and in 2011 an area of 52,900 ha of forest land was fertilized (Skogsstyrelsen, 2012). Atmospheric N inputs range from about 1.5-2 kg N ha^{-1} yr^{-1} in the north to about 8.5 kg N ha^{-1} yr^{-1} in the southwest (Andersson *et al.*, 2011). The decomposition of plant litter and other dead organic material on the soil surface is the subsequent starting point of the release of plant-available N forms into the soil. Depolymerization of proteins through proteolytic activity by mycorrhizal fungi, exudation from plant roots and soil microbes, into peptides and amino acids is followed by their mineralization to NH_4^+. Both NH_4^+ and amino acids, and to some extent peptides and proteins (Soper *et al.*, 2011; Paungfoo-Lonhienne *et al.*, 2008), can be taken up by plants and microorganisms. Ammonium can be further converted to NO_3^- through nitrification and may, under certain circumstances, also be lost to the atmosphere through volatilization. Under anaerobic conditions NO_3^- may also be lost to the atmosphere by denitrification, and due to its negative charge NO_3^- is also highly mobile in the soil which might lead to losses from the system by leaching (Fig. 1).

1.2.1 Soil Nitrogen Availability

Although often highly abundant in soil, N is commonly referred to as the limiting element for plant growth in terrestrial ecosystems such as boreal forests (Tamm, 1991; Vitousek & Howarth, 1991). This seemingly contradictory contention is due to the limited accessibility for plant uptake of many of the vast array of chemical forms of N present in the soil of these ecosystems, as most of the soil N is bound in organic compounds of low availability, or practically unavailable, for plant uptake. The limitation was long thought to lie in slow mineralization rates caused by factors such as low soil temperatures, low pH, anaerobic conditions and high proportions of dead organic matter, rather than the actual N content of the soil (cf. Miller & Cramer, 2004). This conclusion was based on the assumptions that the major N sources for plants are the inorganic N forms NH_4^+ and NO_3^-, and that plants are poor competitors for N against microorganisms (Schimel & Bennett, 2004).

However, during the last two decades research in ecosystems such as the arctic, taiga and alpine, where inorganic N supply by mineralization cannot meet plant demands, have led to the conclusion that plants growing in these ecosystems must have access to other N sources to sustain their annual production (reviewed by Näsholm *et al.*, 2009).

In the light of these findings, a new conceptual model of the soil N cycle has emerged, according to which the limiting step in N-poor ecosystems lies in the depolymerization rates of proteins to peptides and amino acids rather than in slow rates of mineralization (Jones & Kielland, 2012; Jones *et al.*, 2009; Rennenberg *et al.*, 2009; Schimel & Bennett, 2004) (Fig. 1). Recent advances in soil sampling techniques have also revealed that organic N forms dominate the plant-available N pool in boreal forest soils (Inselsbacher & Näsholm, 2012; Inselsbacher *et al.*, 2011).

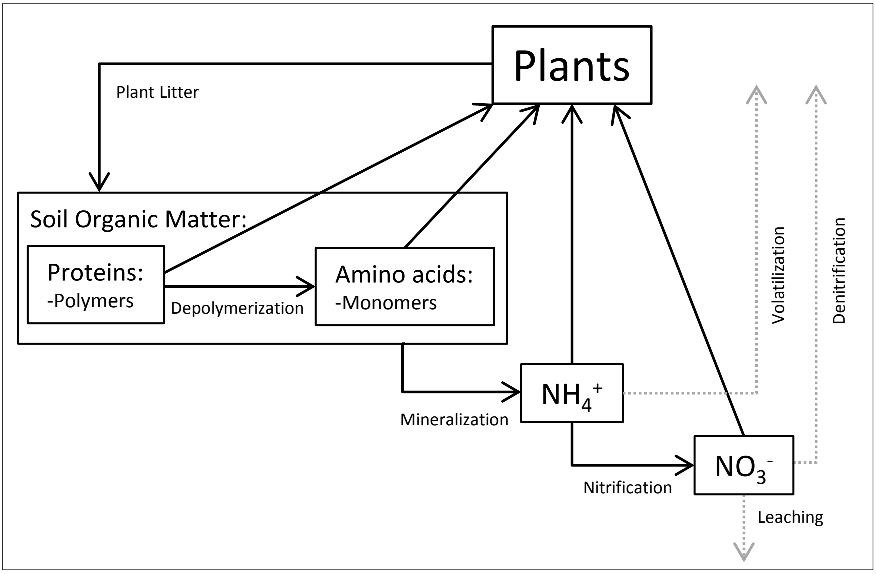

Figure 1. Simplified scheme of the chemical forms of N present in soils and the main processes involved in the release of plant-available N. Dashed lines indicate N losses from the system by volatilization or leaching.

1.3 Competition for Organic Nitrogen

Plants' ability to grow on organic N forms was recognized more than a century ago (Virtanen & Linkola, 1946; Hutchinson & Miller, 1911), but their role as a potentially significant N source for plant growth was long questioned, the underlying argument being that competition for N in the soil is intense among plants and soil microbes and that plants are weak competitors in this context.

Diverse plant species inhabiting virtually all types of ecosystems from the arctic to sub-Antarctic have been shown to take up amino acids (Dannenmann *et al.*, 2009; Gallet-Budynek *et al.*, 2009; Kielland *et al.*, 2006; Nordin *et al.*, 2004; Nordin *et al.*, 2001a; Persson & Näsholm, 2001; Falkengren-Grerup *et al.*, 2000; Schmidt & Stewart, 1999; Näsholm *et al.*, 1998; Raab *et al.*, 1996; Schimel & Chapin, 1996; Kielland, 1994). Moreover, plants have also been shown to have the capacity to take up larger organic compounds, such as peptides (Soper *et al.*, 2011) and proteins (Paungfoo-Lonhienne *et al.*, 2008).

In N-limited ecosystems symbiotic associations between plants and mycorrhizal fungi increase the competitiveness of plants against other soil microorganisms. Conifers growing under natural conditions are mycorrhized, which provides an extended root surface area thereby improving their nutrient acquisition (but see Näsholm *et al.*, 2013). Additionally, ectomycorrhizal fungi have high capacity to take up both inorganic and organic N forms (Chalot & Brun, 1998; Abuzinadah & Read, 1988) and a number of fungal transporters that are active in N uptake have been reported (reviewed by Müller *et al.*, 2007). It has been demonstrated that a wide variety of boreal forest plants, non-mycorrhizal and mycorrhizal, have the ability to use organic N, but this does not exclude the importance of mycorrhizal associations for nutrient capture in natural ecosystems (Persson & Näsholm, 2001).

