Soil nitrogen fluxes and root uptake in the boreal forest: key processes to plant nitrogen nutrition

Olusegun Ayodeji Oyewole
Faculty of Forest Sciences
Department of Forest Ecology and Management
Umeå

Doctoral Thesis
Swedish University of Agricultural Sciences
Umeå 2015

Acta Universitatis agriculturae Sueciae
2015:126

Cover: Microdialysis sampling in a boreal forest soil
(photo: S. Jämtgård)

ISSN 1652-6880
ISBN (print version) 978-91-576-8450-9
ISBN (electronic version) 978-91-576-8451-6

Print: Arkitektopia AB, Umeå 2015

Soil nitrogen fluxes and root uptake in the boreal forest: key processes to plant nitrogen nutrition

Abstract

Nitrogen (N) is essential for growth and net primary production of plants. However, N acquisition by plants is influenced by movement of soil N compounds from bulk soil to plant roots and uptake of N by roots. This thesis is aimed at deepening our knowledge on these key processes involved in plant N acquisition in the N-limited boreal forest. To address this aim, a novel, non-invasive microdialysis technique was employed. Amino acids dominated N fluxes in the boreal forest soils. Further, plant roots were shown to have the capacity to absorb organic and inorganic N present in the measured soil fluxes, but these soil fluxes, rather than root uptake, may limit plant N acquisition. The microdialysis technique was further developed to enable simultaneous estimation of diffusion and mass flow of N in soil. Applying this refinement of the technique in the field showed that mass flow significantly increased flux rates of soil N in the boreal forest ecosystem, and that it also altered the chemical composition of the N fluxes.

The results from the studies presented in this thesis highlight the potential of the microdialysis technique to improve our understanding of the intrinsic processes involved in N acquisition by plant roots. They also suggest that amino acids might comprise an important source of N for plants in the boreal forest ecosystem. The results suggest that mass flow plays an important role for plant N acquisition in the boreal forest, and mass flow might increase the share of nitrate, particularly in nutrient-rich ecosystems. This finding opens a discussion on the role of transpiration in plant N nutrition, with implications for our understanding of how plant N nutrition will be affected by, among other things, elevated CO_2, increased temperatures, and N fertilization.

Keywords: amino acids, diffusion, mass flow, microdialysis, nitrogen availability, nitrogen uptake, plant nutrition, stable isotopes, transpiration

Author's address: Olusegun Ayodeji Oyewole, SLU, Department of Forest Ecology and Management, SE-901 83 Umeå, Sweden
E-mail: Olusegun.Ayodeji.Oyewole@ slu.se

Dedication

To my family and Adebola

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Inselsbacher, E., Oyewole, O.A., Näsholm, T. (2014). Early season dynamics of soil nitrogen fluxes in fertilized and unfertilized boreal forests. Soil Biology & Biochemistry 74, 167-176.

II Oyewole, O.A., Jämtgård, S., Gruffman, L., Inselsbacher, E., Näsholm, T. (2015). Soil diffusive fluxes constitute the bottleneck to tree nitrogen nutrition in a Scots pine forest. *Plant and Soil*. DOI: 10.1007/s11104-015-2680-5.

III Oyewole, O.A., Inselsbacher, E., Näsholm, T. (2014). Direct estimation of mass flow and diffusion of nitrogen compounds in solution and soil. New Phytologist 201, 1056-1064.

IV Oyewole, O.A., Inselsbacher, E., Näsholm, T., Jämtgård, S. Is plant nitrogen acquisition enhanced by transpiration? Estimating nitrogen diffusion and mass flow rates in boreal forest soils (manuscript).

The contribution of Olusegun Ayodeji Oyewole to the papers included in this thesis was as follows:

I Participated in performing the experiment, contributed to the writing and preparation of the manuscript.

II Planning, performance of the experiment, analysis and summary of the results, writing and preparation of the manuscript.

III Planning, performance of the experiment, analysis and summary of the results, writing and preparation of the manuscript.

IV Planning, performance of the experiment, analysis and summary of the results, writing and preparation of the manuscript.

Abbreviations

Abbreviations are explained when they first appear in the text.

1 Introduction

1.1 Background

This thesis addresses the importance of processes involved in movement of soil Nitrogen (N) to plant roots. This is important for plant N acquisition and overall plant N nutrition. Nitrogen is an important component of all living organisms, because it is the building block for the essential macromolecules (protein and DNA) that sustain life. It is also an essential constituent of chlorophyll; the light capturing molecule in plant leaves that is important for the growth and net primary production of plants.

Nitrogen gas (N_2) is the most abundant compound in the atmosphere constituting about 78 % of the total volume, but plants lack the capacity to utilize atmospheric N directly. Atmospheric N becomes available for plant use through a range of processes. Nitrogen can be deposited into ecosystems in particulate, dissolved and gaseous forms during rainfall, lightning and wind-blown particles. Biological fixation of N_2 by N-fixing bacteria and chemical fixation of N through the Haber-Bosch process (industrial production of inorganic N fertilizers) also result in N addition to ecosystems. In addition, decomposition of dead plant biomass and other N contained in soil organic matter release organic and inorganic N compounds into soils. Inorganic N compounds include NH_4^+, NO_2^- and NO_3; while the organic ones include protein, peptides, nucleic acids, amino acids, amino sugar, nucleotides etc. These N compounds possess different chemical properties and can be converted from one form to another in the soil N cycle.

1.2 The soil N cycle

A simplified depiction of the soil N cycle includes N addition to soil; transformation of one N compound to another; uptake of N compounds by plant and soil microbes; release of N compounds by plant roots and soil microbes; and loss of N compounds from ecosystems (Figure 1).

1.2.1 Nitrogen addition to soils

Biological N fixation plays a significant role in N addition to soil. N-fixation involves breakdown of the triple bond of N_2 and its reduction into NH_4^+ by the enzyme nitrogenase produced by N-fixing microbes. Some examples of N-fixing microbes include *Rhizobium*, *Frankia* and *Azotobacter* - which are in symbiotic association with plant roots; and free-living N-fixing *Nostoc*, *Anabaena*, and *Rhodospirillium* (Chapin et al. 2011). Atmospheric N can also be deposited in soils, e.g., during lightning and rainfall, and through anthropogenic activities. These anthropogenic activities include application of NH_4^+ fertilizer which can lead to volatilization of NH_3, and subsequent production of NH_4^+ in the atmosphere (which can be deposited into downwind ecosystems during rainfall). Another example is combustion of fossil fuel and biomass burning that may result in emission of NO_x (which can be deposited as NO_3^- during rainfall).

1.2.2 Production of N compounds and processes involved in their production

Soil microbes release N contained in dead organic matter by as complex organic N, e.g., proteins, nucleotides, or chitin. This thesis focuses on proteinaceous N compounds because they are the dominant N compounds in soils (Schulten and Schnitzer, 1998). Complex proteinaceous N compounds can be broken down into monomeric dissolved organic N (DON; e.g. amino acids) by exoenzymes through a depolymerization process. Depolymerization of complex organic N into monomeric DON is considered the rate-limiting step in the soil N cycle (Schimel and Bennett, 2004).

Soil microbes require external carbon sources for their growth. Whenever microbes are carbon-limited, they utilize carbon from DON and in the process release NH_4^+ into soils. The process involved in the production of NH_4^+ from DON is known as ammonification. Ammonium can be oxidized by nitrifying bacteria to NO_2^- and subsequently into NO_3^- during the process of nitrification. In the boreal forest ecosystem, mineralization of DON to NH_4^+ and finally to NO_3^- are considered slow processes (Vitousek and Howarth, 1991).

1.2.3 Fates of N compounds in soil

The organic N, NH_4^+, and NO_3^- produced during the above processes can be taken up and assimilated by plants (Näsholm et al. 1998; Lipson and Monson, 1998; Kielland, 1994; Kronzucker et al. 1997; Kamminga-van Wijk and Prins, 1993). Soil microbes can also absorb these N compounds and use them as N sources. Monomeric N compounds can also exchange with other ions on anion and cation exchange sites of soil particles. Nitrate and NH_4^+ can be exchanged at the anion and cation exchange sites of soil particles respectively, while amino acids can be exchanged at both exchange sites depending on pH of the soil solution. Exchange of N compounds to the charged surfaces of soil particles could reduce the concentration of available N compounds in soil solution.

1.2.4 Loss of N compounds from ecosystems

Dissolved organic N, NH_4^+, and NO_3^- can be leached into groundwater and stream water. Leaching of N compounds could result in reduction in the concentration of available N compounds in the soil solution and available for plant uptake leading to soil acidification and eutrophication of water bodies (Tilman et al. 2002). Another major loss pathway is the release of gaseous N compounds (e.g. NH_3, N_2, N_2O and NO) into atmosphere. Production of these gaseous N compounds occurs during volatilization of NH_3, nitrification, and denitrification processes. Denitrification is the chemical or biological reduction of NO_3^- to N_2 and NO_X by the denitrifying bacteria.

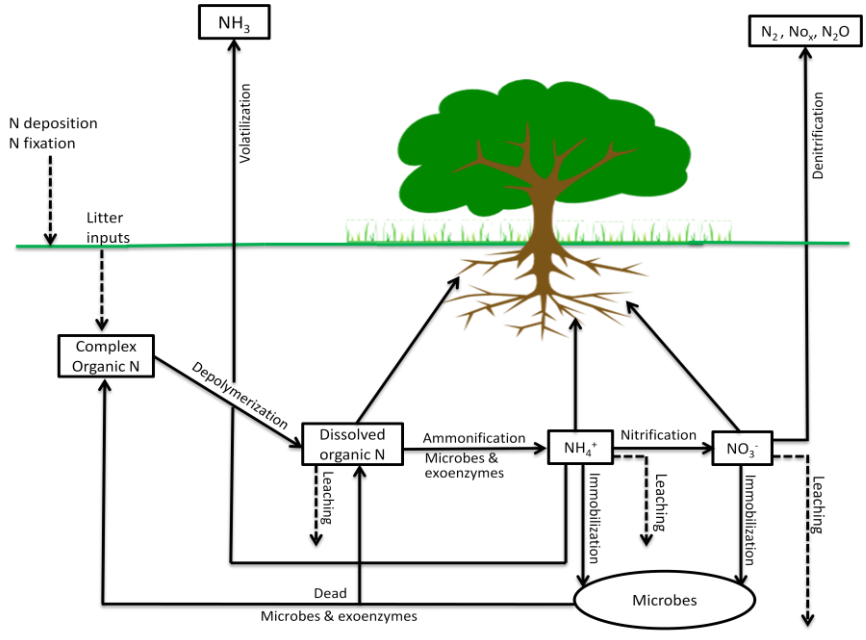

Figure 1. A simplified soil N cycle showing N inputs to soil; transformation of one N compound to another; fates of N compounds; and loss of N compounds from the ecosystem. Rectangular boxes show various N compounds; the oval box represents microbes; solid lines depict the transformation processes; and broken lines represent N inputs and N losses from the soil.

