Nitrogen Enrichment of a Boreal Forest

Implications for Understory Vegetation

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Implications for Understory Vegetation

The aim of this thesis was to investigate how nitrogen (N) enrichment influences ecophysiological processes involved in driving changes in understory species composition in Swedish boreal forests. Studies were performed in a long-term N experiment started in 1996 including the following treatments: control, N additions (12.5 and 50 kg N ha^{-1} yr^{-1}) and recovery (50 kg N ha^{-1} yr^{-1} for five years and then no N addition). Firstly, I studied plant-available N forms deposited with throughfall precipitation, and estimated uptake by mosses and lichens of this N. Regardless of the N treatments, rainwater contributed c. 2 kg N ha^{-1} yr^{-1} and snowmelt c. 0.3 kg N ha^{-1} yr^{-1} to the vegetation. The ground-living bryophyte *Hylocomium splendens* and the epiphytic lichen *Platismatia glauca* took up both organic (glycine) and inorganic (NH_4^+ and NO_3^-) N from the precipitation. The uptake did not significantly differ between the N treatment plots. On the 50 kg N ha^{-1} yr^{-1} plots the abundance of *H. splendens* decreased by 81% following eight years of N additions. The ecophysiological response of *H. splendens* to this N treatment included accumulation of arginine, but no significant changes in its soluble carbohydrate or chlorophyll contents were detected. Secondly, I studied N competition between *Vaccinium myrtillus* and *Deschampsia flexuosa*. I found no significant effects of the long-term N treatments on plant uptake of four different N forms (NH_4^+, NO_3^-, glycine and peptides). Both plants acquired N from NH_4^+, NO_3^- and glycine, but no substantial uptake from peptides was found. When N uptake of the two species was related to the plant biomass, *D. flexuosa* acquired all N forms more efficiently than *V. myrtillus*, but the difference between the species in this respect was greatest for NO_3^-. Finally, results of long-term (12 years) monitoring of the understory vegetation on control, 12.5 and 50.0 kg N ha^{-1} yr^{-1} plots demonstrated that two natural enemies (the fungal pathogen *Valdensia heterodoxa* and the herbivorous larval form of *Operophtera* spp.) exerted strong control over the abundance of the dominant plant, *V. myrtillus*. The study highlights the need for long-term studies to fully capture biotic interactions that influence vegetation dynamics. In summary, changes in N supply may have profound effects on quantitative and qualitative aspects of plant N availability, plant N uptake, plant biochemistry as well as interactions between plants and their natural enemies

Keywords: Amino acids, Boreal forest, *Deschampsia flexuosa*, *Hylocomium splendens*, Natural enemies, Nitrogen, Resource partitioning, Soluble carbohydrates, Throughfall precipitation, *Vaccinium myrtillus*.

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Till Mista I & II

I´d rather go skiing...

Contents

List of Publications

This thesis is based on the work described in the following Papers, referred to by the corresponding Roman Numerals in the text:

I Forsum Å., Dahlman L., Näsholm T. and Nordin A. (2006). Nitrogen utilization by *Hylocomium splendens* in a boreal forest fertilization experiment. *Functional Ecology* 20, 421-426.

II Forsum Å., Laudon H. and Nordin A. (2008). Nitrogen uptake by *Hylocomium splendens* during snowmelt in a boreal forest. *ÉcoScience* 15, 315-319.

III Forsum Å. and Nordin A. Nitrogen form preferences and competition between *Vaccinium myrtillus* and *Deschampsia flexuosa* in a nitrogen enriched boreal forest. (Manuscript).

IV Nordin A., Strengbom J., Forsum Å. and Ericson L. Complex biotic interactions drive vegetation change in a nitrogen enriched boreal forest. (Submitted).

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1 Introduction

1.1 The natural limitation of N

Nitrogen (N) is present in all living organisms and is a building block of proteins and DNA molecules, and hence essential for life. N is difficult for most living organisms to access, even though it is an abundant molecule on earth, since the largest pool of N is present in the atmosphere as inert N_2. This nonreactive pool of N is mainly available to vascular plants through free-living and symbiotic N_2-fixing microbes (Marschner 1995), or through oxidation by oxygen or ozone in the presence of lightning or ultraviolet radiation. Conversion back to its gaseous form occurs through denitrification and the burning of biomass (Taiz & Ziegert 1998).

Plant growth in most terrestrial ecosystems is considered to be N-limited, despite that the ecosystems often contain vast amounts of N. The majority of this N is inaccessible to plants, being immobilized in structures such as plant litter, standing living plant biomass, and microbial biomass (Tamm 1991). This organically bound N becomes available to plants when organic compounds are mineralized into ammonium (NH_4^+), which in turn can undergo nitrification to nitrate (NO_3^-) by hetero- or auto-trophic nitrifying microorganisms (Taiz & Ziegert 1998). However, mineralization rates in boreal ecosystems are slow, due to low temperatures and acidic soils (Swift *et al.* 1979, Van Cleve & Alexander 1979), and consequently the availability of NH_4^+ and NO_3^- is low (Tamm 1991). The previously prevailing assumption that $NH_4^+$and NO_3^- are the most important N forms available to plants has been revised in the last decade. An increasing number of studies have shown that organic N forms, primarily in the form of amino acids, are taken up by plants in boreal, (Näsholm *et al.* 1998, Nordin *et al.* 2001, Persson & Näsholm 2001), arctic (Chapin *et al.* 1993, Kielland 1997, Nordin *et al.* 2004, Kielland *et al.* 2007), and alpine ecosystems (Raab *et al. 1999*, Lipson *et al.* 1999). It is now becoming widely accepted that amino acids contribute substantially to the N economy of boreal and arctic plants (Lipson & Näsholm 2001, Schimel & Bennett 2004).

1.2 Human alteration of the global N cycle

Inputs of biologically available N forms to the biosphere have increased substantially over the last century due to anthropogenic activities. Industrial fixation of N in the production of fertilizer for agricultural purposes, the cultivation of N_2-fixing crops, and the combustion of fossil fuels have all increased inputs of biologically reactive N forms to the global N cycle (Vitousek *et al.* 2002). Compared to natural N_2 fixation, these human activities have more than doubled the inputs of reactive N to the global N cycle (Galloway *et al.* 2004). During such activities, N is lost to the atmosphere as nitric oxide and nitrogen dioxide (NO and NO_2 respectively, collectively termed NO_x), ammonia (NH_3), and nitrous oxide (N_2O). NO_x is created during the combustion of fossil fuels and emissions of NH_3 and N_2O occur through the production and use of fertilizers, with farmyard manure being another important source of NH_3. N lost to the atmosphere, can be transported long distances (particularly as NO_x) before brought back to terrestrial and aquatic ecosystems via wet (precipitation in the form of rain and snow) and dry (windborne gaseous particles) deposition. This atmospheric transport and deposition results in a considerable re-distribution of biologically available N from emission "hot-spots" (i.e. agricultural and densely populated regions) to remote regions with undisturbed ecosystems naturally adapted to very low N inputs.