However, increasing evidence that plants have the capacity to take up and use intact organic N sources, and that they can successfully do so even in N-limiting conditions, has led to a reevaluation of plant-microorganism competition for N, including both spatial and temporal aspects of N availability (Kuzyakov & Xu, 2013; Hodge *et al.*, 2000). In recent years there have been important contributions to elucidation of the importance of organic N nutrition, including the identification of transporters mediating direct root uptake of amino acids and peptides (Lehmann *et al.*, 2011; Svennerstam *et al.*, 2011; Komarova *et al.*, 2008; Svennerstam *et al.*, 2008; Lee *et al.*, 2007; Svennerstam *et al.*, 2007; Hirner *et al.*, 2006).

1.4 Plant Nitrogen Acquisition and Assimilation

1.4.1 Provision of Nitrogen to the Plant Root

In order for plants to take up nitrogenous compounds from the soil solution these compounds have to come into contact with the root surface. This occurs through three major mechanisms: mass flow, diffusion and interception of which the first two are most important. Mass flow of nutrients is caused by the movement of water and solutes from the soil towards the root surface and is driven by the water potential gradient resulting from plant transpiration. When plant demands exceed those delivered by mass flow, which is often the case in low-productivity soils such as those of boreal forests, a concentration gradient between the root surface and the surrounding bulk soil builds up, which drives the diffusion of compounds towards the root. Root interception occurs when roots grow through areas where nutrients are located in the soil (Lambers *et al.*, 1998). However, interception accounts for a smaller fraction of the nutrients taken up, and is only of quantitative importance for the uptake of nutrients that have relatively low mobility in the soil solution (Miller & Cramer, 2004).

The diffusion rate of a specific N compound in the soil depends not only on its concentration gradient in the soil but also its diffusion coefficient. This diffusion coefficient is determined by factors such as the size and charge of the compound, and the temperature, moisture content and buffer capacity of the soil. In water the diffusion coefficients are similar for NO_3^- and NH_4^+; but they differ substantially in soil, being 2.8 x 10^{-1} cm^2 d^{-1} for NO_3^- and about 100-fold less for NH_4^+ in sandy loam for instance, i.e. NO_3^- can diffuse about 1 cm d^{-1}, while NH_4^+ diffuses about 1 mm d^{-1} (Owen & Jones, 2001). The diffusion coefficients of amino acids vary greatly due to differences in size and charge (cationic, neutral or acidic), but Owen & Jones (2001) found that three studied amino acids (lysine, glycine and glutamate) diffused more slowly than NO_3^- in the soil. However, Inselsbacher & Näsholm (2012) recently published results from a study in 15 boreal forest stands using a novel noninvasive method based on microdialysis, which in contrast to traditional lysimeter techniques samples soil through induced diffusion and thus mimics root acquisition through diffusion. They found that supply rates of the amino acids glycine and glutamine were higher than that of NH_4^+, while supply rates of NO_3^- were about 30% lower than those of NH_4^+ and similar to those of various other amino acids. Arginine (the focal amino acid in this thesis) was not detectable with this method, but KCl extractions showed that arginine was primarily present in the exchangeable N pool, indicating that together with diffusion interception may be important for roots to encounter this amino acid in forest soils. Nevertheless, labeling experiments with arginine have shown that plants have the ability to acquire this N source in comparable quantities to those of other amino acids and NH_4^+, from both peat substrate and in field settings (Dannenmann *et al.,* 2009; Persson *et al.,* 2003; Öhlund & Näsholm, 2001). Thus, in spite of very low diffusion rates of arginine in the soil, plants are *de facto* able to efficiently capture this N source.

1.4.2 Root Uptake and Assimilation of Inorganic Nitrogen

Uptake of the inorganic N forms NO_3^- and NH_4^+ is a well-characterized process and numerous genes encoding transport proteins involved in their root uptake have been identified (for reviews, see Glass, 2009; Miller & Cramer, 2004; Williams & Miller, 2001).

The uptake of NO_3^- across the plasma membrane is an active process mediated by carrier proteins which also cotransport protons (H^+) from the soil solution. Therefore, uptake is dependent on energy from adenosine-5’-triphosphate (ATP) to energize active extrusion of H^+ into the surrounding soil and thus maintain the H^+ gradient across the plasma membrane (reviewed by Miller & Cramer, 2004). Three classes of NO_3^- uptake systems have been detected in various plants. Two are high-affinity systems (HATS) that operate at low N concentrations, one of which is constitutively expressed, even in the absence of NO_3^-, while the other is induced when roots are exposed to low concentrations of NO_3^-. The third is a low-affinity transport system (LATS) that operates at high external NO_3^- concentrations (Glass, 2009; Williams & Miller, 2001; Kronzucker *et al.,* 1995). Further, an inducible LATS seems to be present in the model plant Arabidopsis, hence there are four systems with distinct kinetic and expression patterns in this species (reviewed by Glass, 2009).

The transport systems responsible for uptake of NH_4^+ are also divided into HATS and LATS, both constitutively expressed. High-affinity uptake of NH_4^+ is an active uniport process (Ludewig *et al.,* 2002), but low-affinity transport can be passively mediated by channel proteins, thus the energy requirements for NH_4^+ uptake are therefore generally lower than for NO_3^- uptake, since the former is driven by the negative membrane potential of the cell (reviewed by Miller & Cramer, 2004; Williams & Miller, 2001).

Following uptake, NO_3^- has to be reduced to NH_4^+ before assimilation into amino acids. The first step in this process is the reduction of NO_3^- to nitrite (NO_2^-), a reaction catalyzed by the enzyme nitrate reductase (NR). Following this initial step, nitrite reductase (NiR) catalyzes further reduction to NH_4^+. These are energy-intensive reactions that in all require donation of eight electrons, and thus the consumption of approximately 8-12 ATP molecules (cf. Bloom *et al.,* 1992). Ammonium originating from root uptake or NO_3^- reduction is assimilated into amino acids by the Glutamine synthetase/ Glutamate-2-oxoglutarate aminotransferase (GS/GOGAT) pathway, yielding the amino acids glutamine and glutamate as the primary products. This process is the starting point for the synthesis of all other amino acids and is associated with both energetic costs in the form of two electron donations and consumption of an ATP molecule and costs in the form of structural carbon skeletons. In terms of carbon costs, Bloom et al. (1992) found that uptake and subsequent assimilation of NO_3^- and NH_4^+ account for 23% and 14%, respectively, of total root carbon catabolism and suggested the additional energy requirements associated with the use of NO_3^- may limit growth under NO_3^- nutrition. However, other studies on the actinorhizal plant species grey alder (*Alnus incana* (L.) Moench) have suggested the carbon economy of N_2-fixing plants was not adversely effected in terms of net photosynthesis, root respiration or biomass accumulation compared to non-nodulated alders grown on NO_3^- or NH_4^+ (Sellstedt, 1986; Sellstedt & Huss-Danell, 1986).