1.3 How plant roots encounter soil nitrogen

Plant roots encounter soil nutrients through the contact between the root surfaces and the nutrients. The contact can occur by the movement of the nutrients from the bulk soil to the root surfaces through diffusion and mass flow; and by the root growth into the nutrients location in the rhizosphere through root interception (Nye, 1967; Lambers et al. 2008; Tinker and Nye, 2000; Comerford, 2005; Marschner, 1995; Nye and Marriott, 1969; Chapin, 1980; Jungk and Claassen, 1997). Diffusion and mass flow are considered the two main processes involved in the movement of nutrients e.g. N compounds from the bulk soil to the root surfaces (Nye and Tinker, 1977; Nye and Marriot, 1969; Nye, 1979; Chapin, 1980; Tinker and Nye, 2000; Cramer et al. 2008, 2009) (Figure 2). Soil nutrients captured through root interception have been considered negligible and is usually ignored in the calculation of total nutrient uptake (Jungk and Claassen, 1997; Chapin et al. 2011; Lambers et al. 2008).

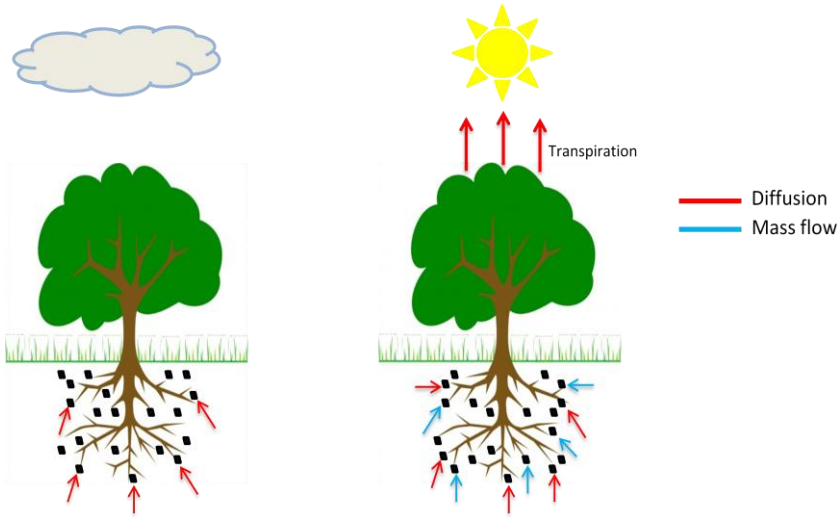

Figure 2. Diffusion is the main process involved in net transport of nutrients from bulk soil to root surfaces for a tree that is not transpiring. For a transpiring tree, both diffusion and mass flow transport nutrients from bulk soil to root surfaces. Furthermore, these two processes interact so that diffusion may be stimulated by mass flow.

Diffusion occurs as a consequence of concentration gradients arising from the active uptake of N (and other mineral nutrients) at the root surface. According to Fick's law, diffusion of nutrients is a function of the concentration gradients and the diffusion coefficient (*cf.* Tinker and Nye, 2000; equation 1).

$$F_D = -D * (\partial C / \partial \chi) \qquad (1)$$

where F_D is diffusion, D is the diffusion coefficient ($cm^2\ s^{-1}$), C is the concentration of nutrient per volume of soil (g cm^{-3} of soil), χ is distance from the root (cm), and $\partial C/\partial \chi$ thus describes the concentration gradient from the root surface into the surrounding soil. The minus sign indicates that movement of nutrients proceeds down the concentration gradient.

The diffusion coefficient is an important determinant of soil N movement. It can be calculated from F_D and $\partial C/\partial \chi$, which can be measured experimentally in the soil. The diffusion coefficient determines the concentration gradient caused by diffusion. The smaller the value of D the steeper the concentration gradient (Jungk and Claassen, 1997). According to Tinker and Nye (2000) and

Comerford (2005) D of soil N includes factors that affect N diffusibility in the bulk soil to the root surfaces (2).

$$D = D_L * \theta * f * (1 / b) \quad (2)$$

D_L is diffusion coefficient in water (cm^{-2} s^{-1}), θ is the volumetric water content (cm^3 water cm^{-3} of soil), f is the impedance factor of the soil (defined below) and b is the soil buffer power (defined below).

The volumetric water content is an important determinant in the soil nutrient movement to the plant roots. It regulates diffusion by determining the cross-sectional area available for diffusion; determining the path length of diffusion by controlling f and contributing to the soil b (Comerford, 2005).

The impedance factor is defined as the ratio of the length of the straight-line path of movement of mineral nutrients to the actual path (Comerford, 2005; Tinker and Nye, 2000). It includes all processes that decrease the mobility of the adsorbed solute from the mobility it would have in free solution (Tinker and Nye, 2000). A decrease in f reduces the diffusive flux by increasing the actual path of the nutrient, and also by decreasing the concentration gradient in the water-filled soil pores (Jungk and Claassen, 1997).

Soil buffer power is the capacity of soil exchangeable pools to replenish the soil solution as nutrients are absorbed (Chapin, 1980). It is generally expressed as (3):

$$b = (\Delta C / \Delta C_L) \quad (3)$$

C is concentration of the ions participating in diffusion (i.e. ions in solutions plus those bound to the solid phase that can be released into the ambient solution) (g cm^{-3} of solution), and C_L is concentration of the ions in soil solution (g cm^{-3} of soil).

Diffusion of N from the bulk soil to plant root surfaces is also affected by the soil temperature (Inselsbacher and Näsholm, 2012b). According to Einstein-Stokes equation, diffusion is directly proportional to temperature (4).

$$F_D = (k_B T) / (6\pi\eta r) \quad (4)$$

k_B is the Boltzmann's constant, T is the absolute temperature (K), η is the viscosity and r is the radius of the molecule (cm).

Mass flow is mass transport of water and dissolved nutrients from the bulk soil to the root surfaces, driven by plant transpiration. It has been calculated as the product of the measured whole-plant transpiration and the soil N concentrations (5).

$$F = w * c \quad (5)$$

F is mass flow, w is transpiration rate (cm s^{-1}) and c is concentration of the nutrients in the bulk soil solution (g cm^{-3} of soil).

The relative importance of diffusion and mass flow processes to plant N nutrition depends on plant species characteristics, plant-soil interactions (Jungk and Claassen, 1997), and soil conditions (Barber, 1995; Comerford, 2005).

1.4 Importance of diffusion and mass flow to plant N nutrition

Diffusion is believed to be the main driver for N fluxes to the roots in nutrient-poor soils, while mass flow is believed to dominate in nutrient-rich soils (Barber, 1995; Comerford, 2005; Smethurst, 2000), but the roles these two processes play in plant N nutrition is controversial. For instance, some models that are based on theoretical assumptions (Yanai, 1994; BassiriRad et al. 2008) suggest that mass flow is not important in soil N fluxes to the roots, because the total N flux to the root would be similar either in presence or absence of mass flow. The models suggest that mass flow decreases diffusion of soil N to the root by flattening or reversing the concentration gradient between the soil solution and the root surfaces. In contrast, a model by Nye and Marriott (1969) suggests that mass flow may be important in nutrient movement to the roots, and that mass flow may facilitate diffusion. Further, several experimental studies suggest that mass flow is an integral part of the total N fluxes to plant roots, and that mass flow plays a prominent role in plant N nutrition (Strebel and Duynisveld, 1989; Plhák, 2003; Cramer et al. 2008).

Studies have also shown that soil N availability may affect water uptake by roots (Matimati et al. 2014; Cramer et al. 2009; Raven, 2008; Gorska et al. 2008; Wilkinson et al. 2007; Kupper et al. 2012). This is because a change in NO_3^- concentration around the root causes sudden change in the root hydraulic properties, resulting in increase in water uptake by the roots from the NO_3^- rich

patches (Gorska et al. 2008; Gloser et al. 2007). Therefore, there is a need to understand the contribution of mass flow to the N flux to the roots; and also understand the effect(s) of mass flow on diffusion.

Traditionally, the total flux of N to plant roots is measured as the sum of diffusion and mass flow:

$$N_{up} = F + F_D \quad (6)$$

N_{up} is the total flux of N to plant roots, F is mass flow (g cm^2 s^{-1} of soil), and F_D is diffusion (cm^2 s^{-1}).

The contribution of mass flow to total N flux is traditionally calculated as stated earlier (Equation 5). Therefore, diffusion is calculated as the difference between total N flux to the roots (over some period of time) and mass flow (Jungk and Claassen, 1997; Lambers et al. 2008):

$$F_D = N_{up} - F. \quad (7)$$

This assessment of the relative contributions of diffusion and mass flow to plant N nutrition using the total N uptake measurement is indirect, and it is also difficult to separate diffusion from mass flow. Further, the total N uptake measurement approach fails to explain the possible interaction(s) between mass flow and diffusion (Nye and Marriott, 1969), and it does not account for the uncertainties in the assessment of transpiration and concentration of N in soil solution. In addition, diffusion of N to the roots could be estimated theoretically from the measurements of soil characteristics such as diffusion coefficient, volumetric water content, impedance factor and soil buffer power, as stated in equations 1 - 4. However, it is difficult to measure these soil characteristics, thereby complicating the calculation of soil N diffusion from soil characteristics. Hence, there is need for a technique that is relatively direct and more robust for the estimation of the contributions of the two processes to plant N nutrition.