The global N cycle is closely linked to the climate system and the global carbon (C) cycle (Vitousek *et al.* 1997). The highest global surface temperatures, since measurements began in the 1850s (IPCC 2007), have been recorded during eleven out of the last twelve years (1995-2006). There is consensus in the scientific community that the cause of the apparent temperature rise is anthropogenic release of so-called greenhouse gases (GHG). This change in climatic conditions is predicted to result in a 2-6 C° rise in the average global temperature by the end of the 21th century (IPCC 2007), which is expected to affect the global N and C cycles in numerous ways. For example, higher temperatures in boreal and arctic ecosystems are likely to enhance soil mineralization rates, thus increasing the soil supply of inorganic N forms (Schmidt *et al.* 2002). Furthermore, N availability plays a crucial role in controlling key interactions of the global C cycle, and the amount of C fixed by plants is generally restricted by the N supply (Gruber & Galloway 2008). Today, increases in N availability due to N deposition are believed to be responsible for the increase in terrestrial C sequestration found worldwide (Magnani *et al.* 2007). Therefore, increasing N availability in N-limited ecosystems to increase C sequestration is sometimes considered as a method of mitigating climate change. Consequently, there is an urgent need for an understanding of the effects of N addition on the biogeochemical N cycle of terrestrial ecosystems, and its consequences for biodiversity.

1.3 Nitrogen enrichment of boreal forests

N inputs to boreal forests have increased over the last 50 years, due to atmospheric N deposition and commercial forest fertilization. In Sweden, atmospheric N deposition occurs across a gradient that ranges from 15 kg N ha^{-1} $year^{-1}$ in the southwest, to 1-2 kg N ha^{-1} $year^{-1}$ in the far north (Fig. 1). Commercial forest fertilization is a silvicultural practice that started in the early 1960's and was extensively practiced for c. 30 years, with c. 200 000 ha fertilized each year during the mid 1970's (Fig. 2). Interest in commercial forest fertilization declined in the early 1990's, mainly due to the recognition of limited knowledge regarding the environmental side-effects of the practice (Nohrstedt & Westling 1995). However, increasing demand for forestry products over recent years has resulted in renewed interest in forest fertilization, and in 2007 about 36 000 ha was fertilized in Sweden (Fig. 2). The most common fertilization procedure is to add 150 kg N ha^{-1} 10 – 15 years before the final forest harvest. However, there is growing interest in increasing the number of fertilizer applications during a rotation period, and also in fertilizing young forests (< 10 years old).

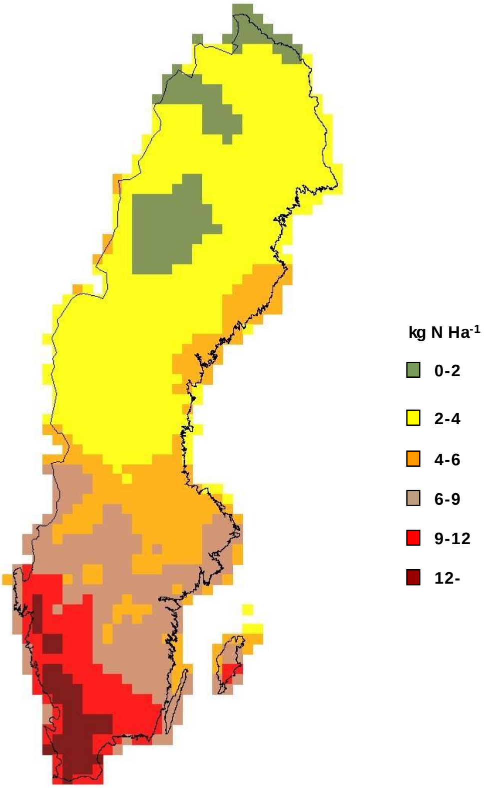

Figure 1. Atmospheric N deposition (NH_X and NO_x) over Sweden in 2005 (kg N ha^{-1}) (SMHI 2008)

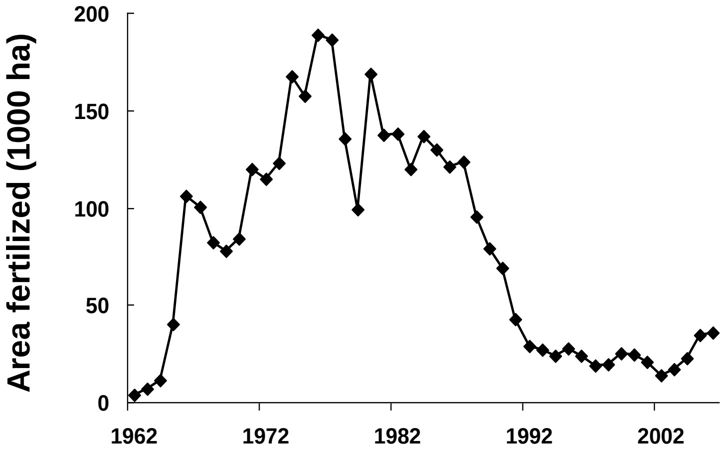

Figure 2. Area of forest land subjected to commercial forest fertilization in Sweden during the years 1962-2006 (Skogsstyrelsen 2007)

The ecosystem structure and function of boreal forests have evolved under conditions of strict N limitation. Thus, the N-limited nature of boreal forests has favored N-preserving plant traits, such as slow growth rates and biomass turnover (Grime 1977, Chapin 1980). These strategies have been successful in low N-availability environments, but become disadvantageous if the N availability increases (Chapin 1980, Tamm 1991). In N-rich environments, plants with such traits are often replaced by nitrophilous species with faster growth rates (Tilman 1988). Instead of using additional N for growth, the slow-growing species accumulate N in their tissues and consequently achieve higher internal N concentrations (Chapin *et al.* 1986, Lähdesmäki *et al.* 1990, Näsholm & Ericsson 1990, Lipson *et al.* 1996, Nordin & Näsholm 1997). Besides the direct effect that increased N availability exerts on the inter-specific competitive balance, indirect effects include modification of the interactions between plants and the herbivores (Huntly 1991) or pests and parasites (Harper 1990, Strengbom *et al.* 2002, Mitchell 2003) that feed on them (i.e. natural enemies). A small increase in host plant quality may affect pathogenic fungal or herbivore populations (Crawley 1993, White 1993, Marschner 1995). N-induced vegetation change can therefore be mediated by increased attacks by natural enemies reducing the dominant species' leaf area and thereby increasing understory light availability for relatively fast-growing competitive species (Aerts *et al.* 1990, Strengbom *et al.* 2002). However, several natural enemies often share the same host plant. Thus, in order to asses the effect on N addition on the vegetation the interaction between different natural enemies also needs to be understood.

2 Objectives

Overall, the studies presented in this thesis aimed to increase our understanding of the effects of N enrichment on ecophysiological processes that influence species composition of the understory vegetation in a Swedish spruce-dominated boreal forest.