1.4.3 Root Uptake and Assimilation of Organic Nitrogen

The acquisition of amino acids has similarities to that of inorganic N sources and their uptake has been described as an active proton-coupled process (Rentsch *et al.*, 2007). Both HATS and LATS that participate in the process have been identified in Arabidopsis (Svennerstam *et al.*, 2011; Svennerstam *et al.*, 2008; Lee *et al.*, 2007; Svennerstam *et al.*, 2007; Hirner *et al.*, 2006). Further, high affinity arginine uptake has been observed in barley (*Hordeum vulgare*) (Jämtgård *et al.*, 2008) and Arabidopsis (Svennerstam *et al.*, 2011), indicating that this N source might be important for N nutrition at field-relevant concentrations.

Given the diversity of amino acids with regards to size, charge (cationic, neutral or acidic), polarity and concentrations in the soil, it seems intuitively likely that more transporters should be involved in the direct uptake of these compounds than in NO_3^- and NH_4^+ uptake. However, Svennerstam et al. (2011) have provided convincing evidence that only two distinct transporters are important for the uptake of amino acids in Arabidopsis: amino acid permease 5 (AAP5), which is responsible for the uptake of cationic amino acids including arginine, and lysine-histidine-like transporter 1 (LHT1), which is responsible for the uptake of neutral and acidic amino acids. These two transporters have distinct, complementary affinities to the tested amino acids. Moreover, double mutants defective in both AAP5 and LHT1 have strongly reduced uptake capacities for all tested amino acids; corroborating the critical role of these transporters in amino acid uptake.

Few studies have investigated the metabolic pathways of amino acids in general, and arginine in particular, immediately following root uptake (but see Funck *et al.*, 2008). However, generally, when amino acids enter the root pool they may be directly used as substrates for protein synthesis, deaminated or transported to sink tissues (Miller & Cramer, 2004). Arginine is considered a storage compound in many forest species including conifers (Nordin *et al.*, 2001b; Edfast *et al.*, 1996; Näsholm *et al.*, 1994) and storage proteins typically contain high proportions of arginine (Müntz, 1998). In conifers, arginine is catabolized to ornithine, NH_4^+ and CO_2, through activities of the enzymes arginase and urease. Ornithine is subsequently metabolized to other amino acids, while the NH_4^+ is re-assimilated by the GS/GOGAT pathway (Cánovas *et al.*, 2007).

In contrast to N in the forms of NO_3^- or NH_4^+, N in amino acids is obviously already incorporated into organic molecules. This may at least in the short term lead to energetic savings, given that amino acids can be used as a direct substrate for further biosynthesis of proteins (Zerihun *et al.*, 1998) or, alternatively if catabolized, energy and carbon skeletons from amino acids may be used for other physiological processes in the plant.

1.5 Nitrogen Availability and Plant Biomass Distribution

Plant growth and development depend on a variety of environmental factors including light, temperature, water and nutrient availability. As N is one of the nutritional elements required in the largest quantities, N availability is highly important for plant production in general. However, N, together with C, also plays a critical role in the relative distribution of biomass between plant parts. Notably, as well described in the literature, plant N availability and the internal N concentration affect the partitioning of biomass between roots and shoot (Bown *et al.*, 2010; Bauer & Berntson, 2001; Ericsson, 1995; Ingestad & Ågren, 1991; Wilson, 1988; Brouwer, 1962a; Brouwer, 1962b). Plants respond to limited N availability by allocating relatively more new biomass to roots than to shoots, although at a decreased rate. Hence, N deficiency results in increases in root:shoot or root:plant ratios.

The underlying molecular and physiological mechanisms of such responses are not entirely clear. However, N deficiency results in an accumulation of starch and sugars in leaves followed by an increase in translocation of sucrose to roots. In contrast, accumulation of NO_3^- in the shoot is negatively correlated with C allocation to roots. Hence, N-deprived plants allocate a larger proportion of C to root structures, which may explain the observed increase in root:shoot ratios (reviewed by Hermans *et al.*, 2006). Plant hormones have also been suggested to be involved in the regulation of biomass partitioning. For instance the production and export of root-derived cytokinins to shoots are decreased in N-deprived plants, while the addition of these hormones is associated with a retention of photoassimilates in shoots (reviewed by Beck, 1996).

It was early recognized that root growth is affected by the availability of photoassimilates and it was hypothesized that shoot structures had first priority to carbon derived from photosynthesis. However, Shank (1945) and numerous other authors suggested that nutrients and water are critical for allocation of biomass, with the idea that when any of these resources are limiting, a larger fraction of these resources is retained in roots, with increasing root:shoot ratios as a result. Brouwer, (1962b) suggested that the internal balance between C and N in plants is the key determinant of biomass distribution between plant parts, based on the hypothesis that nutrients, i.e. N, and water, are acquired by roots, which thus have first priority for these resources, while C is acquired by the shoot, which accordingly has first priority for this resource. Consequently, when either of these resources is in short supply, allocation of that specific resource to sink tissues will decrease in favor of the source tissue. Hence, impaired shoot growth due to N deficiency limits the use of carbohydrates in the shoot, resulting in a subsequent increase in allocation to below-ground structures where it enhances root growth because root growth is commonly limited by carbohydrate supply (Brouwer, 1962a; Brouwer, 1962b). In his work Brouwer (1962b) further developed the idea of a functional equilibrium i.e. plants will “strive” towards an optimal root:shoot ratio specific to the developmental phase of the plant, which he showed by either depriving maize plants (*Zea mays*) of N, hence increasing the root:shoot ratio over time or adding N and thereby decreasing the root:shoot ratio. When N was added again to deprived plants, they rather quickly restored the same root:shoot ratio as their untreated counterparts. Importantly, it was recognized that such seedling responses were caused by alterations in the internal concentrations of C and N in the plants, and only indirectly as a result of a change in the external N concentration.

The work of Brouwer, and others before him, later formed the basis for what is known as the “Thornley model” which mathematically describes the control of root:shoot ratio, taking into consideration the supply, transport and utilization of N and C by roots and shoots, respectively (Thornley, 1972). This model seems to explain effects of most changes in the availability of light, C, water and N, despite obvious simplifications, regarding for instance, the transport of photoassimilates and N being unidirectional and only caused by concentration gradients within the plant (cf. Ericsson, 1995; Wilson, 1988). Numerous models have been subsequently developed in attempts to explain the control of root:shoot ratios, including simple empirical, allometric models, i.e. models built by fitting equations to observations that are not intended to explain any underlying mechanisms. Others have included, to varying degrees, mathematical functions intended to describe various biological processes associated with plant growth, amongst them the functional equilibrium, Thornley’s, other optimization or steady-state and hormonal models (Ågren & Franklin, 2003; Thornley, 1998; Thornley, 1995; Dewar, 1993; Gleeson, 1993; Ingestad & Ågren, 1991; Wilson, 1988; Ågren & Ingestad, 1987; Thornley, 1972). Some more recent publications have also presented forest stand-level N and C allocation models (Valentine & Mäkelä, 2012; Mäkelä *et al.*, 2008; Franklin, 2007).