1.5 Assessing soil nitrogen availability

Assessments of soil N availability have been based mostly on soil N concentrations and N turnover rates (Marschner, 1995; Leadley et al. 1997). However, soil N concentrations may not give a true reflection of N that is

available for plant uptake. This is because only a few of the N compounds in the bulk soil are available for plant use, while most of them are not accessible or available for plant nutrition e.g. high molecular-weight N compounds. Therefore, a plant growing in soil with high N concentration may suffer starvation; while another plant that is growing in soil with low N concentration may grow well (Schachtman et al. 1998; Öhlund, 2004). Soil N availability should take into account N concentration in bulk soil; release of N from solid phase (i.e. in the soil) to solution phase (through desorption); movement of soil N to roots and mycorrhizal hyphae; and N uptake by plant roots (Comerford, 2005).

Nitrogen compounds can exist in solid forms, and as solutes in the soil solution. In this thesis, the focus is on amino acids, NH_4^+ and NO_3^-. Amino acids that are present in the soil can exist in three pools: (a) free amino acid (FAA): dissolved in the soil solution where they are readily available for uptake by the plant; (b) exchangeable amino acids: bound to the charged surfaces of organic matter or clay particles; or (c) chemically bound as peptide- and protein-bound amino acid (BAA). FAA might account for a small fraction of the total amino acid pool, while a large fraction of amino acid might be present as BAA (Andersson and Berggren, 2005; Yu et al. 2002; Schulten and Schnitzer, 1998; Senwo and Tabatabai, 1998; Jämtgård et al. 2010; Farrell et al. 2011). Unlike FAA, BAA may either be accessible and used as a N source by plants (Paungfoo-Lonhienne et al. 2008, 2012) or it may not be easily accessible and available for plant uptake. Hence, BAA might serve as the largest reservoir and a possible replenishment source for FAA (Jämtgård et al. 2010).

1.6 Nitrogen uptake by plant roots

Several studies have demonstrated that plants possess the capability to take up and use a wide variety of organic and inorganic N compounds (Näsholm et al. 1998, 2000; Warren, 2013; Paungfoo-Lonhienne et al 2008, 2012; Farrell et al. 2013; Harrison et al. 2007; Jones and Darrah, 1993; Kielland, 1994; Stoelken et al. 2010; Weigelt et al. 2005; Kronzucker et al. 1997; Kamminga-van Wijk and Prins, 1993; Streeter et al. 2000). Nitrogen uptake in plants is mediated by high-affinity transport systems (HATS) and low-affinity transport systems (LATS) (Näsholm et al. 2009; Nacry et al. 2013). High-affinity transport systems mediate N uptake at low soil N concentration, and LATS at high soil N concentration. Transporters present in the epidermal and cortex cells of roots, and mycorrhizal hyphae mediate the uptake of NH_4^+, NO_3^- and amino

acids (Lehmann et al. 2011; Lee et al. 2007; Hirner et al. 2006; Svennerstam et al. 2007, 2008; Näsholm et al. 2009; Nacry et al. 2013).

Plant species have different uptake capacities for organic and inorganic N compounds (Metcalfe et al. 2011; Pfautsch et al. 2009; Sauheitl et al. 2009; Thornton and Robinson, 2005; Öhlund and Näsholm, 2004; Jones and Darrah, 1993; Gruffman et al. 2014). For instance in some conifer species, uptake of NH_4^+ was shown to be higher than uptake of NO_3^- (Stoelken et al. 2010; Kronzucker et al. 1997; Kamminga-van Wijk and Prins, 1993), while the uptake of amino acids was found to be similar to NH_4^+ uptake (Gruffman et al. 2014; Persson et al. 2006). These uptake capacities for different N compounds may be affected by the internal N status of the plant, for instance N uptake will be down-regulated at a high internal N status (Persson and Näsholm, 2002; Öhlund and Näsholm, 2004; Gruffman et al. 2014). Plant uptake capacities for N compounds may also be affected by the external soil N concentrations (Stoelken et al. 2010) and the presence of different N compounds. For example, NH_4^+ inhibits the uptake of NO_3^- in some conifers (Kamminga-van Wijk and Prins, 1993).

Despite the overwhelming evidence supporting the movement of soil N to the roots and the roots' uptake capacities for these N compounds, our knowledge about the amount of soil N supplied to the roots in relation to the amount the roots actually take up is limited. Hence, a combination of both root uptake capacities measurements with soil N diffusive fluxes measurements will enable us to identify the limiting process for plant N acquisition in the boreal forest ecosystem.

2 Objectives

This thesis is aimed at increasing our knowledge of plant N nutrition in the N-limited boreal forest ecosystem. The studies presented in this thesis focus on soil N availability; fluxes of N from the bulk soil to plant roots; and N uptake by the roots.

Recently, a novel technique has been used for *in-situ* monitoring of soil N fluxes (microdialysis; Inselsbacher et al. 2011). Paper I was aimed at using the microdialysis technique to monitor N fluxes in a fertilized and a non-fertilized boreal forest soil at the onset of the growing season.

Previous studies have demonstrated that plant species have different uptake capacities for organic and inorganic N compounds, but there is a knowledge gap between N fluxes from the bulk soil to the roots and the root uptake capacities for these N compounds. In paper II, the aim was to compare the diffusive fluxes of soil N compounds in boreal forest soils with the root uptake capacities for these N compounds.

The traditional approach for the estimation of diffusion and mass flow of N compounds is indirect and associated with some challenges. We speculated that the microdialysis technique may be further developed to include direct estimation of mass flow, and that the contributions of mass flow and diffusion to plant nutrition could be estimated simultaneously. Hence, paper III was aimed at using the microdialysis technique to estimate the contributions of diffusion and mass flow in the laboratory.

Paper IV was aimed at using a modified microdialysis technique to estimate diffusion and mass flow of N, and also to give insights into the role of each process for plant N nutrition in two boreal forest soils with contrasting fertility.

3 Methodological considerations

3.1 Choice of soil sampling technique

Determination of N availability for plant acquisition involves the estimation of N concentration and chemical composition in soil. Traditionally, soil N concentration and chemical composition have been estimated using: soil extraction (Jones and Willett, 2006; Rousk and Jones, 2010; Chen et al. 2005); centrifugation (Yu et al. 2002; Chen and Xu, 2008; Giesler et al. 1996); and tension lysimeters (Andersson, 2003; Andersson and Berggren, 2005; Jämtgård et al. 2010). Soil extraction and centrifugation techniques cause disturbance to the *in-situ* soil structure. There could also be under- or over-estimation of soil N concentration and chemical composition when soil extraction or centrifugation techniques are employed. These arise from production or decomposition of N compounds in the samples prior to chemical analyses. Production or decomposition of N compounds in the samples can occur during sample preparation (e.g. soil sieving, soil homogenization and filtration); sample handling; temperature; and the time lags between soil sampling and chemical analyses (Jones et al. 2005; Chen and Xu, 2008; Rousk and Jones, 2010; Inselsbacher, 2014; Lipson et al. 2001).

Various types of lysimeters are available for soil sampling. A rhizon lysimeter with pore size of 0.1 µm diameter (Andersson, 2003; Andersson and Berggren, 2005; Jämtgård et al. 2010) causes smaller disturbance to the *in-situ* soil structure when compared with soil extraction and centrifugation techniques. The small pore size of this lysimeter prevents microbial degradation of N compounds, in the process reducing possible production or decomposition of N compounds in the samples prior to chemical analyses (Andersson, 2003; Andersson and Berggren, 2005; Jämtgård et al. 2010). The major limitation to the lysimeter technique is the reliance on high soil moisture content. Further,

the results from this technique mainly represent N concentration from the largest water filled soil pores; and therefore it may not give a true reflection of N concentration in the bulk soil.

In earlier studies (Jämtgård et al. 2010; Inselsbacher et al. 2011; Inselsbacher and Näsholm, 2012a), differences in the soil N pool composition were observed when soil extraction and lysimeter techniques were compared. For instance, NO_3^- dominated the soil N pool when lysimeters were used (Jämtgård et al. 2010), while NH_4^+ was dominant when soil extraction was used (Inselsbacher et al. 2011; Inselsbacher and Näsholm, 2012a). Another major challenge is the inability to understand whether both free and exchangeable N compounds (from the water and salt extraction samples respectively) are available for plant N nutrition. Further, estimation of N flux rates in the soil to plant roots using the traditional approach could result in under- or over-estimation of N availability for plant uptake. Hence, the need exists for a technique that causes minimal disturbance to soil structure; and limits over- and under-estimation of soil N concentration. Microdialysis (Figure 3), a technique originally developed in neuroscience, was recently introduced as a tool for monitoring soil N compounds (Inselsbacher et al. 2011, Inselsbacher and Näsholm, 2012a & b; Paper I). This technique allows for continuous sampling of soil N and it can also detect small changes in diffusion of soil N (Inselsbacher et al. 2011, Inselsbacher and Näsholm, 2012a & b). Microdialysis has the potential to give a better reflection of N flux rates in soils than soil N concentrations, because of the small size of the dialysis probe membrane (which causes minimal disturbance to the *in-situ* soil structure). This technique also minimizes the risk of decomposition and production of soil N compounds as an effect of sampling. This is because unlike the soil extraction technique, there is no sample preparation processes, such as soil sieving, soil homogenization and soil filtration. Therefore, microdialysis was adopted for monitoring soil N fluxes; and for direct estimation of induced diffusive and mass flow fluxes of soil N in the studies presented in this thesis.