The specific questions addressed were:

- Which plant-available N forms are deposited with throughfall precipitation during the vegetation period and during snowmelt in an N-enriched ecosystem?
- Is N in throughfall precipitation used by ground-living forest bryophytes and epiphytic lichens?
- What is the ecophysiological response of the bryophyte *Hylocomium splendens* to increased N supply?
- Are species-specific N form preferences important for the competitive interaction between *Vaccinium myrtillus* and *Deschampsia flexuosa* subjected to increased N supply?
- Do biotic interactions drive vegetation dynamics in an N-enriched boreal forest and do they operate differently in the long-term than in the short-term?

3 Material and Methods

3.1 The experimental site

The experiments presented in this thesis were performed within the Svartberget experimental forest (64º14´N, 19º46´E, 70 km NW of Umeå), northern Sweden, in the middle boreal zone (Ahti *et al.* 1968). The background deposition of atmospheric N in this region has been estimated to c. 3 kg N ha^{-1} yr^{-1} (Phil-Karlsson *et al.* 2003, I & II). The experimental site is a late successional Norway spruce (*Picea abies* L.) forest of *Vaccinum Myrtillus* type (Kalliola 1973). *Hylocomium splendens* (Hedw.) B.S.G. and *Pleurozium schreberi* (Brid.) Mitt. are the dominating bryophytes compromising 50 and 30% of the bottom layer moss mats, respectively. Other major bryophytes are *Ptilium crista-castrénsis* (Hedw.) De Not, *Dicranum polysetum* (Sw.) and *Dicranum scoparium* (Hedw.). The ericaceous dwarf shrub *Vaccinum myrtillus* L. dominates the understory vegetation, and other important species are *V. vitis-idea* L., *Linnea borealis* L. and *Deschampsia flexuosa* (L.) Trin.. Common tree-living lichens include *Platismatia glauca* (L.) W.L.Culb. & C.F.Culb. and *Hypogymnia physodes* (L.) Nyl..

An N addition experiment was set up in 1996, and square plots of 1000 and 5000 m^2 (only 1000 m^2 for control plots) were assigned to different N treatments: control (0 kg N ha^{-1} yr^{-1}), low N addition (12.5 kg N ha^{-1} yr^{-1}) and high N addition (50 kg N ha^{-1} yr^{-1}) in a randomized block design (n = 6 for each N treatment). Experimental N additions began in 1996 and were repeated each year by adding granulated NH_4NO_3 by hand at the onset of the growing season (late May or early June). In 2001 fertilized plots (sized 5000 m^2) were split in halves, and the following years N was only added to half of the original plots. The other halves were left without N addition, and are referred to as recovery plots in this thesis.

3.2 The study species

This thesis focuses on a few foundation plant species of boreal mesic spruce-dominated forests: *V. myrtillus*, *D. flexuosa, H. splendens* and *P. glauca*. *V. myrtillus* is an ericaceous dwarf-shrub with wide geographic distribution. A large number of organisms are associated with it (Niemelä *et al.* 1982). *V. myrtillus* may also serve as a model plant for many slow-growing boreal species adapted to low N availability. The grass *D. flexuosa* can inhabit N poor ecosystems, but often responds positively to increased N availability, and may serve as a model plant for species favored by increased N supply. *H. splendens* is an abundant boreal forest bryophyte in mesic forests. Its importance for boreal ecosystem functioning was acknowledged by Tamm (1953) already in the 1950´s. Of the forest mosses common in boreal coniferous forest, *H. splendens* has been identified as particularly sensitive to high N input (Hallingbäck 1992, Olsson & Kellner 2006, Pitcairn *et al.* 2006). Decreased *H. splendens* abundance following N fertilization has been observed long after (nearly 50 years) the termination of fertilizations (Strengbom *et al.* 2001). *P. glauca* is one of the most common lichens found on branches in full-grown and old-growth spruce forests.

Among the studied species there are also organisms that serve as natural enemies to the studied plants. *Operophtera brumata* L. and *O. fagata* Scharfenberg are two closely related polyphagous moths, which in Swedish boreal forests share the same biology. *V. myrtillus* is the main host plant for *Operophtera* spp. larvae in coniferous boreal forests. *Operophtera* spp. eggs hatch in late May or early June and the larvae feed until mid-July when they drop to the ground and form pupae. Adult moths start to emerge in mid-September. Wingless females are then located by the males, and after mating both female and males die and the species survive the winter as eggs. Outbreaks of *Operophtera* spp. larvae are common in many ecosystems. For instance, larval outbreaks have been reported to cause severe defoliation of *V. myrtillus* in British moorlands (Kerslake *et al.* 1996). Furthermore, larvae feeding on host plants with improved quality (e.g. fertilized *V. myrtillus*) grow better (Strengbom *et al.* 2005). *Valdensia heterodoxa* Peyronel is a pathogenic fungus commonly found on *V. myrtillus*. The fungus overwinters as sclerotia in the veins of *V. myrtillus* leaves that are infected and shed during the summer (Norvell & Redhead 1994). The sclerotia produces ascospores in the following early summer, which then infects new *V. myrtillus* leaves. Conidia are produced on leaves during the summer and are visible as a brown spot disease. If severe, it may result in premature leaf loss and visible defoliation patches in the *V. myrtillus* cover (Strengbom *et al.* 2002). *Valdensia heterodoxa* occurs naturally in the boreal forest, and has been observed to increase in abundance after N additions and as a response to atmospheric N deposition (> 6 kg N ha^{-1} yr^{-1}) (Nordin *et al.* 1998, Strengbom *et al.* 2002, 2003).

3.3 Monitoring of species abundances

Abundances of the different plant species (except of *P. glauca*) was scored in July each year (except for 2001) with the point intercept method (see Strengbom *et al.* 2002 for details). Nine randomly placed subplots (except for the first year of the experiment when there were only five) in each N treatment plot were scored with a frame sized 0.20 x 0.60 m with 30 random points. Data from the nine subplots were summarized within each N treatment plot before further analysis. *Operophtera* spp. larval density was scored the third week of June each year. This was done within five permanently marked circular 0.1 m^2 subplots in each of the 1000 m^2 N treatment plots. *Valdensia heterodoxa* disease incidence was scored on *V. myrtillus* leaves collected in late August each year. From each N treatment plot 500 *V. myrtillus* leaves were randomly collected, brought to the laboratory, dried and checked for disease symptoms.

3.4 Monitoring of N in throughfall precipitation

Throughfall precipitation in the form of rain was collected from each N treatment plot using two LDPE 1000-ml bottles, that were placed centrally on the plot c. 1 m above the ground. In order to minimize the risk of transformation of N forms in the samples by microbial activity an acid holding solution of HCl (17 mM) was used. The precipitation collectors were emptied after each major rain event. The pH of the solution increased as it was diluted with rainwater, but samples were always collected to the laboratory before the pH exceeded 3.0.