The concept of root:shoot ratio has been criticized because many studies and models have failed to account for what is called ontogenetic drift, i.e. the root:shoot ratio changes over time due to the developmental stage and hence the size of the plant. Observed differences in root:shoot ratios at a certain time point may therefore reflect differences in plant size rather than differences in distribution patterns between plant parts (Reich *et al.*, 2002; Wilson, 1988).

In their extensive work on steady-state nutrition of forest tree species during the 1980’s and 1990’s, Ingestad and co-workers found that changes in root:shoot ratios can be ascribed to changes in the plants’ internal nutrient status, and that the relative growth rates of different plant parts will be equal when a steady state of the internal nutrient status is obtained (Ericsson & Ingestad, 1988; Ingestad & Ågren, 1988; Ingestad & Lund, 1986). Consequently, it was pointed out that in order to study effects of nutrient availability on biomass allocation between plant parts, the nutrient status should be maintained constant over a sufficient time period for plants to reach a steady state (Ericsson, 1995; Ingestad & Ågren, 1991). It was also found that an increased allocation of resources to roots in response to restricted nutrient supply does not result in a concomitant increase in uptake efficiency (i.e. uptake rate x unit root growth rate^{-1}); instead, resources are used to increase the root growth rate. Hence, in a nutrient-restricted soil where the diffusion rates of nutrients limit uptake, plants use these resources to explore new environments and thereby increase the probability of nutrient capture (cf. Ingestad & Ågren, 1988).

In accordance with the above rationale, plants with similar total biomasses and internal N concentrations should be used in “snap shot” comparisons of root:shoot ratios arising from growth on different N sources, in order to avoid differences caused by ontogenetic drift and/or plant N status, both of which may confound assessments of effects of N sources (Fig. 2). We currently lack knowledge of how N forms influence biomass distribution in forest trees, and results from the few studies that have examined effects of different inorganic N sources, i.e. NO_3^- and NH_4^+, singly or in combination, are not consistent in this respect (Bown *et al.*, 2010; Heiskanen, 2005; Bauer & Berntson, 2001). However, the study by Bown et al. (2010) on distribution patterns of radiata pine (*Pinus radiata*), which includes allometric corrections for ontogenetic drift, suggested that N supply but not N source affected biomass allocation over time.

Previous studies have largely ignored the possibility that the use organic N forms may influence biomass distribution, and few studies have compared the effects of inorganic and organic N sources in this respect. However, Soper et al. (2011) found that plants growing on amino acids and peptides distributed relatively more biomass to roots than to shoots. It has also been shown that absorbed amino acid N is allocated to shoots at a slower rate than that of NO_3^- indicating that it has a similar allocation pattern, or possibly even more root-specific pattern, than that of NH_4^+ (Persson *et al.*, 2006; Schmidt & Stewart, 1999; Papers I & II). As will be discussed later, the results from the studies this thesis is based upon strongly suggest that organic N does affect biomass distribution so that conifers as well as Arabidopsis distribute relatively more biomass to root structures, despite similar total biomasses and N contents (Papers I & IV, Fig. 2).

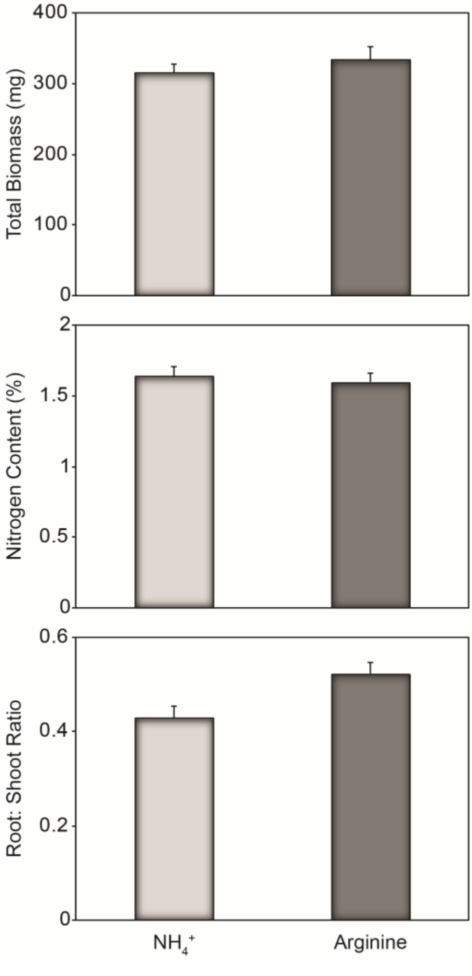

Figure 2. Total biomass, nitrogen content and root:shoot ratio of Scots pine seedlings cultivated on NH_4^+ or arginine. Arginine-treated seedlings have significantly higher root:shoot ratios, while the other seedling traits are similar between treatments (One-way ANOVA, $P = 0.009$, data transformed when required to meet ANOVA assumptions).

1.6 Nitrogen in Forest Nursery Fertilizers

Due to the long rotation times in boreal forests and high costs of forest regeneration it is of the utmost importance for the forest industry to produce and deliver high quality seedling material. In order to do so forest nurseries apply large amounts of N over relatively small areas (Juntunen & Rikala, 2001). Although the amounts of fertilizers used in forest seedling production are relatively small compared to those used in agriculture they still may have a substantial environmental impact, at least locally, especially when nurseries are located in areas close to ground water reservoirs or rivers (Juntunen *et al.*, 2002). Results from a questionnaire survey concerning production practices and the use of fertilizers in Swedish forest nurseries (collectively accounting for two thirds of the total seedling production in Sweden) showed that the N addition per produced seedling was 100 mg N per containerized seedlings and 1 g N per bareroot seedling (Nyström *et al.*, 2001). In total, Swedish forest nurseries use approximately 50 tons of N annually for their production, of which about 50% is leached into the surrounding soil. Another study found that 25-70% and 17-80% of the N supplied to containerized seedlings and bareroot seedlings, respectively, in three conifer nurseries was leached, and that most of the N was lost in the early phase of cultivation (Hannerz & Rosenberg, 2001). Such losses could probably be decreased by using more efficient cultivation practices such as demand-driven fertilization techniques (Rytter *et al.*, 2003; Juntunen *et al.*, 2002).