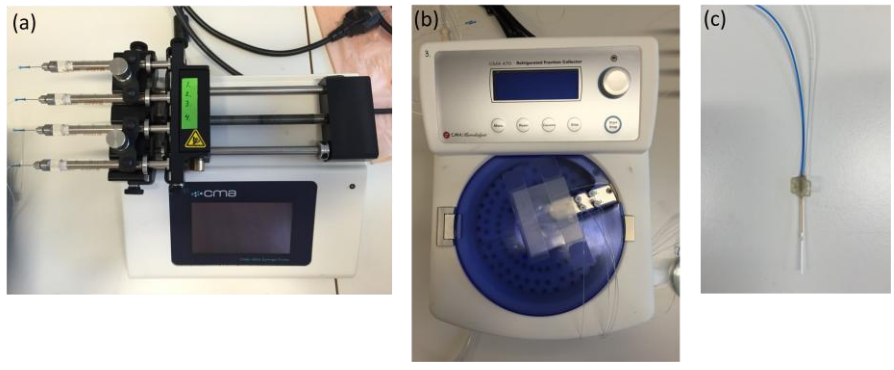

Figure 3. A typical microdialysis setup consisting of (a) a syringe infusion pump (CMA 400) equipped with four syringes, (b) a fraction collector (CMA 470) and (c) microdialysis probes (CMA 20) with a 10 mm long polyarylethersulphone membrane (molecular cutoff, 20 kDa; 400 µm inner and 500 µm outer diameters).

In the studies presented in this thesis, microdialysis probes with a 10 mm long polyarylethersulphone membrane (molecular cutoff, 20 kDa; 400 µm inner and 500 µm outer diameters) were used. The small size of this membrane would not result in large-scale disturbance to *in-situ* soil structure when inserted in soils. A larger probe would give higher recovery, which simplifies chemical analysis of N compounds, but I wished to minimize disturbance to soil structure, hence the use of this small probe. Further, the low molecular weight cut-off of the membrane prevents leakage of perfusate with higher molecular weight into soils. For instance, a perfusate containing solution of Dextran 40 (molecular weight of 40 kDa) was kept inside the dialysis probes because it has bigger size than the molecular weight cut-off of the probe membranes (Paper III and IV). The molecular weight cut-off of the dialysis probe also allows N compounds with lower molecular weight (<20 kDa) and prevents those with higher molecular weight (>20 kDa) to pass across the membranes into the dialysates. Low microdialysis pump flow rates were used in this thesis in order to achieve higher relative recoveries (but also resulting in lower absolute recoveries: measured in nmol N cm^{-2} h^{-1}) of N compounds in the dialysate (Figure 4; Inselsbacher et al. 2011). In Papers I and II, a pump flow rate of 5 µl min^{-1} was used, but in Papers III and IV a flow rate of 1 µl min^{-1} was used. This switch in the pump flow from 5 µl min^{-1} to 1 µl min^{-1} allows for measurable increase in dialysate volume due to mass flow.

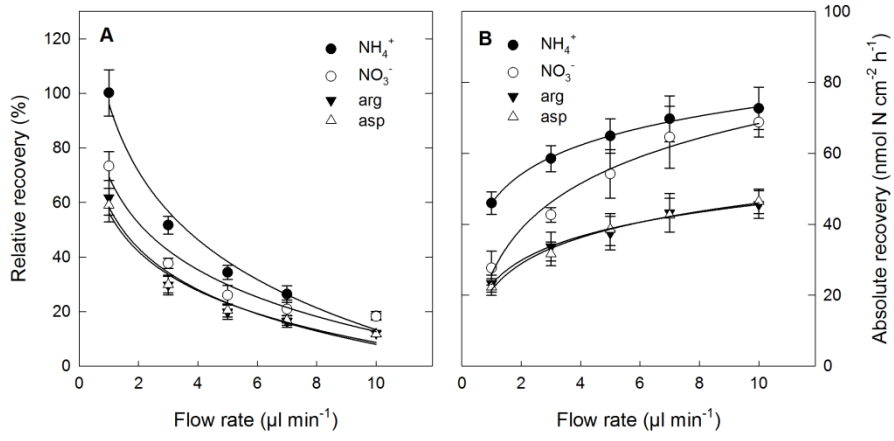

Figure 4. The relationship between the pump flow rates and (A) the relative recoveries of NH_4^+, NO_3^-, arg and asp (mean of 7 concentrations (0.05 – 4 mmol N l^{-1}) and n = 7 at each concentration) from standard solutions and (B) the absolute recoveries of NH_4^+, NO_3^-, arg and asp (each 100 µmol N l^{-1} n = 7) from standard solutions.

Like other soil sampling techniques, microdialysis has limitations. For instance, roots can regulate water and nutrient uptake, but the dialysis probe membranes lack capacity to do this. Plant roots can grow through soil and encounter soil nutrients in the process, while dialysis probes are stationary in soil and are prone to the formation of depletion zones for soil N compounds around them. Unlike plant roots, microdialysis probes lack the capacity to exude nutrients; and also have no capacity for active uptake of soil nutrients. Mycorrhizal and non-mycorrhizal roots exude exoenzymes that affect the supply rate of N compounds in the soil (Hartmann et al. 2009). However, microdialysis probe membranes lack the capacity to mimic this function of the roots. Some studies (Inselsbacher et al. 2011; Inselsbacher and Näsholm, 2012a; Papers I - III) have suggested that the microdialysis technique underestimates the availability of some amino acids (e.g. arginine and lysine) despite their availability in the boreal forest soil (Nordin et al. 2001). Further, a microdialysis set-up is costly; hence its use may be limited in large scale soil studies. The microdialysis technique is also less suitable for determining soil N concentrations. This is because it measures N flux rates in soils rather than concentrations. The concentrations of individual N compounds in soils could be calculated theoretically from their respective flux rates and their relative recovery rates in standard solutions. However, the estimated recovery rates in soil may be different from those established for solutions.

3.2 Measurements of nitrogen uptake by roots

This thesis also aimed at understanding plant uptake capacities for different N compounds, in relation to soil N fluxes to the roots. We now know a lot about plant uptake of NH_4^+ and NO_3^- but little is known about amino acids. In Paper II, NO_3^-, NH_4^+, gly and arg were chosen because their respective uptake has been found to be mediated by different N transporters (Hirner et al. 2006; Svennerstam et al. 2007, 2008; Lee et al. 2007; Frommer et al. 1993). In addition, gly and arg were used in previous studies (Gruffman et al. 2014; Öhlund and Näsholm, 2001, 2004; Persson et al. 2003), and arg is a prominent amino acid in boreal forest soil (Nordin et al. 2001).

In the study present in this thesis, isotopic labeling was used to estimate root uptake of N compounds (Figure 5). Incubation solutions were prepared at low and high concentrations, in order to assess the activity of high-affinity and low-affinity root transport systems respectively. The incubation solutions contained four combinations of one labelled and three unlabeled N compounds. This enabled studies of root uptake from complex mixtures of N sources, a situation that is relevant for roots growing in soil. The uptake of intact arg molecules by roots in incubation solution was determined by comparing the relationship between excess ^{13}C and excess ^{15}N in the root samples to that of dual labeled arg (1.5).

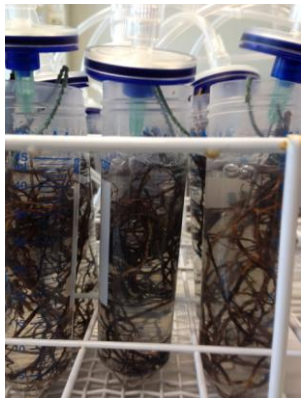

Figure 5. Measurement of roots' uptake capacities for dual labeled N compounds from incubation solution.

3.3 Choice of perfusate for inducing mass flow

Based on an earlier study (Rosdahl et al. 2000), Dextran (a polysaccharide) was chosen for lowering the osmotic potential of the perfusate and thus inducing mass flow of water and solutes across the dialysis probe membranes (Papers III and IV). Following a series of experiments testing the suitability of Dextran 20, Dextran 40 and Dextran 70 (with molecular weight of 20 kDa, 40 kDa and 70 kDa respectively); a solution containing Dextran 40 was chosen because it formed a clear solution when dissolved in MilliQ water, and was most effective in inducing mass flow without leaking into soil or standard solution.

Dextran in the dialysates obtained from the mass flow experiments were found to interfere with the derivatisation of amino acids prior to analysis of these compounds. Hence, Dextran was precipitated with ethanol by adding 100 µl of 98 % ethanol to 100 µl of the sample (*cf.* Behravan et al. 2003). The mixture was spun down with a centrifuge, and the supernatant was collected and processed for chemical analysis for NH_4^+, NO_3^- and amino acids. In addition, tests were performed to determine the potential effects of dextran precipitation with ethanol on the relative recoveries of NH_4^+, NO_3^- and amino acids. This was done by comparing concentrations of these compounds in standard solutions mixed with Dextran 40 and treated with ethanol to the original standard solutions.

4 Results and Discussion

In recent studies (Inselsbacher et al. 2011; Inselsbacher and Näsholm, 2012a and b), a novel microdialysis technique was used to estimate induced diffusive fluxes of N compounds. In this thesis, this microdialysis technique was used to estimate induced diffusive fluxes of soil N compounds (Paper I and II). The technique was then further developed to include estimation of mass flow of soil N (Paper III) and this method was applied in a field study, comparing two forests of contrasting fertilities (Paper IV). Induced diffusive fluxes of soil N were also compared with root´s uptake capacities for soil N, in order to determine the limiting factor for plant N acquisition (Paper II). The main results from these studies are presented below.

4.1 Paper I

The aim of the study in Paper I was to monitor induced diffusive fluxes of N in a fertilized and a non-fertilized boreal forest soil at the onset of the growing season. Microdialysis probes were inserted into the organic soil layer of control and fertilized sites and the induced diffusive fluxes of N across probe membranes were estimated. Similar to Inselsbacher et al. (2011) and Inselsbacher and Näsholm (2012a), induced diffusive fluxes of N were dominated by amino acids, which represented 82 % and 67 % of total N flux in control and fertilized soils respectively. The remainder of the flux was almost entirely NH_4^+; fertilization increased the proportional NH_4^+ fluxes to 32 % from 17 % of total N. The induced diffusive flux of NO_3^- was very low in both control and fertilized soils (1.3 % of total N fluxes in both soils). Higher fluxes of NH_4^+ in fertilized soil might have resulted from longer retention of NH_4^+ in the soil (due to its higher capacity to bind to soil particles), while low fluxes of NO_3^- in the fertilized soil could have resulted from high mobility of NO_3^- (Chapin et al. 2011), or higher plant or microbial uptake. The contribution of

amino acids to plant N nutrition appeared to be especially high at the onset of growing season.