A test was performed to examine the effects of the acid holding solution on precipitation N forms. Separate solutions were used to test the preservative effect of acid pH (<3) on inorganic (NH_4^+ and NO_3^-), and organic N (a 20 µM N mix of six amino acids). The solutions were stored at room temperature for 14 days. The results showed no significant changes in NH_4^+ concentrations after the storage (Table 3). In contrast, the concentration of NO_3^- decreased slightly, with 84% of the initial concentration remaining in the solution after 14 days (Table 3). Analyses of the amino acid solution revealed significant changes only in the concentration of glutamine, and 35% of the initial concentration remained after storage (Table 3).

Table 1. Percentages of N compounds (NO_3^-, NH_4^+, glutamine, asparagine, glutamic acid, arginine, glycine and ornithine) remaining after 14 days in an acid holding solution (pH < 3) stored at room temperature.

	% Remaining
NO_3^-	84
NH_4^+	100
Glutamine	35
Asparagine	100
Glutamic acid	100
Arginine	100
Glycine	100
Ornithine	100

Troughfall precipitation in the form of snowmelt was collected from three acid washed lysimeters sized 1.44 m^2 in the experimental forest adjacent to the N addition experiment. The lysimeters were installed on the ground prior to the winter and placed c. 10 m apart from each other (for details see Laudon *et al.* 2004). Snowmelt from the lysimeters was collected in plastic bags connected to them with plastic pipes.

3.5 Measuring soil N

Water extraction of soil samples were performed to determine soil concentrations of soluble forms of plant available N. Soil samples from the experimental site were collected at three different occasions. In the summer of 2004, prior to the ^{15}N-labeling experiment described in Paper III, soil samples were collected from control plots and from 12.5 kg N ha^{-1} yr^{-1} plots (data presented in Paper III). In the summer of 2005, soil samples were collected from all the N treatment plots (data presented in this thesis).

At all sampling occasions one soil sample was collected from each N treatment plot. For this a 0.14 m diameter corer was used, and samples were taken from the mor layer. Samples were put in plastic bags and kept on ice while transported to the laboratory. At the laboratory, the coarse roots were carefully removed as well as the most recent litter, and the remaining sample was homogenized. Each homogenized sample was divided into two subsamples of *c*. 12 g fresh mass (FM). One subsample was left for dry mass (DM) ratio determination of the soil, and the other one was mixed with 60 mL of ultrapure water for one hour. The slurries were then immediately filtered through GF 30 glass fibre filters using a vacuum pump. The resulting extracts were frozen (-20 C°) until analysed.

3.6 Measuring plant N uptake

The use of ^{15}N-labeled N compounds makes it possible to study plant uptake of the compounds. Under most natural conditions a mixture of different N compounds are available to plants and therefore the N forms used in all experiments described in this thesis were combined in solutions so that each N form made up an equal proportion of the total amount of N in the solution. In each mixture only one of the N forms was labeled with ^{15}N.

In total three ^{15}N uptake experiments are presented in this thesis. In the first experiment natural rain events were simulated by using a mixture of three N forms (NH_4^+, NO_3^- and glycine) in concentrations similar to those found in rainwater. In July 2003, we applied the mixture during twenty wetting events (by spraying) to *H. splendens* and to *P. glauca* on control plots, 50 kg N ha^{-1} yr^{-1} plots and recovery plots. The second experiment was performed during spring 2004 to study *H. splendens* N uptake during snowmelt. The same N mixture was added to the snowpack on the different N treatment plots one month prior to the initiation of snowmelt. The third experiment was performed in the summer of 2004 on control and 12.5 kg N ha^{-1} yr^{-1} plots. The targeted species were *V. myrtillus* and *D. flexuosa*. In this experiment a fourth N form, peptides, was added to the N mixture, and the mixture was injected into the soil. In all three experiments the plant material was washed in 0.5 mM $CaCl_2$ after harvest, to remove ^{15}N-labeled solutes not taken up by the plants but adhering to plant tissue surfaces.

3.7 Chemical analyses

When studying how plants respond to increased N supply it can be useful to study biochemical markers indicating major metabolic changes. Since the C and N metabolism in plants are closely intertwined I have focused on major compounds containing these two elements:

- *Free amino acids* are well known to be used by plants for both seasonal N storage and storage of N taken up in excess of the demands for growth (see for example Chapin *et al.* 1986, Lähdesmäki *et al.* 1990, Näsholm & Ericsson 1990, Lipson *et al.* 1996, Nordin & Näsholm 1997).

- *Chlorophylls* are the pigments used in photosynthesis, and are primarily responsible for harvesting the light energy that is used in carbon assimilation (Taiz & Zeigert 1998). Chlorophyll concentrations often reflect a plant’s light and N availability, and also indicate its potential carbohydrate production capacity, which in turn provides energy for processes like growth and N uptake.

- S*oluble carbohydrates.* Besides energy, carbohydrates provides C skeletons for amino acid synthesis in the plant. In theory, excessive amino acid synthesis may therefore compete with growth supporting processes (Baxter *et al.* 1992, Nordin & Gunnarsson 2000, Paulissen *et al.* 2005). Although the protocol used for analyzing soluble carbohydrates detected sugars, sugar alcohols, and starch, the plant analyzed in this thesis, *H. splendens*, contained only detectable concentrations of sugars.

All extractions and analyses of amino acids from plant material were performed by RP-HPLC using gradient elution according to Nordin and Gunnarsson (2000). Amino acids in precipitation and soil extracts were also analyzed with RP-HPLC (Nordin *et al.* 2001). Nitrate in rain water was analyzed using the NO_3^-/NO_2^- colorometric assay kit no:780001 from Cayman chemicals, while NO_3^- in soil extracts was analyzed by ion chromatography. NH_4^+ from plant material, in rainwater and in soil extracts were analyzed by RP-HPLC. NH_4^+ in snowmelt was analyzed by the phenol-hypochlorite method. NO_3^- in snowmelt was analyzed by an auto-analyzer with cadmium reduction. Chlorophyll from plant material was determined with a spectrophotometer according to Palmqvist and Sundberg (2001). Soluble carbohydrates were analyzed by ion chromatography. ^{15}N

abundance of ^{15}N labeled plant material (as well as N % and C % of this material) was analyzed using Continuous Flow Isotope Mass Spectrometry (CF-IRMS).

4 Results and Discussion

The long-term N addition experiment within the Svartberget experimental forest used for the studies included in this thesis is located in a forest type common in north and central Scandinavia (spruce-dominated overstory and *V. myrtillus* dominated understory). The back-ground deposition of atmospherically transported N is relatively low (c. 3 kg N ha^{-1} yr^{-1}). Thus, the experiment is suitable for studying initial as well as long-term effects of N enrichment on ecosystem processes. The N doses applied (12.5 and 50 kg N ha^{-1} yr^{-1} as NH_4NO_3) were chosen so the lower dose is comparable to the levels of atmospheric N deposition over parts of south-Scandinavia, while the higher N dose simulates a more extreme rate of N enrichment than currently experienced in Scandinavia. In addition, the experiment includes plots where N additions have been terminated, which provides an opportunity to investigate ecosystem recovery from high N input.