Commercially available inorganic fertilizers typically contain about 40% NH_4^+-N and 60% NO_3^--N, although many conifer species have well-established and strong preferences for NH_4^+ over NO_3^- (Miller & Hawkins, 2007; Persson *et al.*, 2006; Öhlund & Näsholm, 2001; Kronzucker *et al.*, 1997; Kronzucker *et al.*, 1996; Buchmann *et al.*, 1995; Kamminga-van Wijk & Prins, 1993; Papers II & III). Moreover it has been shown that the recovery of NO_3^- is low both in the seedlings and the growth substrate of containerized seedlings (Öhlund & Näsholm, 2002). The obvious solution would, therefore, be to increase the fraction of NH_4^+ in fertilizers in order to decrease the apparent risks of NO_3^- leaching. However, this has been shown to cause problematic acidification of the growth substrate and NH_4^+ toxicity in seedlings (Britto & Kronzucker, 2002; Öhlund & Näsholm, 2001).

2 Objectives

The main objectives of the studies this thesis is based upon were to increase our understanding of how different chemical forms of N affect the growth and biomass distribution of conifer seedlings, and hence their establishment and performance in the field. In order to realize these aims a series of studies were performed, ranging from highly controlled experiments on the model plant Arabidopsis in sterile growth conditions (Paper I) to field trials with conifer seedlings cultivated under forest nursery conditions (Paper IV). Nitrogen availability and the internal N concentration have previously been shown to influence biomass partitioning between roots and shoots. In Study I we sought to elucidate if also the chemical form of N affects the distribution of biomass between roots and shoots and if different chemical forms of supplied N are distributed differently between plant parts. In Studies II & III we performed uptake studies on Scots pine seedlings in greenhouse- and climate chamber conditions. The purpose of Study II was to compare the dependence of the acquisition and assimilation of inorganic and organic N forms on energy from current photoassimilates transported from the shoot to the roots. In Study III we investigated effects of the internal N concentration and co-occurrence of organic and inorganic N sources on root N acquisition. Finally, the purpose of the field trials that resulted in Paper IV was to evaluate how an altered biomass distribution in conifer seedlings affects their ability to successfully establish in the field.

3 Materials and Methods

3.1 Choice of Study Systems

As mentioned in the former section, the main objective of the present thesis work has been to obtain greater knowledge of how different N forms affect the biomass distribution and growth of conifer seedlings. However, to fully understand the mechanisms underlying such effects it is essential to minimize external factors that could interfere with the uptake of nitrogenous compounds, such as mycorrhizal associations and soil microbial activity. Therefore, we decided to study biomass distribution and growth in detail in the model plant Arabidopsis, *Arabidopsis thaliana* (ecotype Col-0), in sterile conditions (Study I) and then extended the studies to include Scots pine (*Pinus sylvestris* (L.)) grown in greenhouse conditions where external factors are less controlled (Studies II & III). In Study IV Scots pine and Norway spruce (*Picea abies* (L.) Karst.) seedlings were cultivated in a commercial conifer nursery and subsequently planted in the field to investigate the potential effects of organic N nutrition in a practical setting. Following this strategy we aimed to combine studies of high precision with those of relevant practical importance.

3.2 Choice of Nitrogen Sources

The work mainly focused on the uptake characteristics and effects of four different N sources on the growth, biomass distribution and internal allocation patterns. These four N sources were the inorganic N forms NH_4^+ and NO_3^-, and two amino acids: arginine and (in Study I) glutamine. Arginine was chosen as a model amino acid partly because it had been used in previous work on conifers (Öhlund & Näsholm, 2004; Persson *et al.*, 2003; Öhlund & Näsholm, 2002; Öhlund & Näsholm, 2001) and partly because of its chemical properties. Arginine is a N-rich molecule containing four N atoms, which collectively contribute 32% of its molecular weight. It is positively charged and thus acts as a basic cation in the acidic pH of boreal forests soils, binding to negatively charged soil particles, which restricts its mobility. This, together with conifer seedlings' very high affinities for and high uptake rates of arginine, makes it suitable as a N source and as an alternative to conventional fertilizers in cultivation systems such as conifer nurseries where N is provided in large quantities and leaching of negatively charged NO_3^- may be of concern. However, although arginine has been shown to be a suitable N source for conifers, at high (mM) concentrations it inhibits growth of Arabidopsis (Forsum *et al.*, 2008). Therefore, in Study I glutamine was chosen as the organic N source and arginine was included only in traceable amounts to study its internal distribution.

3.3 Growth Conditions and Methodological Aspects

3.3.1 Growth Systems

In Study I, Arabidopsis was grown on agar plates in sterile growth conditions with N-free half-strength Murashige and Skoog (MS) medium (Murashige & Skoog, 1962) and the selected N sources added to suit our purposes. A sterile split-root experimental system was developed that enabled us to study the movement of nitrogenous compounds between plant parts without interference from external factors (Fig. 3). In the conifer studies (II & III) seedlings were cultivated in pots in climate chamber or greenhouse conditions with controlled light quality and light/dark cycles and in Study IV containerized seedlings were initially cultivated in a commercial forest nursery and subsequently transferred to the field. Fertilizer compositions were based on commercial fertilizers commonly used in forest nurseries, but with different N sources (Studies II, III & IV). In Study II, girdling was used as a tool to gradually deprive roots of current photoassimilates in order to study energy-dependent processes associated with root uptake of different N sources.

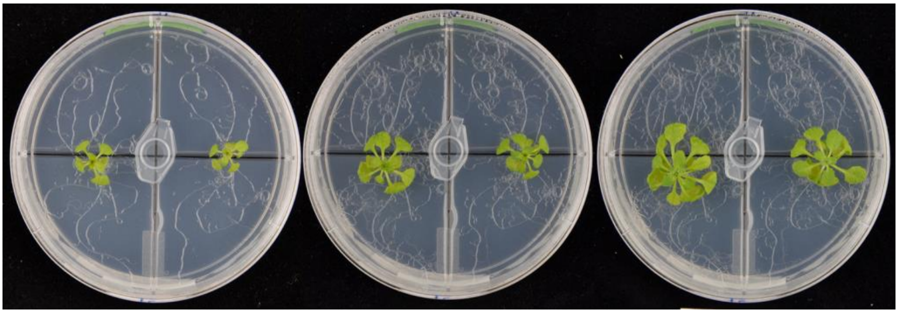

Figure 3. Arabidopsis plants 3, 7 and 10 days (left to right) after transfer to the split-root experimental system. Each compartment contained 3mM N, provided as 1.5 mM glutamine (upper compartments) or 3mM NO_3^- (lower compartments). Photograph courtesy of Camila Aguetoni Cambuí.