Free and exchangeable N in both control and fertilized soils were also determined using water and KCl extraction techniques. In both extracts and both soils, NH_4^+ was the dominant N compound (94 % of total N pool), while NO_3^- and amino acids were very low. The total N pool was higher in KCl extracts than in water extracts. Six amino acids were detected in the water extracts and all but three amino acids were detected in KCl extracts. The observed differences in soil N concentrations between microdialysis and soil extraction techniques might have resulted from disturbance of the soil structure and soil sample handling during soil extraction. Therefore, soil extraction techniques may not reflect N concentration in undisturbed soils (Thomsen and Schjonning, 2003; Johnson et al. 2005).

Temporal shifts in soil N fluxes were studied over a short time-scale (100 minutes). There were no temporal shifts in fluxes of NH_4^+ and NO_3^- and most amino acids in the soil of the control site during the short time study. In contrast, time-dependent temporal shifts in fluxes of NH_4^+ were detected in soil from the fertilized site. Since there was no increase in fluxes of NO_3^-, nitrification should not have been responsible for decline in NH_4^+ fluxes. Microbial immobilization and root uptake were not responsible for decline in fluxes of NH_4^+, because these processes would have accounted for lower fluxes of NH_4^+ at the start of sampling. However, rapid decline in fluxes of NH_4^+ could have resulted from the formation of a diffusion shell (i.e. insufficient replenishment of NH_4^+ after diffusion) in the soil surrounding the membrane surfaces (Tinker and Nye, 2000; Leitner et al. 2010; Inselsbacher et al. 2011). This result suggests that mineralization of amino acids to NH_4^+ could not replenish the pool of NH_4^+ in the fertilized soils.

Temporal shift in NO_3^- flux was also studied over a long time-scale (25 days) after pulse-addition of either water (10 l m^{-2}) or NO_3^- (10 l m^{-2} with a concentration of 1 g N l^{-1}) to both soils. The addition of water had no effect on fluxes of NO_3^- in either soil. In contrast, NO_3^- addition resulted in a strong increase in NO_3^- fluxes, but the effect was much stronger in the fertilized soil. The duration of the NO_3^- pulse was also much longer in the soil of the fertilized plot. This could have resulted from lower immobilization rates of NO_3^- in fertilized soil than control soil (Högberg et al. 2011). This result implies that NO_3^- was available in the soil organic layer for plant uptake for less than three weeks after fertilizer application.

4.2 Paper II

Plant roots have different uptake capacities for organic and inorganic N compounds (Öhlund and Näsholm, 2004; Metcalfe et al. 2011; Jones and Darrah, 1993; Kielland, 1994; Streeter et al. 2000; Pfautsch et al. 2009; Weigelt et al. 2005; Thornton and Robinson, 2005; Harrison et al. 2007; Gruffman et al. 2014; Sauheitl et al. 2009). With the traditional techniques it is difficult to compare instantaneous N fluxes to the roots with root uptake capacities. This is because the traditional techniques give estimates of soil N concentrations and not N fluxes to the roots. Microdialysis offers the possibility of comparing fluxes of N compounds to the roots with root uptake capacities for N compounds. Hence, Paper II was aimed at understanding the relationship between N fluxes to the roots and root uptake capacities for organic and inorganic N compounds in order to identify the limits for tree N acquisition. This study combined measurements of induced diffusive fluxes of N in control and fertilized boreal forest soils with the laboratory measurements of the detached root uptake capacities for N from 50 and 500 µM incubation solutions at the onset and end of a growing season. The solution concentrations were chosen in order to measure maximum root uptake rates, more particularly, to target high-affinity transport systems (HATS) and low-affinity transport systems (LATS) respectively.

Amino acids dominated induced N fluxes in both control and fertilized soils at both the onset and the end of a growing season (47 – 80 % of total N flux). The contributions of gly, NH_4^+ and NO_3^- to total N in both soils and seasons were similar. In detail, NH_4^+ had the largest contribution (15 - 41 %), followed by gly (5 - 12 %), NO_3^- (1.5 - 6 %; except in fertilized soil at the end of growing season where NO_3^- contributed 27 %), and arg was below detection limit at both soils and in both seasons. These results suggest that organic and inorganic N are available for plant uptake, but the shares of amino acids to plant N nutrition are higher at the onset of growing season than at the end of the growing season. Diffusive fluxes of soil N were higher at the onset than at the end of the growing season. This could have resulted from the predominant freeze-thaw cycle at the onset of the growing season, which affects turnover of soil N (Ivarson and Sowden, 1966; Lipson and Monson, 1998). However, fertilizer was applied only two weeks before the early-season sampling, which almost certainly modified the fluxes.

Roots displayed highest uptake rates for arg followed by NH_4^+ and gly, while NO_3^- uptake was very low from both 50 and 500 µM incubation solutions at both the onset and the end of the growing season. Roots from the fertilized plot

showed lower uptake of gly and NH_4^+ from both incubation solutions and lower uptake of NO_3^- from the 50 µM incubation solution. High internal N status of roots from the fertilized soil might be responsible for this, resulting in down-regulation of high-affinity NH_4^+ and NO_3^- transporters (Rawat et al. 1999; Vidmar et al. 2000; Nazoa et al. 2003) and neutral/acidic amino acids transporters.

The induced soil N fluxes were compared with measured root uptake capacities from 50 µM incubation solution. Although the concentration of 50 µM is most likely high compared to the concentrations of N compounds in the boreal forest soils, this concentration is still relevant for determining the root's maximum uptake capacities for organic and inorganic N compounds. Root uptake rates of N compounds were higher (6 – 290 times) than diffusive fluxes at both seasons (except for NO_3^- at the end of the growing season when its fluxes was higher than root uptake in fertilized soil). This implies that roots have excess capacity to acquire N compounds that arrive at their surfaces, and that diffusive flux of soil N probably is the limiting step to tree N acquisition. This corroborates earlier studies that used indirect traditional techniques in nutrient poor ecosystems (Chapin, 1980); and results from a model study on plant nutrient uptake (Raynaud and Leadley, 2004). The results from the current study shows that in addition to depolymerisation of high molecular weight organic N (Schimel and Bennett, 2004), induced diffusive flux of soil N to plant roots could be the rate-limiting step.

4.3 Paper III

In Paper III, a method was developed for simultaneous estimation of diffusion and mass flow of N compounds in standard solution and soil solution. This was aimed at determining the contributions of diffusion and mass flow to plant N nutrition and possible interactions between the two processes. In the first experiment, various perfusates with different osmotic potentials and different pump rates were tested in order to determine their capabilities to induce mass flow of water across the dialysis probes from standard solution and soil solution. A perfusate of 20% (w/v) Dextran 40 with osmotic potential of -0.1 MPa at the room temperature, and a pump flow rate of 1 µl min^{-1} induced a measureable mass flow of water from both standard solution and soil. Estimated rates of water flux towards roots have fallen between 0 – 10^{-7} m s^{-1} (Tinker and Nye, 2000; BassiriRad et al. 2008). However, my results show that the velocity of radial flux of water across dialysis probe membrane was 3.0 x

10^{-7} m s^{-1} in standard solution and 1.8 x 10^{-7} m s^{-1} in soil. These values correspond to water velocities at high transpiration rates.

Estimation of diffusive flux of N from standard solution and soil was thus achieved by using water as perfusate (Inselsbacher et al. 2011; Inselsbacher and Näsholm, 2012a, b; Paper II), while simultaneous estimation of mass flow and diffusive fluxes of N from both standard solution and soil was achieved using 20% Dextran 40 as perfusate. In standard solution, total fluxes of NH_4^+, NO_3^- and total amino acids across probe membranes were respectively, 58 %, 63 % and 34 % higher when Dextran 40 was used as perfusate than when water was used. In soil, total fluxes of NH_4^+ and total amino acids across probe membranes were 30 % and 72 % higher respectively, when Dextran 40 was the perfusate than when water was used. The flux rate of NO_3^- was below the detection limit when water was the perfusate, while the flux rate of NO_3^- was c. 3.9 nmol m^{-2} s^{-1} when Dextran 40 was used as perfusate.

Separating total fluxes of N into diffusive and mass flow flux, the contributions of mass flow to total fluxes of N were 19 % and 20 % for NH_4^+ and NO_3^-, respectively; and 25 % and 24 % for total amino acids and total N respectively in standard solution. In the soil, the contributions of mass flow to total fluxes of N were 6 %, 12 % and 14 % for NH_4^+, total amino acids and total N respectively. Mass movement of water (containing dissolved N compounds) across the dialysis membrane could be responsible for these results (i.e. direct effect of mass flow fluxes). The contributions of diffusive flux to total fluxes of N were significantly lower in soil than in standard solution. This might be due to chemical interactions between N compounds in soil solution and the solid phase of soils, biological interactions along the uptake path, or the tortuosity of soils. However, there was strong effect of mass flow on diffusive flux of N compounds in soil, but not in solution. I speculate that the indirect effect of mass flow on diffusion might be responsible for the observed increase in N diffusive fluxes in soil. This effect might have resulted in the formation of steeper concentration gradients between membrane surfaces and the soil, and in the process increased diffusive flux of N.