4.1 Plant-available N forms in an N enriched boreal ecosystem

4.1.1 In rain

Precipitation that reaches the forest floor underneath a forest canopy is termed throughfall precipitation (Parker 1983), which provides a pathway for nutrient input and transfer in forest ecosystems. Solutes in throughfall precipitation originate from tree leachates and deposition of atmospherically transported compounds. In Europe, measurements of throughfall N have been taken within the frameworks of various environmental monitoring programs, and have mainly focused on inorganic N forms. In Sweden, throughfall concentrations of NH_4^+ and NO_3^- have been extensively monitored for decades (see http://www.ivl.se/miljo/projekt/kron/). Few studies have, however, been made to explore whether N enrichment of an ecosystem influences the quantity and quality of throughfall N or whether plant available organic N forms, such as amino acids, make a substantial contribution to precipitation N. In this thesis it was demonstrated that in the Svartberget experimental forest the long-term N addition treatments had no significant influence on throughfall precipitation quantity or quality of N during the vegetation period (I). This indicates that N additions were not large enough to cause increased N leaching from the tree canopy. In addition, the results from Paper I show that of the N in throughfall precipitation (c. 2 kg N ha^{-1}), collected as rain over a single vegetative season (late May to early October), amino acids, NH_4^+ and NO_3^- accounted for 17 %, 78 % and 5 %, respectively (I, Table 2).

Table 2. Concentrations (µg N dm^{-3}) of plant-available N forms in throughfall precipitation as rain (25/5 - 3/10 2003) and snowmelt (10/4 - 29/4 2004). Values are means (n = 3-6) ± 1 S.E. (The table is a combination of rain data presented in Paper I and snowmelt data presented in Paper II).

Rain	NH_4^+	NO_3^-	*Amino acids*
25/5-26/6 2003	*8.5±0.5*	*4.6±0.3*	*2.6±0.4*
27/6-10/7 2003	*33.4±2.7*	*1.8±0.1*	*6.8±1.0*
11/7-31/7 2003	*43.2±4.5*	*1.7±0.1*	*12.7±2.1*
1/8-20/8 2003	*31.3±3.7*	*1.3±0.1*	*8.3±1.7*
21/8-3/10 2003	*32.4±2.5*	*2.1±0.3*	*3.0±0.3*
Snowmelt			
10/4-14/4 2004	*1.9±0.1*	*28.0±4.2*	*0.6±0.1*
15/4-17/4 2004	*1.5±0.1*	*12.3±2.3*	*0.6±0.1*
18/4-19/4 2004	*1.0±0.5*	*7.7±1.0*	*0.3±0.0*
20/4-24/4 2004	*1.4±0.5*	*9.4±1.2*	*0.4±0.2*
25/4-28/4 2004	*1.7±0.1*	*12.6±0.7*	*0.3±0.2*
29/4- 2004	*1.7±0.2*	*9.4±1.5*	*0.4±0.1*

Possible amino acid sources in precipitation include agricultural inputs, oceanic injections of aerosols, and leakage from surrounding biota (Neff *et al.* 2002, Milne & Zika 1993). Since micro-organisms in the atmosphere can easily digest amino acids they are likely to be locally emitted and deposited (Neff *et al.* 2002). For example, amino acid concentrations in precipitation and aerosols over the ocean outside Florida have been observed to reach values of 13-15 µM, compared to 0.3-0.5 µM over the large city Miami, which is further away from the presumed oceanic source (Mopper & Zika 1987). A major source of amino acids in forest throughfall precipitation could be the surrounding vegetation, since organic N compounds may leach from leaves or needles (Carlise *et al.* 1966, Parker 1983). Supporting this idea, higher concentrations of arginine and glutamine (the major amino acids in spruce needles during budburst) were found in throughfall precipitation at the Svartberget experimental forest during the early summer tree budburst and shoot elongation period (I).

4.1.2 In snowmelt

A large part (c. one third) of the annual precipitation in boreal regions is normally added and accumulated as snow (Löfvenius *et al.* 2003). The potential plant-available N accumulated in the snowpack is released over a relatively rapid period during spring snowmelt. Several studies have reported considerable concentrations of NH_4^+ and NO_3^- (Reynolds 1983, Hiltbrunner *et al.* 2005), along with dissolved organic N (DON) (Petrone *et al.* 2007), in snow. The proportion of DON represented by plant-available organic N (i.e. amino acids) has not been well studied. Paper II presents results from a study in which the release of N forms directly available to plants (NH_4^+, NO_3^- and amino acids) was monitored during snowmelt in the Svartberget experimental forest. The results demonstrated that the snowpack contained about 0.3 kg N ha^{-1} as NH_4^+, NO_3^-, and amino acids, and that the contribution of amino acid N was minor (c. 3%). Instead, NO_3^- dominated the snowmelt N pool, amounting to 83% of the plant-available N, in contrast to the throughfall precipitation results from the vegetation growth period, in which NH_4^+ was the dominant N form (I, Table 2). The high concentration of NO_3^- in snowmelt may result from airborne NO_3^- deposition or microbial activities in the snow during snowmelt. Snow can host various organisms, such as snow algae, snow fungi, and eubacteria (Jones 1999a). Moreover, micro-organisms that utilize snow as a growth medium have been found, in some studies, to prefer NH_4^+ as an N source (Jones & DeBlois 1987, Delmas *et al.* 1996), leaving NO_3^- to be released with snowmelt.

4.1.3 In soil

In N limited ecosystems, in arctic and boreal regions, degradation of organic N is generally a slow process. This result in a large proportion of organic N in the soil compared to NH_4^+ and NO_3^- (Kielland 1995, Näsholm *et al.* 1998, Nordin *et al.* 2001). The majority of organic compounds found in boreal soils consist of various complex polymers (proteins, peptides, DNA, RNA, chitin and lignin) and monomers (nucleic acids, amino sugars and amino acids) (Lipson & Näsholm 2001). Although the capacity of plants to take up organic N in the form of amino acids has been known for several decades (Virtanen & Linkola 1946, Melin & Nilsson 1953), the ecological significance of this capacity has previously been considered to be minor (Taiz & Ziegert 1998). Instead, the prevailing assumption has been that plants have to rely on mineralization of organic compounds into inorganic N forms before N can be assimilated (Tamm 1991). However, in the last decade it has become apparent that amino acids may provide a directly accessible N source for a variety of plants in boreal (Näsholm *et al.* 1998, Nordin *et al.* 2001), arctic (Kielland 1994, Kielland 1997, Nordin *et al.* 2004, Kielland *et al.* 2007), and alpine ecosystems (Raab *et al.* 1999, Lipson *et al.* 1999). Amino acid concentrations have been found to be substantial, or even higher, than mineral N concentrations in arctic and boreal soils (Näsholm *et al.* 1998, Nordin *et al.* 2001, Kielland *et al.* 2007). In addition, the amino acid pool is a highly dynamic soil N pool that turns over several times per day (Kielland 1995, Jones 1999b, Jones & Kielland 2002, Kielland *et al.* 2007).