3.3.2 Assessing Nitrogen Uptake

In Studies I, II & III Arabidopsis and Scots pine seedlings were cultivated on, or exposed to, nutrient solutions with isotopically labeled N sources. Scots pine seedlings were transferred to a hydroponic system, where they were allowed to acclimate for three days before uptake assessments (Studies II & III). Nutrient solutions were changed daily and gently bubbled with air at all times. Study II was designed as a pulse-chase experiment, in which seedlings were allowed to take up different isotopically labeled N-containing solutions for two hours then transferred to identical but unlabeled solutions for 24 hours, allowing detection of post-uptake allocation of the different N sources between plant parts, while seedlings were harvested immediately after the two hour incubation in Study III (Fig. 4). The labeled N forms used in the experiments included universally labeled U-[$^{13}C_6$], [$^{15}N_4$]-L-arginine, α-^{15}N-glutamine, $^{15}NO_3^-$ and $^{15}NH_4^+$. Samples were analyzed by Elemental Analysis-Isotope Ratio Mass Spectrometry (EA-IRMS), for high precision determination of ^{15}N and (for arginine) ^{13}C contents, thereby enabling the supplied N sources to be traced and quantified within plant parts. A drawback of this technique, compared to depletion studies followed by analysis with High Performance Liquid Chromatography, HPLC, is that it does not account for possible efflux of the different N sources, i.e. the isotope technique can only be used to assess gross uptake, while the depletion technique can be used to assess net uptake. However, previous analyses have shown that arginine effluxes in short-term uptake studies, such as II & III are negligible (Jämtgård *et al.*, 2008).

Figure 4. Scots pine seedlings during uptake of U-[$^{13}C_6$], [$^{15}N_4$]-L-arginine (Paper III). Needles were attached to sterile filters and coupled to an aquarium pump for aeration.

3.3.3 Quantification of Uptake of Intact Amino Acids

Microbial activity may lead to the mineralization of amino acids. Thus, when assessing amino acid uptake by plants it is essential to distinguish between N taken up in the form of intact amino acids and N taken up as mineralized forms. This is not possible using amino acids that are labeled only at their N positions. However, use of universally labeled amino acids (U-[$^{13}C_6$], [$^{15}N_4$]-L-arginine) provides a tool to quantify intact uptake using the relationship between excess ^{13}C and excess ^{15}N in the plant (Näsholm *et al.*, 1998); the theoretical C_6:N_4 slope of regression for 100% uptake of intact arginine is 1.5 (Fig. 5). This method was used to quantify intact uptake in Studies II and III. However, since plants have substantially higher C contents than N (a few percent), and the natural abundance of ^{13}C in plant tissues is relatively high, attempts to quantify intact uptake are not straightforward, partly because dilution of the ^{13}C tracer may result in underestimates (Näsholm & Persson, 2001). Moreover, in long-term studies with universally labeled N sources losses of ^{13}C through respiration may shift the ^{13}C:^{15}N relationship and in a closed sterile system, such as that used in Study I, ^{13}C may also be lost by transpiration and recaptured by photosynthesis, possibly leading to ambiguous results. In Study II relationships between ^{13}C:^{15}N actually led to steeper slopes than those predicted by the theoretical relationship, probably indicating that some of the N was effluxed, while C was retained for energetic purposes in the carbohydrate-limited seedlings.

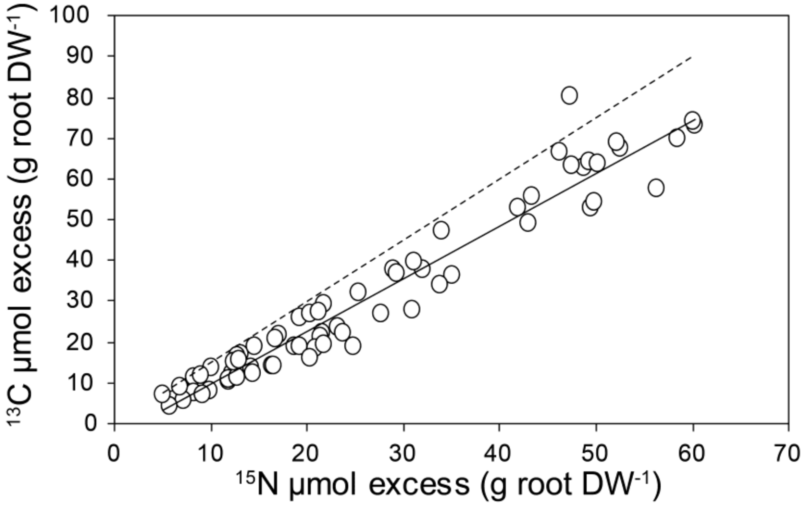

Figure 5. The relationship between excess ^{13}C and excess ^{15}N in Scots pine seedlings labeled with U-[$^{13}C_6$], [$^{15}N_4$]-L-arginine. The dashed line indicates the theoretical slope of regression (1.5) for 100% uptake of intact arginine, while the solid line indicates the slope of regression obtained from the recorded data, 1.30 (R^2= 0.95), indicating that at least 87% of the arginine was taken up in its intact form.

4 Results and Discussion

The major findings from the studies included in the present thesis are presented and discussed with the purpose to link results from individual studies to each other as well as to the main objectives of the thesis. Particular emphasis is placed on discussing the role of organic N for biomass distribution elaborating on possible underlying mechanisms as well as possible advantages for plants of the ability to adjust root:shoot ratios in response not only to N availability but also N forms.

4.1 Paper I

The main aims of the studies described in Paper I were to investigate whether the chemical form of available N affects biomass distribution between the roots and shoot and if different chemical forms of N acquired simultaneously from the growth media are distributed differently between plant parts.

In a first experiment we tested how growth and biomass distribution of Arabidopsis plants were affected by mixtures of NO_3^- and the amino acid glutamine, relative to those of pure NO_3^- and NH_4NO_3. The root mass fractions were consistently higher when the organic N source glutamine was included in the growth media, while the total biomass and N contents of the plants were similar. A second experiment was designed to elucidate whether the increased biomass distribution to roots of plants provided with the organic N source coincided with a high degree of that particular N source in the roots. Of the total N present in roots of plants grown on a mixture of NO_3^- and glutamine, 24% was derived from NO_3^--N while 76% was derived from glutamine-N. In plants grown on a mixture of NO_3^- and NH_4^+, 39% of the N was derived from NO_3^--N and 55% from NH_4^+-N. In plants grown on NO_3^- with arginine added in traceable amounts, arginine-N was more than twice as abundant in roots as in shoots. These results suggested that N derived from the organic N forms glutamine and arginine is retained to a significantly higher degree in roots than

NH_4^+-N and, in particular, NO_3^--N. This is accompanied by increased distribution of biomass to roots, which raised the question how absorbed N moves between plant parts. Using a split-root experimental system, we tested the possibility that overrepresentation of glutamine-N in roots resulted solely from incorporation of glutamine-N at the site of acquisition or if it was to some extent determined for root growth in general. Nitrogen derived from NO_3^- dominated the shoot N pool, and glutamine-N was less abundant in shoots than in roots. In the part of the root system that was supplied with glutamine, 87% of the N in that root part was derived from glutamine and 9% from NO_3^- allocated from the other part of the root system. Moreover, glutamine-derived N was also to a larger extent translocated to the part of the root system that was exposed to NO_3^-, where 70% of the N pool was NO_3^--derived and 25% was glutamine-derived. These results suggested that organic N is incorporated, to a high degree, at the site of acquisition and that reallocation of N between root parts is to a higher degree occurring for absorbed organic N than inorganic N.