4.4 Paper IV

In Paper IV the method developed in paper III was used to estimate diffusion and mass flow of N and also to give insights into the role of each process for plant N nutrition in a nutrient-poor Scots pine and a nutrient-rich Spruce forest site. At the nutrient poor Scots pine site, the soil organic layer is 5 - 15 cm

deep, pH (H_2O) is 3.8 and soil moisture content was 0.9 g g^{-1} DW. In contrast, at the nutrient-rich spruce site, the organic layer is approximately 5 cm deep, pH (H_2O) is 5.4 and soil moisture content was 1.2 g g^{-1} DW. MilliQ water was used as perfusate for estimating induced diffusive flux of N compounds (Inselsbacher et al. 2011; Inselsbacher and Näsholm, 2012a, b; Papers I and II). A perfusate of 10 % (w/v) Dextran 40 was used to induce mass flow. Both perfusates were pumped through the microdialysis membranes at 1 µl min^{-1}. The Dextran solution had osmotic potential of -0.04 MPa at room temperature. Ten % (w/v) Dextran 40 was chosen because we aimed at lowering the velocity of radial flux of water across probe membranes, which were at the higher end of a range suggested by Tinker and Nye (2000) and BassiriRad et al. (2008) when 20 % (w/v) Dextran 40 was used (Paper III). However, the velocities of radial flux of water across probe membranes were 2.33 * 10^{-7} ms^{-1} and 2.15 * 10^{-7} ms^{-1} at the Scots pine and Norway spruce forest sites respectively, and were similar to results obtained in Paper III. These values correspond to values for water fluxes at high transpiration rates (Tinker and Nye, 2000; BassiriRad et al. 2008).

In spite of the many signs of contrasting soil fertility between the two sites, no differences in diffusive fluxes of N were observed. In contrast, mass flow resulted in a strong increase in induced total fluxes in both soils, but the effect was stronger in the nutrient rich soil. Specifically, the Dextran perfusate induced 53, 3 and 5 times higher total flux of NO_3^-, total amino acids, and total N than the water perfusate in the nutrient poor Scots pine soil. Similarly, the Dextran perfusate induced 45, 4 and 11 times higher fluxes of NO_3^-, total amino acids, and total N than the water prefusate in the nutrient-rich Norway spruce soil. These differences in induced total fluxes of N when water and Dextran 40 were used could be explained by NO_3^-, which in relative numbers increased similarly at both sites, but in absolute numbers increased much more in the nutrient rich site. The predominant effect of mass flow on NO_3^- could be associated with its high mobility. It has been suggested in earlier studies that mass flow is a major process for movement of NO_3^- to plant roots (Marschner, 1995; Lambers et al. 2008; Chapin et al. 2011). Results from Paper IV thus corroborate results from Paper III and I speculate that mass flow had direct and indirect effects on induced fluxes of N compounds.

In this study, depletion of N compounds in soils surrounding probe membranes was not investigated. However, possible depletion of N compounds in both forest soils was suspected, in particular when water was used as perfusate. This is because the flux rate of total N at the second sampling period was lower than

that observed during the first sampling period in both forest soils. This might have resulted from lack of replenishment of N in the bulk soil and subsequent formation of depletion zones around the probes. However, when Dextran 40 was used there were no changes in the total flux rate of total N at the first and second sampling times. I speculate that the indirect effect of mass flow on diffusion might have been responsible for this. This indirect effect might have resulted in the formation of steeper concentration gradients between membrane surfaces and the soil.

5 Conclusions and future perspectives

The results presented in this thesis focus on the processes involved in N acquisition by boreal forest plants. A novel, non-invasive, microdialysis technique was employed to estimate fluxes of organic and inorganic N in boreal forest soils. The microdialysis technique was further developed from a method that could only estimate diffusion of N compounds to one that can now also be used to estimate mass flow of N in soils. Further by comparing microdialysis measurements directly to root uptake studies, the relative importance of soil fluxes and root uptake capacities as limiting processes to plant N nutrition in the boreal forest ecosystem can be assessed.

My results suggest that the microdialysis technique gives reproducible results with relatively low temporal and spatial variation in induced N flux rates during soil sampling. The technique is, however, less suitable for determining soil N concentrations, because it measures induced flux rates rather than concentrations. Concentrations of individual N compounds could theoretically be extrapolated from their respective flux rates and relative recovery rates, as measured in standard solutions. However, the relative recovery rates of N compounds in soils cannot be determined and may be different from those established for solutions. Hence, using microdialysis to estimate soil solution concentrations is more problematic than using it to estimate fluxes. Microdialysis technique could be further developed in several areas and applied in other ecosystems. For instance, the knowledge gained from using the microdialysis technique to study N fluxes in N-limited boreal forest soil could also be extended to studying the fluxes of other N compounds (*cf.* Warren, 2013) and other major soil nutrients e.g. study of soil P fluxes in ecosystems of different P availabilities (Lambers et al. 2010; Oliveira et al. 2015).

The studies reported in this thesis revealed that amino acids are the dominant N compounds among the studied N forms in the studied boreal forest soils. The induced diffusive fluxes of soil N are responsive to fertilization: pulse-addition of NO_3^- solution strongly increased flux rates of NO_3^- across dialysis probe membranes. Using a solution containing Dextran 40 as perfusate, mass flow of water across the dialysis probe membranes was induced. Mass flow of water across the probe membranes was responsive to Dextran 40 concentration and reproducible effects on fluxes of soil N were achieved. These results support the claim that microdialysis can be used to simulate some of the crucial processes underpinning soil N turnover and plant N acquisition. Results from the studies presented in this thesis also showed that the maximum root uptake rates of N exceed induced diffusive fluxes of N to the roots. Further, it was shown that mass flow substantially increases N fluxes in soils and also alters the chemical composition of the N fluxes to a much greater contribution of NO_3^-. Considering both the significant increases in N fluxes due to mass flow and diffusion of soil N, future studies may investigate if flux rates for some N compounds may exceed their maximum root uptake rates.

Given that the results achieved through the microdialysis measurements are valid for a growing plant it follows that mass flow and hence plant transpiration are crucial for plant N acquisition. This result is in stark contrast to predictions from models investigating the importance of mass flow for plant N nutrition (Yanai, 1994; BassiriRad et al. 2008), but it is in line with some recent experimental studies (Cramer et al. 2008, Matimati et al. 2014; Gorska et al. 2008). The results presented in this thesis suggest that a growing plant may experience vastly different N availabilities depending on the rate of transpiration. This implies that transpiration may not only be the unfortunate downside of photosynthesis but that it may have an important role in plant N nutrition. Hence, future studies should test whether results from these microdialysis measurements can be validated through experiments on plants.

Knowledge gained through this study could assist us to optimize N use efficiency and enhance forest growth through the addition of sufficient inorganic N fertilizer without leaching of N compounds into groundwater. The possibility appearing through the use of microdialysis to compare soil N flux rates with root uptake capacities is relevant in this context.

The results presented in the thesis are also relevant for our predictions of how increased atmospheric CO_2 concentrations and how increased temperatures and altered precipitation may affect plant growth. Specifically, lowered

transpiration resulting from elevated CO_2 may have negative effects on plant N acquisition (McDonald et al. 2002; Conroy, 1992; Conroy and Hocking, 1993) and hence on plant growth. Therefore, experiments testing the importance of mass flow on acquisition of different N forms are warranted.

In summary, the microdialysis technique shows great promise. It has potential to give insights towards understanding the intrinsic processes involved in N acquisition by plant roots and overall plant N nutrition. These insights will be especially important in the boreal forest ecosystem, but they should be much more general.

References

Andersson, P. (2003). Amino acid concentration in sampled soil water: effects of sampling and storage using lysimeters with small pore-size and sterile pre-evacuated sampling tubes. *Communications in Soil Science and Plant Analysis* 34: 21-29.

Andersson, P., Berggren, D. (2005). Amino acids, total organic and inorganic nitrogen in forest floor soil solution at low and high nitrogen input. *Water, Air & Soil Pollution* 162: 369-384.

Barber, S.A. (1995). Nutrient uptake by plant roots growing in soil. *In: Soil nutrient bioavailability – A mechanistic approach. 2nd Ed.* John Wiley & Sons, Inc. New York, USA.

BassiriRad, H., Gutschick, V., Sehtiya, H.L. (2008). Control of plant nitrogen uptake in native ecosystems by rhizospheric processes. In: Bruulsema T, Ma L, Ahuja LR, eds. *Quantifying and understanding plant nitrogen uptake for systems modelling*. Boca Raton, Florida, USA: CRC Press, 71-93.

Behravan, J., Bazzaz, B.S..F., Salimi, Z. (2003). Optimization of dextran production by *Leuconostoc mesenteroides* NRRL B-512 using cheap and local sources of carbohydrates and nitrogen. *Biotechnology and Applied Biochemistry* 38: 267-269.

Chapin, F.S., III., Matson, P.A., Vitousek, P.M. (2011). *Principles of terrestrial ecosystem ecology*. Springer, New York, New York, USA.

Chapin, F.S., III. (1980). The mineral nutrition of wild plants. *Annual Review of Ecology and Systematic* 11: 233-260.

Chen, C.R., Xu, Z.H. (2008). Analysis and behavior of soluble organic nitrogen in forest soils. *Journal of Soils and Sediments* 8: 363-378.

Chen, C.R., Xu, Z.H., Zhang, S.L., Keay, P. (2005). Soluble organic nitrogen pools in forest soils of subtropical Australia. *Plant and Soil* 277: 285-297.

Comerford, N.B. (2005). Soil factors affecting nutrient bioavailability. In: BassiriRad, H. (Ed.) *Nutrient acquisition by plants an ecological perspective. Ecological Studies*, Vol. 181. Springer-Verlag Berlin Heidelberg, Germany, pp 1-14.

Conroy, J.P. (1992). Influence of elevated atmospheric CO_2 concentrations on plant nutrition. *Australian Journal of Botany* 40: 445-456.

Conroy, J., Hocking, P. (1993). Nitrogen nutrition of C_3 plants at elevated atmospheric CO_2 concentrations. *Physiologia Plantarum* 89: 570-576.

Cramer, M.D., Hoffmann, V., Verboom, G.A. (2008). Nutrient availability moderates transpiration in *Ehrharta calycina*. *New Phytologist* 179: 1048-1057.

Cramer, M.D., Hawkins, H.J., Verboom, G.A. (2009). The importance of nutritional regulation of plant water flux. *Oecologia* 161: 15-24.