Since the plant-accessible N pool in boreal forest soils generally is low and dominated by amino acids, addition of inorganic N as NH_4^+ and NO_3^- may have direct as well as indirect effects on soil N form availability. A direct effect is increased availability of the added N forms, i.e. NH_4^+ and NO_3^-. Indirect effects include changes in soil N turnover due to the higher N availability. For example, N addition decreases the soil C:N ratio, increases the bacteria:fungi ratio, and increases soil mineralization and nitrification rates (Frey *et al.* 2004, Booth *et al.* 2005, Högberg *et al.* 2007a, 2007b).

In the N addition experiment at Svartberget I examined water extractable amino acids, NO_3^- and NH_4^+ in soils on the N treatment plots on two different occasions in 2005, one in early July and one in mid-September (Fig. 3). The number of sampling points was one sample per 1000 m^2 plot, which was probably too little to sufficiently account for the within plot heterogeneity, and consequently replicate samples includes a large portion of variance that cannot be explained by the N treatments. One-way ANOVAs on log-transformed values revealed significant effects of N treatment on NO_3^- and NH_4^+ concentrations in July (Fig. 3). This higher availability of inorganic N may have resulted from the fertilization event in late May, with soluble N remaining in the soil until the time of sampling in July. However, the fertilization effect diminished over the season, and soil

N concentrations in the N addition plots in September were similar to those in control plots (Fig. 3), and those found in studies of other (unfertilized) boreal soils (Näsholm *et al.* 1998, Nordin *et al.* 2001). However, measurements of pool sizes do not capture differences in flow-rates, and the rates of soil N mineralization can still be different between the N treatment plots (i.e. Chen & Högberg 2006). Unfortunately, studies of N mineralization rates have not yet been performed within the Svartberget N experiment.

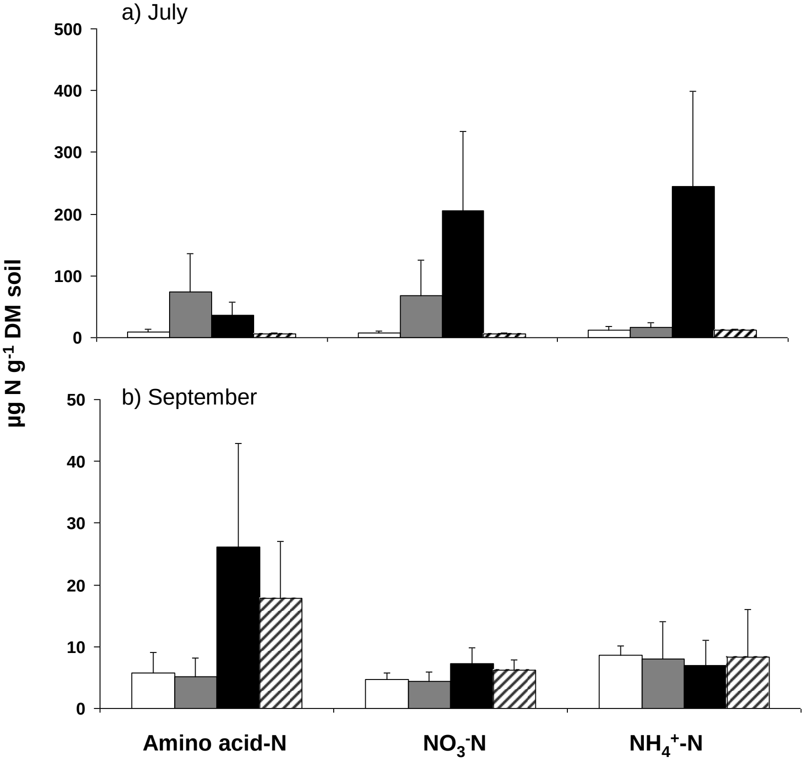

Figure 3. Concentrations of water-extractable amino acid N, NO_3^- and NH_4^+ (µg N g^{-1} DM soil) in soils from plots subjected to three different N treatments: control plots (white bars, 0 kg N ha^{-1} yr^{-1}), low N addition plots (grey bars, 12.5 kg N ha^{-1} yr^{-1}), high N addition plots (black bars, 50 kg N ha^{-1} yr^{-1}) and recovery plots (striped bars, 50 kg N ha^{-1} yr^{-1} for five years and then no N addition for four years). Soils were sampled during 2005 on July 7 and September 19. One-way ANOVAs revealed significant treatment effects for NO_3^- (p=0.048, MS=1.588, F=3.172, df=3) and NH_4^+ (p=0.035, MS=1.508, F=3.517, df=3) in July. Means (n = 6) ± 1 S.E. Note the different scales on the Y- axes.

4.2 Plant responses to N enrichment

4.2.1 Cryptogam uptake of troughfall precipitation N

Ground-living bryophytes and/or lichens often form continuous mats covering the ground in boreal forests, and epiphytic lichens can form substantial biomass within the tree canopy. Hence, in boreal forests cryptogams are the first recipients of throughfall rain precipitation and the nutrient pulse released during snowmelt. Two ^{15}N uptake experiments were performed to explore cryptogam uptake of different organic and inorganic N forms in throughfall precipitation and to investigate whether N enrichment influenced this uptake.

Inorganic N uptake by bryophytes and lichens is relatively well studied (for examples, see Crittenden 1996, 1998, Kielland 1997, Jauhiainen *et al.* 1998, Eckstein & Karlsson 1999). However, fewer studies have explored whether amino acids may contribute to the N supply of cryptogams (but see Simola 1975, Kielland 1997, Dahlman *et al.* 2004, Palmqvist & Dahlman 2006). The results presented in Paper I and in this thesis clearly demonstrated that *H. splendens* and *P. glauca* have the capacity to acquire the intact form of the amino acid glycine during the vegetation growth period (Fig. 4). In Paper II it was demonstrated that *H. splendens* was able to acquire intact glycine also from snowmelt in early spring (Fig. 4). Glycine uptake was quite substantial in relation to inorganic N uptake. For *H. splendens* glycine uptake corresponded to 67 % of the uptake of the most acquired N form from rain (NH_4^+). In snowmelt, when accounted for dilution by the N present in the snowpack, glycine uptake on control plots corresponded to 27% of the most acquired N form from snowmelt (NO_3^-) (II). For *P. glauca* glycine uptake corresponded to 80% of the most acquired N form from rain (NH_4^+) (Fig. 4).