Although the above results may provide insights into the effects of organic N on biomass distribution between plant parts, the mechanisms underlying such responses cannot be deduced from them. However, the primary site of assimilation differs between NO_3^- and glutamine; when NO_3^- availability is limited larger proportions of NO_3^- reduction and assimilation is located to roots, and as the NO_3^- availability increases shoot assimilation becomes more important (Andrews, 1986). Hence, a significant proportion of the absorbed NO_3^- may be directly transported to the shoot. In contrast, organic N has been suggested to be preferentially metabolized in roots (Persson *et al.*, 2006; Schmidt & Stewart, 1999). This could explain the observed overrepresentation of NO_3^- in shoots and parallel overrepresentation of glutamine-N in roots. Moreover, if root growth is limited by carbohydrate supply the use of glutamine for root growth may be advantageous from an energetic perspective (Bloom *et al.*, 2003; Zerihun *et al.*, 1998).

4.2 Paper II

In light of the findings from Study I, in the following study we examined the requirement for energy from recent photoassimilates transported from the shoot to the roots of Scots pine seedlings for the acquisition of arginine, NH_4^+ and NO_3^-. Seedlings were girdled at different time points to gradually deplete carbohydrate reserves, and this was followed by a pulse-chase study with isotopically labeled N sources.

Uptake of N originating from arginine was highly efficient, despite a steep decrease in carbohydrates and starch contents, while the uptake of both NH_4^+

and NO_3^- decreased significantly, suggesting that arginine-treated seedlings were able to benefit from carbon provided by the N source for energy-dependent processes associated with uptake, reduction and assimilation (Zerihun *et al.*, 1998). Analysis of the internal allocation between plant parts also showed that arginine-N was almost exclusively residing in roots (95-96%) 24 hours after tracer addition in both control seedlings and girdled seedlings, supporting the results from Study I, while there was a gradual increase in reallocation of N to shoots in NH_4^+- and NO_3^- treated seedlings. This was especially evident in seedlings supplied with NO_3^-; the allocation pattern of NO_3^--N was opposite in ungirdled seedlings to that of seedlings that had been girdled for seven days. In control plants, 72% of the acquired NO_3^--N resided in the roots while girdled seedlings transported 71% to shoots. As previously mentioned, assimilation of NO_3^- predominantly occurs in roots when external N concentrations are low (Andrews, 1986; Smirnoff *et al.*, 1984). Our results provide further information on the cause of a spatial shift of NO_3^- assimilation from roots to shoots, and suggest that NO_3^- assimilation in roots is highly dependent on recent photoassimilates. The stepwise reduction of NO_3^- to NH_4^+ in leaves may lead to energetic savings as relative to respiration-driven reduction in roots (cf. Andrews, 1986), which may explain the dramatic shift in reallocation of absorbed NO_3^--N to shoots.

4.3 Paper III

When N availability is low, and specifically when internal N concentrations are low, higher proportions of biomass are distributed to root growth. Studies I & II indicated that such a shift in biomass distribution may also depend on the chemical form of the N source, irrespective of whether N is in short supply. Therefore, in Study III we examined effects of the internal N status of the plant on short-term acquisition of the individual N forms arginine, NH_4^+ and NO_3^- in Scots pine seedlings. Additional aims were to investigate how the co-occurrence of other N forms in the uptake solution affects acquisition of individual N forms and if previous exposure to arginine affects its subsequent uptake, which would help elucidate the relative importance of these N forms for plant nutrition in nursery cultivation of seedlings and in the field. Seedlings were pre-treated with a low organic-, high organic- or high inorganic-N solution to obtain plant material with variable N status to enable studies of the importance of previous exposure to organic N on its uptake characteristics.

Uptake of arginine-N was higher in seedlings previously exposed to a high organic-N pre-treatment, suggesting that arginine uptake is positively, rather than negatively, affected by a high internal N status. Moreover, it suggests that uptake involves an inducible high-affinity transport system (iHATS), such as AAP5, previously reported to mediate high affinity uptake in Arabidopsis (Svennerstam *et al.*, 2011). In contrast to arginine uptake, uptake of NH_4^+ and NO_3^- was negatively affected by a high internal N status, regardless of whether the N forms were provided as single N sources or in mixtures, indicating that seedlings down-regulated uptake of these N forms in response to high internal N status. This was especially evident for NO_3^- uptake, in accordance with earlier studies (Glass *et al.*, 2002; Öhlund & Näsholm, 2001). Generally, uptake rates of arginine-N and NH_4^+-N was in the same order and about 10-fold higher than that of NO_3^--N, corroborating earlier findings that many conifer species have low NO_3^- uptake rates (Miller & Hawkins, 2007; Öhlund & Näsholm, 2001; Gessler *et al.*, 1998; Kronzucker *et al.*, 1995). In commercial seedling cultivation in Sweden, seedlings are supplied with high concentrations of inorganic N, commonly in a ratio of about 60% NO_3^- to 40% NH_4^+, despite low recoveries of the former. In contrast, recovery of arginine has been shown to be high in seedlings and growth substrates of containerized seedlings (Öhlund & Näsholm, 2002). Nitrate uptake in seedlings with a low internal N content was down-regulated in the presence of NH_4^+, but not in the presence of arginine in the high concentration range, which may have important implications for commercial conifer seedling cultivation practices.

4.4 Paper IV

The aim of the study reported in this paper was to investigate whether the chemical form of available N affects the growth and morphology of conifers (specifically Norway spruce and Scots pine) during commercial seedling production, and if so to what extent these effects influence seedling performance in the field. Hence, this study was performed to verify if the morphological and physiological characteristics observed in Studies I-III would also be displayed in a more practical setting.

The N sources (a commercial inorganic N fertilizer or an arginine-based fertilizer) had no significant effect on the shoot biomass or total seedling biomass. Conventionally treated spruce seedlings had slightly lower N status than the target value (2%) aimed for by the forest nursery, while conventionally treated pine seedlings had slightly higher than target N contents. Regardless of these differences, arginine-grown seedlings of both species had larger root biomasses, resulting in a significant shift in the root:shoot ratios, suggesting that not only the internal N concentration of seedlings but also the chemical form of available N influenced biomass distribution between plant parts. The N source used for seedling growth in the nursery had a significant effect on the current-year shoot length of Norway spruce seedlings, resulting in better growth of arginine-grown seedlings a year after transplantation, despite a smaller initial shoot length. This highlights the importance of good quality seedlings, with well-developed root systems, for successful establishment in the field.