Farrell, M., Hill, P.W., Farrar, J., DeLuca, T.H., Roberts, P., Kielland, K., Dahlgren, R., Murphy, D.V., Hobbs, P.J., Bardgett, R.D., Jones, D.L. (2013). Oligopeptides represent a preferred source of organic N uptake: a global phenomenon. *Ecosystems* 16: 133-145.

Farrell, M., Hill, P.W., Farrar, J., Bardgett, R.D., Jones, D.L. (2011). Seasonal variation in soluble soil carbon and nitrogen across a grassland productivity gradient. *Soil Biology & Biochemistry* 43: 835-844.

Frommer, W.B., Hummel, S., Riesmeier, J.W. (1993). Expression cloning in yeast of a cdna-encoding a broad-specificity amino-acid permease from *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences of the United States of America* 90: 5944-5948.

Giesler, R., Lundström, U.S., Grip, H. (1996). Comparison of soil solution chemistry assessment using zero-tension lysimeters or centrifugation. *European Journal of Soil Science* 47: 395-405.

Gloser V, Zwieniecki MA, Orians CM, Holbrook NM. (2007). Dynamic changes in root hydraulic properties in response to nitrate availability. *Journal of Experimental Botany* 58: 2409 -2415.

Gorska A, Ye Q, Holbrook NM, Zwieniecki MA. (2008). Nitrate control of root hydraulic properties in plants: Translating local information to whole plant response. *Plant Physiology* 148: 1159-1167.

Gruffman, L., Jämtgård, S., Näsholm, T. (2014). Plant nitrogen status and co-occurrence of organic and inorganic nitrogen sources influence root uptake by Scots pine seedlings. *Tree Physiology* 34: 205-213.

Harrison, K., Bol, R., Bardgett, R.D. (2007). Preferences for different nitrogen forms by coexisting plant species and soil microbes. *Ecology* 88(4): 989-999.

Hartmann, A., Schmid, M., van Tuinen, D., Berg, G. (2009). Plant-driven selection of microbes. *Plant Soil* 321: 235-257.

Högberg, P., Johannisson, C., Yarwood, S., Callesen, I., Näsholm, T., Myrold, D.D., Högberg, M.N. (2011). Recovery of ectomycorrhiza after 'nitrogen saturation' of a conifer forest. *New Phytologist* 189: 515-525.

Hirner, A., Ladwig, F., Stransky, H., Okumoto, S., Keinath, M., Harms, A., Frommer, W.B., Koch, W. (2006). Arabidopsis *LHT1* is a high-affinity transporter for cellular amino acid uptake in both root epidermis and leaf mesophyll. *Plant Cell* 18: 1931-1946.

Inselsbacher, E. (2014). Recovery of individual soil nitrogen forms after sieving and extraction. *Soil Biology & Biochemistry* 71: 76-86.

Inselsbacher, E., Näsholm, T. (2012a). The below-ground perspective of forest plants: soil provides mainly organic nitrogen for plants and mycorrhizal fungi. *New Phytologist* 195: 329-334.

Inselsbacher, E., Näsholm, T. (2012b). A novel method to measure the effect of temperature on diffusion of plant-available nitrogen in soil. *Plant Soil* 354: 251-257.

Inselsbacher, E., Öhlund, J., Jämtgård, S., Huss-Danell, K., Näsholm, T. (2011). The potential of microdialysis to monitor organic and inorganic nitrogen compounds in soil. *Soil Biology & Biochemistry* 43: 1321 – 1332.

Ivarson, K.C., Sowden, F.J. (1966). Effect of freezing of amino acids in soil. *Canadian Journal of Soil Science* 46: 115-120.

Jämtgård S., Näsholm T., Huss-Danell K. (2010). Nitrogen compounds in soil solutions of agricultural land. *Soil Biology & Biochemistry* 42: 2325-2330.

Johnson, D.W. Verburg, P.S.J., Arnone, J.A. (2005). Soil extraction, ion exchange resin, and ion exchange membrane measures of soil mineral nitrogen during incubation of a tallgrass prairie soil. *Soil Science of America Journal* 69: 260-265.

Jones, D.L., Darrah, P.R. (1993). Influx and efflux of amino-acids from *Zea mays* L roots and their implications for N nutrition and the rhizosphere. *Plant and Soil* 155: 87-90.

Jones, D.L., Willett, V.B. (2006). Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. *Soil Biology and biochemistry* 38: 991-999.

Jones, D.L., Healey, J.R., Willett, V.R., Farrar, J.F., Hodge, A. (2005). Dissolved organic nitrogen uptake by plants – an important N uptake pathway? *Soil Biology & Biochemistry* 37: 413-423.

Jungk, A., Claassen, N. (1997). Ion diffusion in the soil-root system. *Advances in Agronomy* 61: 53-110.

Kamminga-Van, W.C., Prins, H.B.A. (1993). The kinetics of NH_4^+ and NO_3^- uptake by Douglas fir from single N-solutions and from solutions containing both NH_4^+ and NO_3^-. *Plant and Soil* 151: 91-96.

Kielland, K. (1994). Amino acid absorption by arctic plants: implications for plant nutrition and nitrogen cycling. *Ecology* 75: 2373-2383.

Kronzucker, H.J., Siddiqi, M.Y., Glass, A.D.M. (1997). Conifer root discrimination against soil nitrate and the ecology of forest succession. *Nature* 385(2): 59-61.

Kupper P, Rohula G, Saksing L, Sellin A, Lõhmus K, Ostonen I, Helmisaari H-S, Sõber A. (2012). Does soil nutrient availability influence night-time water flux of aspen saplings? *Environmental and Experimental Botany* 82:37-42.

Lambers, H., Brundrett, M.C., Raven, J.A., Hopper, S.D. (2010). Plant mineral nutrition in ancient landscapes: high plant species diversity on infertile soils is linked to functional diversity for nutritional strategies. *Plant and Soil* 334: 11-31.

Lambers, H., Chapin, F.S., III., Pons, T.L. (2008). *Plant physiological ecology*. 2nd Edition. Springer, New York, USA.

Leadley, P.W., Reynolds, J.F., Chapin, F.S. (1997). A model of nitrogen uptake by *Eriophorum vaginatum* roots in the field: Ecological implications. *Ecological Monographs* 67: 1-22.

Lee, Y.H., Foster, J., Chen, J., Voll, L.M., Weber, A.P.M., Tegeder, M. (2007). AAP1 transports uncharged amino acids into roots of Arabidopsis. *Plant Journal* 50: 305-319.

Lehmann, S., Gumy, C., Blatter, E., Boeffel, S., Fricke, W., Rentsch, D. (2011). *In planta* function of compatible solute transporters of the AtProT family. *Journal of Experimental Botany* 62: 787-796.

Leitner, D., Klepsch, S., Ptashnyk, M., Marchant, A., Kirk, G.J.D., Schnepf, A., Roose, T. (2010). A dynamic model of nutrient uptake by root hairs. *New Phytologist* 185:792-802.

Lipson, D.A., Monson, R.K. (1998). Plant-microbe competition for soil amino acids in the alpine tundra: effects of freeze-thaw and dry-rewet events. *Oecologia* 113: 406-414.

Lipson, D.A., Raab, T.K., Schmidt, S.K., Monson, R.K. (2001). An empirical model of amino acid transformations in alpine soil. *Soil Biology & Biochemistry* 33: 189-198.

McDonald, E.P., Erickson, J.E., Kruger, E.L. (2002). Can decreased transpiration limit plant nitrogen acquisitions in elevated CO_2? *Functional Plant Biology* 29: 1115-1120.

Marschner, H. (1995). *Mineral nutrition of higher plants*. 2nd Edition. Academic press limited, London.

Matimati, I., Verboom, G.A., Cramer, M.D. (2014). Nitrogen regulation of transpiration controls mass flow acquisition of nutrients. *Journal of experimental Botany* 65(1):159-168.

Metcalfe, R.J., Nault, J., Hawkins, B.J. (2011). Adaptations to nitrogen form: comparing inorganic nitrogen and amino acid availability and uptake by four temperate forest plants. *Canadian Journal of Forest Research* 41: 1626-1637.

Näsholm, T., Ekblad, A., Nordin, A., Giesler, R., Högberg, M., Högberg, P. (1998). Boreal forest plants take up organic nitrogen. *Nature* 392 (30): 914-916.

Näsholm, T., Huss-Danell, K., Högberg, P. (2000). Uptake of organic nitrogen in the field by four agricultural important plant species. *Ecology* 81 (4): 1155-1161.

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

Nacry, P., Bouguyon, E., Gojon, A. (2013). Nitrogen acquisition by roots: physiological and developmental mechanisms ensuring plant adaptation to a fluctuating resource. *Plant and Soil* 370: 1-29.

Nazoa, P., Vidmar, J.J., Tranbarger, T.J., Mouline, K., Damiani, I., Tillard, P., Zhuo, D.G., Glass, A.D.M., Touraine, B. (2003). Regulation of the nitrate transporter gene *AtNRT2*.1 in *Arabidopsis thaliana*: responses to nitrate, amino acids and developmental stage. *Plant Molecular Biology* 52: 689-703.

Nordin, A., Högberg, P., Näsholm, T. (2001). Soil nitrogen form and plant nitrogen uptake along a boreal forest productivity gradient. *Oecologia* 129: 125-132.

Nye, P.H. (1967). Changes in the concentration of nutrients in the soil near planar absorbing surfaces when simultaneous diffusion and mass flow occur. *Trans. Intern Soc. Soil Sci. Comm* II & IV: 317-328.