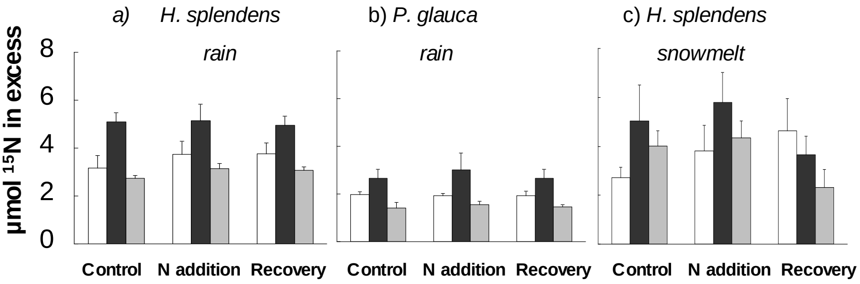

Figure 4. Excess ^{15}N in *Hylocomium splendens* (a) and *Platismatia glauca* (b) tissues after ^{15}N addition as simulated rain events and in *Hylocomium splendens* (c) after ^{15}N addition to the snowpack prior to snowmelt. The bars represent uptake from ^{15}N-labeled glycine (white bars), NH_4^+ (black bars), and NO_3^- (grey bars). The three N forms were combined in mixtures in which one N form was labeled at a time. The cryptogams were growing in three different N treatment plots; control (0 kg N ha^{-1} yr^{-1}), N addition (50 kg N ha^{-1} yr^{-1} for eight years), and recovery (50 kg N ha^{-1} yr^{-1} for five years and then no N addition for three years). Means (n = 5) ± 1 S.E. Note that data on *H. splendens* N uptake from rain was reported in Paper I and data on *H. splendens* N uptake from snowmelt on control plots was reported in Paper II. Remaining data is only reported in this thesis.

Interestingly, N uptake was not affected by long-term N additions in either *H. splendens* or *P. glauca*. Furthermore, internal N concentrations in *P. glauca* were similar irrespective of the N addition treatment (data not shown). These findings indicate that the annual application of fertilizer N to the ground (for a description of the experiment see p. 19 in *Material and Methods*) had no effect on the tree-living lichen. In contrast, ground-living *H. splendens* had significantly higher N concentrations after N addition (control, 8.4 mg N g^{-1} DM; N addition, 18.7 mg N g^{-1} DM; recovery, 11.1 mg N g^{-1} DM) (I). However, despite higher internal N concentrations, *H. splendens* N uptake did not differ between N treatments (I, Fig. 4). This is in contrast to higher plants, where N uptake is regulated according to the whole plant N demand and hence down-regulated as internal N concentrations increase (Vidmar *et al.* 2000).

4.2.2 Ecophysiology of H. splendens

In Paper I it is demonstrated that N addition decreases *H. splendens* abundance and density. Several different physiological mechanisms have been suggested as possible contributors to such N-induced bryophyte decline. The results presented in this thesis indeed support the suggestion by Jauhiainen *et al.* (1998) that bryophyte N uptake is less well regulated than N uptake in higher plants. Unregulated N uptake may result in accumulation of toxic NH_4^+ in moss tissues subjected to high N conditions (Bates 1992). It has been suggested that NH_4^+ toxicity is related to that high internal NH_4^+ concentrations may cause plant cell membranes to dysfunction (Limpens & Berendse 2003, Paulissen *et al.* 2005). Potassium leakage from tissues has been used as a indicator of such membrane dysfunction (Pearce *et al.* 2003, Paulissen *et al.* 2005). NH_4^+ needs to be incorporated into amino acids to avoid toxic accumulation, but this process demands energy and a sufficient availability of soluble C. Therefore, in theory, excessive amino acid synthesis may compete with growth-supporting processes (Baxter *et al.* 1992, Nordin & Gunnarsson 2000, Paulissen *et al.* 2005).

In Paper I, it was demonstrated that arginine dominated the amino acid pool in *H. splendens* from N addition plots, since arginine concentrations were more than 10 times as high as in moss from control plots. This accumulation did, however, have no influence on moss tissue concentrations of sugars as no significant difference was observed between mosses from the two N treatments. Hence, the data suggests that the C supplied through photosynthesis was enough to sustain the pool of sugars as well as the elevated arginine synthesis in *H. splendens* at the N addition plots.

In conjunction to the N uptake study in Paper II, we performed a study of how increased N supply may interfere with the physiological responses of *H. splendens* to snowmelt. Whether high N supply may interfere with moss metabolic responses to the sudden transition from being under the snow to being in bright light are not well studied. Woolgrove & Woodin (1996) showed that the bryophyte *Kiaria starkei* (a typical snow-bed bryophyte) was capable of photosynthetic activity immediately after snow removal and that tissue concentration of carbohydrates more than doubled during the transition from being under the snow to being in full light. To reveal whether the N status of *H. splendens* had any effects on the metabolic events following snowmelt, we studied selected N and C compounds in the moss during a time sequence following snowmelt (Fig. 5).

Interestingly, at the time of snowmelt (day 0) the amino acid concentrations of *H. splendens* was not significantly different between the N treatment plots, while at the end of the observation period (day 22) amino acid concentrations were significantly higher in moss on N addition plots than in moss on control or recovery plots (Fig. 5). This increase of the amino acid pool over time following snow melt was mainly due to increasing arginine concentrations (Fig. 5). Because no N was applied to the mosses during this time period, the increase in arginine concentrations must have occurred via re-allocation of N from senescing to viable parts of the moss. Alternatively, degradation of N compounds (proteins) followed by arginine synthesis may have occurred within the green segments. In support for the former alternative, substantial translocation of N from decaying parts to top segments has previously been reported for *H. splendens* (Eckstein 2000).

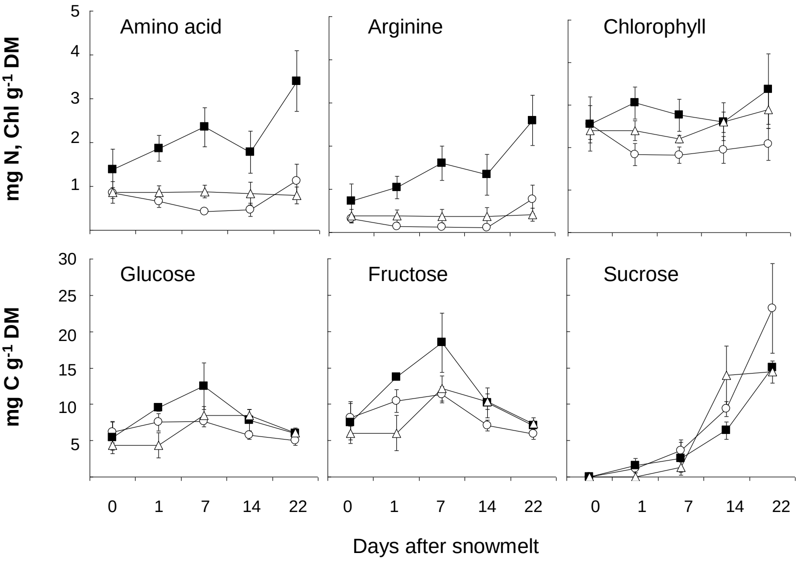

Figure 5. Concentrations (mg N g^{-1} DM, mg Chl g^{-1} DM and mg C g^{-1} DM) of N and C compounds in *H. splendens* from three different N treatments: Control o (0 kg N ha^{-1} yr^{-1}), N addition ■ (50 kg N ha^{-1} yr^{-1}) and Recovery Δ (50 kg N ha^{-1} yr^{-1} for five years and then no N addition for three years) 0, 1, 7, 14, and 22 days after snowmelt. Means (n = 1-5) ± 1 S.E. Note the different scales on the Y-axes.