4.5 The Functional Equilibrium May Vary for Different N Sources

While it is well documented that N availability, and more specifically the internal N concentration, plays a major role in the distribution of biomass between plant parts, the contribution of different chemical forms of N to this effect has been largely ignored in earlier studies (but see Soper *et al.*, 2011; Bown *et al.*, 2010; Heiskanen, 2005; Bauer & Berntson, 2001). Taken together, the results presented in this thesis indicate that organic N affects biomass partitioning in plants, inducing them to distribute larger proportions of biomass to root structures than plants with similar total biomasses and N contents provided with inorganic N forms. A fundamental assumption of the functional equilibrium hypothesis, as described in section 1.5, is that there is a relationship between the availability of a resource and the plant biomass fraction of the organ responsible for acquiring this resource (Brouwer, 1962a; Brouwer, 1962b). The larger root:shoot ratio of plants growing on low N compared to plants growing on high N is thus, according to this hypothesis, a functional adjustment of the plant. However, soil N availability is primarily a question of supply rates of N to root surfaces, and such rates are intimately connected to the chemical nature of those N sources. Critical parameters in this context are the effective diffusion coefficients of various N sources, which generally increase in the order amino acids < NH_4^+ < NO_3^-, implying that plants will experience a relatively higher N availability, and need a smaller root surface area to sustain a certain rate of N flux for NO_3^- than for NH_4^+ or amino acids (cf. Raven *et al.*, 1992; Paper I). However, although differences in diffusion coefficients between different N forms provide a rationale for that plant biomass distribution could be affected not only by N amount but also by N form; this does not provide indications of the mechanism(s) through which plants can adjust biomass partitioning according to N form. In Study I we addressed this issue by examining the distribution of N from various N sources and found that organic N resided at the site of acquisition to a higher degree than N derived from NO_3^- and that reallocation of organic N was to a high degree root-specific. The site of assimilation is probably important in this context as organic N has been shown to be allocated to shoots at a slower rate than NO_3^- and it has also been suggested that organic N is metabolized in roots (Persson *et al.*, 2006; Schmidt & Stewart, 1999; Papers I & II). These spatial differences in assimilation sites may also reflect the high energetic costs of reducing and assimilating NO_3^- in general, and respiratory-driven root reduction in particular (Andrews, 1986; Paper II), implying that organic N is a more energetically cost-effective N source, especially under energy-limiting conditions (Zerihun *et al.*, 1998; Paper II). This lower energetic investment of roots when acquiring organic N could consequently explain why roots may be able to grow more vigorously on such N forms than on inorganic N.

5 Conclusions and Future Perspectives

The results presented in this thesis show that the amino acid arginine supports growth of Scots pine and Norway spruce seedlings as effectively as commercial inorganic fertilizers currently used in Swedish forest nurseries (Papers II & IV). The results also show that a change of N source, from inorganic to organic, affects seedlings in a multitude of ways. Some of these changes seem to be beneficial for seedling establishment and growth in field as evidenced by Norway spruce seedlings cultivated on arginine, which improved their field performance compared to seedlings cultivated on inorganic fertilizers by a significant increase in current-year shoot length (Paper IV).

Moreover, the results indicate that organic N affects the distribution of biomass between plant parts, so that conifer seedlings and Arabidopsis distribute a significantly larger proportion of the biomass to root structures compared to counterparts with similar size and N contents grown with the inorganic N forms NH_4^+ and NO_3^- (Papers I & IV, Fig. 2). While it is well known that a high internal N status of the plant directs biomass distribution towards shoots, this effect seems to be less pronounced for plants growing on organic N sources. Hence, conifer seedlings and Arabidopsis displayed larger root:shoot ratios despite similar total biomasses and N contents. Detailed studies on Arabidopsis in sterile conditions (Paper I) showed that an increased distribution of biomass to roots coincided with high retention of N derived from organic N sources in roots, corroborating earlier studies suggesting that organic N might be preferentially assimilated in roots (Persson *et al.*, 2006; Schmidt & Stewart, 1999). In contrast, N derived from NO_3^- dominated shoot N contents, indicating that the site of assimilation as well as energetic constraints associated with uptake, reduction and assimilation may influence biomass distribution. This was further corroborated in Study II, in which carbohydrate-deprived seedlings supplied with arginine maintained uptake rates throughout the experimental period and retained the arginine-N in roots while seedlings supplied with NH_4^+ or NO_3^- responded with drastic reductions in uptake rates. Moreover, a spatial shift of inorganic N, especially NO_3^--N, from roots to shoot was observed; suggesting that NO_3^- assimilation in roots is highly dependent on recent photoassimilates. Hence, considering the unaffected short-term uptake rates of arginine and preferential assimilation of organic N in roots, there may be substantial energetic benefits for plants to use organic N sources when carbohydrate supplies are limited.

The findings that uptake rates of arginine-N are in the same order as those of NH_4^+-N (Papers II & III), and that seedlings grow as well on arginine as a sole N source as on inorganic sources (Papers II & IV), suggest that this form of organic N, at least, is a suitable N source for conifers. During cultivation in conifer nurseries seedlings are supplied with high concentrations of inorganic N and the recovery of NO_3^- in seedlings and the growth substrate is low. Seedlings provided with NH_4^+ and NO_3^-, but not those provided with arginine, down-regulated uptake in response to a high internal N status. Moreover, the simultaneous presence of arginine and NO_3^- affected NO_3^- uptake less severely than the simultaneous presence of NH_4^+ and NO_3^-, suggesting that mixtures of arginine and NO_3^- may provide higher uptake efficiencies (Paper III).

With increasing demands on forestry in terms of productivity, seedling quality and fertilization management, research on N nutrition in general and organic N in particular seems more important than ever. The leaching associated with low recoveries of NO_3^--N in conifers has adverse environmental consequences (eutrophication), which viable alternatives to inorganic N-dominated fertilizers may help to mitigate. It is concluded that organic N sources might be suitable for commercial seedling production and provide robust, viable alternatives or complements to fertilizers presently used in forest nurseries. Developing the capacity to customize fertilizer contents to meet the demands of different species and perhaps even tailoring seedling traits in a certain direction, such as predictable root:shoot ratios, seems tempting, for productivity, environmental and economic reasons. To develop such capacity, it remains to be elucidated if different plant species respond to organic N nutrition in the same manner, i.e. by increasing the distribution of biomass to roots, and exactly *how* plants regulate this distribution in response to internal N status and available N forms. Hence, one of the future challenges will be to identify the signals/compounds responsible for sensing and responding to N availability and N form by modulating the root:shoot ratio. It will also be important to determine the effects of organic N sources on photosynthesis and respiration to assess the potential energetic implications associated with their use for plant nutrition. Another area that requires further investigation is the metabolic fate of organic N immediately after acquisition.

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