Nye, P.H., Marriott, F.H. (1969). A theoretical study of the distribution of substances around roots resulting from simultaneous diffusion and mass flow. *Plant and Soil* 30: 459-472.
Nye, P.H., Tinker, P.B. (1977). *Solute movement in the soil-root system*. Blackwell Scientific Publications, Oxford, UK (342pp.).
Nye, P.H. (1979). Diffusion of ions and uncharged solutes in soil and soil clays. *Advances in Agronomy* 31:225-272.
Öhlund, J., Näsholm, T. (2004). Regulation of organic and inorganic nitrogen uptake in Scots pine (*Pinus sylvestris*) seedlings. *Tree Physiology* 24: 1397-1402.
Öhlund, J. (2004). Organic and inorganic nitrogen sources for conifer seedlings: abundance, uptake and growth. *Acta Universitatis Agriclturae Sueciae, Silvestria* 312. Doctoral Thesis, Swedish University of Agricltural Sciences.
Öhlund, J., Näsholm, T. (2001). Growth of conifer seedlings on organic and inorganic nitrogen sources. *Tree Physiology* 21: 1319-1326.
Oliveira, R.S., Galvão, H.C., de Campos, M.C.R., Eller, C.B., Pearse, S.J., Lambers, H. (2015). Mineral nutrition of *campos rupestres* plant species on contrasting nutrient-impoverished soil types. *New Phytologist* 205: 1183-1194.
Paungfoo-Lonhienne, C., Lonhienne, T.G.A., Rentsch, D., Robinson, N., Christie, M., Webb, R.I., Gamage, H.K., Carroll, B.J., Schenk, P.M., Schmidt, S. (2008). Plants can use protein as a nitrogen source without assistance from other organisms. *PNAS* 105(11): 4524-4529.
Paungfoo-Lonhienne, C., Visser, J., Lonhienne, T.G.A., Schmidt, S. (2012). Past, present and future of organic nutrients. *Plant and Soil* 359: 1-18.
Persson, J., Gardeström, P., Näsholm, T. (2006). Uptake, metabolism and distributionof organic and inorganic nitrogen sources by *Pinus sylvestris*. *Journal of Experimental Botany* 57(11): 2651-2659.
Persson, J., Högberg, P., Ekblad, A., Högberg, M., Nordgren, A., Näsholm, T. (2003). Nitrogen acquisition from inorganic and organic sources by boreal forest plants in the field. *Oecologia* 137: 252-257.
Persson, J., Näsholm, T. (2002). Regulation of amino acid uptake in conifers by exogenous and endogenous nitrogen. *Planta* 215: 639-644.
Pfautsch, S., Rennenberg, H., Bell, T.L., Adams, M.A. (2009). Nitrogen uptake by *Eucalyptus regnans* and *Acacia* spp. – preferences, resource overlap and energetic costs. *Tree Physiology* 29: 389-399.
Plhák, F. (2003). Nitrogen supply through transpiration mass can limit nitrogen nutrition of plants. *Plant Soil Environment* 49 (3): 473-479.
Raven, J.A. (2008). Transpiration: how many functions? *New Phytologist* 179: 905-907.
Rawat, S.R., Silim, S.N., Kronzucker, H.J., Siddiqi, M.Y., Glass, A.D.M. (1999). *AtAMT1* gene expression and NH_4^+ uptake in roots of *Arabidopsis thaliana*: evidence for regulation by root glutamine levels. *Plant Journal* 19: 143-152.
Raynaud, X., Leadley, P.W. (2004). Soil characteristics play a key role in modeling nutrient competition in plant communities. *Ecology* 85: 2200-2214.
Rosdahl, H., Hamrin, K., Ungerstedt, U., Henriksson, J. (2000). A microdialysis method for the in situ investigation of the action of large peptide molecules in human skeletal muscle: detection of local metabolic effects of insulin. *International Journal of Biological Macromolecules* 28: 69-73.
Rousk, J., Jones, D.L. (2010). Loss of low molecular weight dissolved organic carbon (DOC) and nitrogen (DON) in water and 0.5 M K_2SO_4extracts. *Soil Biology and Biochemistry* 42: 2331-2335.
Sauheitl, L., Glaser, B., Weigelt, A. (2009). Uptake of intact amino acids by plants depends on soil amino acid concentrations. *Environmental and Experimental Botany* 66: 145-152.
Schachtman, D.P., Reid, R.J., Ayling, S.M. (1998). Phosphorus uptake by plants: From soil to cell. *Plant Physiology* 116: 447-453.
Schimel, J.P., Bennett, J. (2004). Nitrogen mineralization: challenges of a changing paradigm. *Ecology* 85 (3): 591-602.
Schulten, H.R., Schnitzer, M. (1998). The chemistry of soil organic nitrogen; a review. *Biology and Fertility of Soils* 26: 1-15.

Senwo, Z.N., Tabatabai, M.A. (1998). Amino acid composition of soil organic matter. *Biology and Fertility of Soils* 26: 235-242.
Smethurst, P.J. (2000). Soil solution and other analyses as indicators of nutrient supply: a review. *Forest Ecology and Management* 138:397-411.
Stoelken, G., Simon, J., Ehlting, B., Rennenberg, H. (2010). The presence of amino acids affect inorganic N uptake in non-mycorrhizal seedlings of European beech (*Fagus sylvatica*). *Tree Physiology* 30: 1118-1128.
Strebel, O., Duynisveld, W.H.M. (1989). Nitrogen supply to cereals and sugar beet by mass flow and diffusion on a silty loam soil. *Z. Pflanzenernähr. Bodenk.*, 152: 135-141.
Streeter, T.C., Bol, R., Bardgett, R.D. (2000). Amino acids as a nitrogen source in temperate upland grasslands: the use of dual labelled (^{13}C, ^{15}N) glycine to test for direct uptake by dominant grasses. *Rapid Communications in Mass Spectrometry* 14: 1351-1355.
Svennerstam, H., Ganeteg, U., Näsholm, T. (2008). Root uptake of cationic amino acids by Arabidopsis depends on functional expression of amino acid permease 5. *New Phytologist* 180: 620-630.
Svennerstam, H., Ganeteg, U., Bellini, C., Näsholm, T. (2007). Comprehensive screening of Arabidopsis mutants suggests the lysine histidine transporter 1 to be involved in plant uptake of amino acids. *Plant Physiology* 143: 1853-1860.
Thomsen, I.K., Schjonning, P. (2003). Evaluation of a non-destructive technique for inorganic soil N measurement. *Geoderma* 113: 147-160.
Thornton, B., Robinson, D. (2005). Uptake and assimilation of nitrogen from solution containing multiple N sources. *Plant, Cell & Environment* 28: 813-821.
Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R., Polasky, S. (2002). Agricultural sustainability and intensive production practices. *Nature* 418: 671-677.
Tinker, P.B., Nye, P.H. (2000). *Solute movement in the rhizosphere*. Oxford University Press, USA (444pp.).
Vidmar, J.J., Zhuo, D., Siddiqi, M.Y., Schjoerring, J.K., Touraine, B., Glass, A.D.M. (2000). Regulation of high-affinity nitrate transporter genes and high-affinity nitrate influx by nitrogen pools in roots of barley. *Plant Physiology* 123: 307-318.
Vitousek, P.M., Howarth, R.W. (1991). Nitrogen limitation on land and in the sea: How can it occur? *Biogeochemistry* 13: 87-115.
Warren, C.R. (2013). Quaternary ammonium compounds can be abundant in some soils and are taken up as intact molecules by plants. *New Phytologist* 198: 476-485.
Weigelt, A., Bol, R., Bardgett, R.D. (2005). Preferential uptake capacity of amino acids by ten grasses and forbs in relation to soil acidity and nitrogen availability. *Oecologia* 142: 627-635.
Wilkinson, S., Bacon, M.A., Davies, W.J. (2007). Nitrate signalling to stomata and growing leaves: interactions with soil drying, ABA and xylem sap pH in maize. *Journal of Experimental Botany* 58: 1705-1716.
Yanai, R.D. (1994). A steady-state model of nutrient uptake accounting for newly grown roots. *Soil Science Society of America Journal* 58: 1562-1571.
Yu, Z., Zhang, Q., Kraus, T.E.C., Dahlgre, R.A., Anastasio, C., Zasoski, R.J. (2002). Contributions of amino compounds to dissolved organic nitrogen in forest soils. *Biogeochemistry* 61: 173-198.

Acknowledgements

I would like to express my gratitude to God who made this possible.

My heartfelt appreciation to my supervisors: Prof. Torgny Näsholm, Dr. Erich Inselsbacher and Dr. Sandra Jämtgård for giving me the opportunity to work with them in the past four years. I highly appreciate your support, motivation and encouragement. I have learnt a lot from you and I believe these lessons will assist me immensely in my future endeavours.

My sincere gratitude to my opponent, Professor Richard Bardgett and the members of the thesis evaluation committee; Professors Anita Sellstedt, Tryggve Persson and Reiner Giesler for their valuable comments on this thesis.

Special thanks to Prof. John Marshall for his valuable comments on this thesis. I really appreciate the encouragement and support of the present and past members of the N group: Ulrika, Hyungwoo, Nils, Margareta, Iftikhar, Linda, Johanna, Camila, Henrik, Mattias, Lasse, Dörte and Veronika.

I appreciate all my colleagues here in SLU for your assistance and kind gesture. Special thanks to Bright Kumordzi, Robert, Andres, Tejshree & Johannes, Daniel Lussetti, Ogonna Obudulu, Rose-Marie, Georg, Junbin, Andy, Jordan, Guillaume, Ben Jackson, Anna-Maria, Clydecia, Poy, and other PhD colleagues and Post-Docs at the department. The kind support and assistance of the administrative staff of the Department: Ulf, Karin, Elisabeth, Ann-Kathrin, is hereby appreciated.

Thanks to Olusola Alabi, Ikena Obi, Evans Adjei, Mr. Ali, Amin Kamiru, Chidi, Jude, Gbotemi Adediran and Seun Omoniyi. Special thanks to my special friends: Dapo Fawehinmi, Muyiwa Ojo, Sunkanmi Aliu, Opeyemi Ojo,

Adebola Orosakin, Tunde Afilaka, Omolola Martins, Gbenga Omoku, Adu Johnson and Taiwo Fadare.

My sincere appreciation to the families of Agbaje, Sesan and Oluyadi for their support.

Special thanks to my parents - Mr & Mrs. Oyewole my siblings for their tremendous support and encouragement over the years.

My heartfelt gratitude to my fiancée - Adebola Agbaje. Thank you for your love, support, words of encouragement and above all, great company.