H. splendens chlorophyll concentrations were similar, regardless of N treatment on day 0 (snow covered) (Fig. 5). Furthermore, the transition into light did not cause any significant changes in *H. splendens* chlorophyll concentrations during the three-week study period. This is in contrast to findings reported by Woolgrove and Woodin (1996) of a ten-fold increase in *K. starkei* chlorophyll concentrations two weeks after snow removal. N addition treatments had no significant effects on moss tissue sugar concentrations following snowmelt (Fig. 5). However, sugar pool composition in the moss changed during the observation period (Fig. 6). At snowmelt (day 0), *H. splendens* contained the simple sugars glucose and fructose, but no sucrose (Fig. 5). Following snowmelt sucrose concentrations rapidly increased, and at the end of the observation period (day 22) sucrose dominated the sugar pool. These findings suggest that increased light following snowmelt resulted in accumulation of soluble C (which was not directly needed in the moss metabolism) in the form of sucrose.

4.2.3 Nitrogen competition between V. myrtillus and D. flexuosa

It has been suggested that arctic and boreal plant species within the same plant community may partition the N resources between them, thereby reducing inter-specific competition for N and facilitate species co-existence (e.g. Kielland 1994, McKane *et al.* 2002, Miller & Bowman 2003, Reynolds *et al.* 2003). Such partitioning may be based on differences in N form preferences between co-existing plant species, and support for this hypothesis includes between-species differences in the relative uptake of different types of N (e.g. Näsholm *et al.* 1998, McKane *et al.* 2002, Kahmen *et al.* 2006). Moreover, the relative abundance of inorganic N forms (NH_4^+ and NO_3^-) in soils has been proposed to be an important determinant for plant species distribution (Diekmann & Falkengren-Grerup 1998). Thus, theoretically, N enrichment of forest soils has the potential to alter the prerequisites for co-existence and competition between species by altering the relative abundance of different N forms.

It is well known that N addition induces vegetation changes in boreal ecosystem (Strengbom *et al.* 2001, Nordin *et al.* 2005). In Paper III we aimed to elucidate if species specific preferences for different N forms could be an important driver behind the observed vegetation change within the Svartberget N addition experiment where the grass *D. flexuosa* has increased in abundance (Nordin *et al.* 2005). We hypothesized that on control plots a high capacity of *V. myrtillus* to use organic N forms would ensure its dominance over *D. flexuosa*, while at N addition plots a high capacity of *D. flexuosa* to use inorganic N (in particular NO_3^-) would support grass proliferation. However, the result from the study presented in Paper III demonstrated that *D. flexuosa* acquired more labeled N than *V. myrtillus* regardless of N form, although the difference in acquisition was largest for NO_3^-. Several studies have pointed out a high uptake capacity for NO_3^- of graminoids (McKane *et al.* 2002, Persson *et al.* 2003, Nordin *et* *al.* 2006). Furthermore, this has been interpreted as a key component in the process whereby grasses out-compete other plants, e.g. after forest clear cutting (Kronzücker *et al.* 1997).

4.2.4 Interactions between plants and their natural enemies

Besides N uptake processes related to interspecific competition for N, plant community structure is also influenced by interactions between plants and their natural enemies. N addition causes increased plant tissue N concentrations, increasing the nutritional value of the plants, which may result in greater damage to them by their natural enemies. In the Svartberget study system all frequent natural enemies were related to the dominant understory species, *V. myrtillus*. The most abundant organisms were the leaf pathogen *Valdensia heterodoxa* causing a brown spot disease on *V. myrtillus* leaves and larvae of *Operophtera brumata and O. fagata* consuming leaves as well as annual shoots of *V. myrtillus*.

In Paper IV a positive effect of N addition both on *Valdensia heterodoxa* and on *Operophtera spp*. larvae was demonstrated. For both the fungus and the larvae this has been shown also in previous studies (Nordin *et al.* 1998, Strengbom *et al.* 2002, 2005, Nordin *et al.* 2006, Strengbom *et al.* 2006). What Paper IV demonstrates in addition to these previous studies, is (1) an interaction between *V. heterodoxa* and *Operophtera* spp. larvae, and (2) the long-term dynamics of the system. Hence, during the study period an outbreak of *Operophtera* spp. larvae lasting for several consecutive years caused severe *V. myrtillus* decline on all N treatment plots and diminished disease incidence of *V. heterodoxa* due to that the fungal substrate (*V. myrtillus* leaves) was consumed by the larvae before they could be infected by the fungus. After the outbreak of larvae was over, it took a year before *V. heterodoxa* disease incidence was back on the same levels as before the outbreak. Paper IV concludes that the part of the effect of N addition on the competitive interaction between *V. myrtillus* and *D. flexuosa* is indirect and mediated by these natural enemies.

4.2.5 The understory species composition after 12 years of N addition

This thesis demonstrates that plant N uptake and plant interactions with their natural enemies are processes that contribute to N induced vegetation change. Twelve years of yearly N additions to the understory vegetation in the Svartberget experimental forest resulted in a strong decline of the previously dominant bryophyte *H. splendens*, while the second most abundant moss, *Pleurozium shcreberi*, was not significantly affected (Fig. 6). For vascular species the graminoid *D. flexuosa* increased from the N additions, while *V. vitis-idaea* decreased (Fig. 6). For the dominant vascular plant, *V. myrtillus*, the abundance in 2007 did not vary significantly due to the N additions (Fig. 6). However, abundance of *V. myrtillus* over the 12-year period was influenced also by biotic interactions (IV). Paper IV as well as other studies performed in the same study system has displayed a negative impact of N addition on *V. myrtillus* abundance (Strengbom *et al.* 2002, Nordin *et al.* 2005). In general for all species impacted by the N additions, the abundance responses to the N treatments were not proportional to the N doses, i.e. the response to the 50 kg N ha^{-1} yr^{-1} treatment was not four times as strong as the response to the 12.5 kg N ha^{-1} yr^{-1} treatment (Fig. 6).

Many of N induced vegetation changes recorded in this long-term N addition experiment have also been observed by other investigators in similar study systems (see for example Hallingbäck 1992, Nilsson *et al.* 2002, Skrindo & Økland 2002). Nilsson *et al.* (2002) reported increased abundance of *D. flexuosa* after N addition as well as after removal of the dominant ericaceous shrub *Empetrum hermaphroditum* in an alpine tundra community. Skrindo and Økland (2002) found decreased abundance of the bryophytes *Dicranum polysetum* and *D. fuscescens* after 6 years of N addition, while the abundance of *P. schreberi* was unchanged. Laboratory studies have confirmed that different bryophyte species may tolerate different levels of N. Salemaa *et al.* (2008) showed that of three forest mosses (*H. splendens*, *D. polysetum* and *P. schreberi*) *H. splendens* had the lowest tolerance for N before growth inhibition occurred, while *P. schreberi* had the highest.

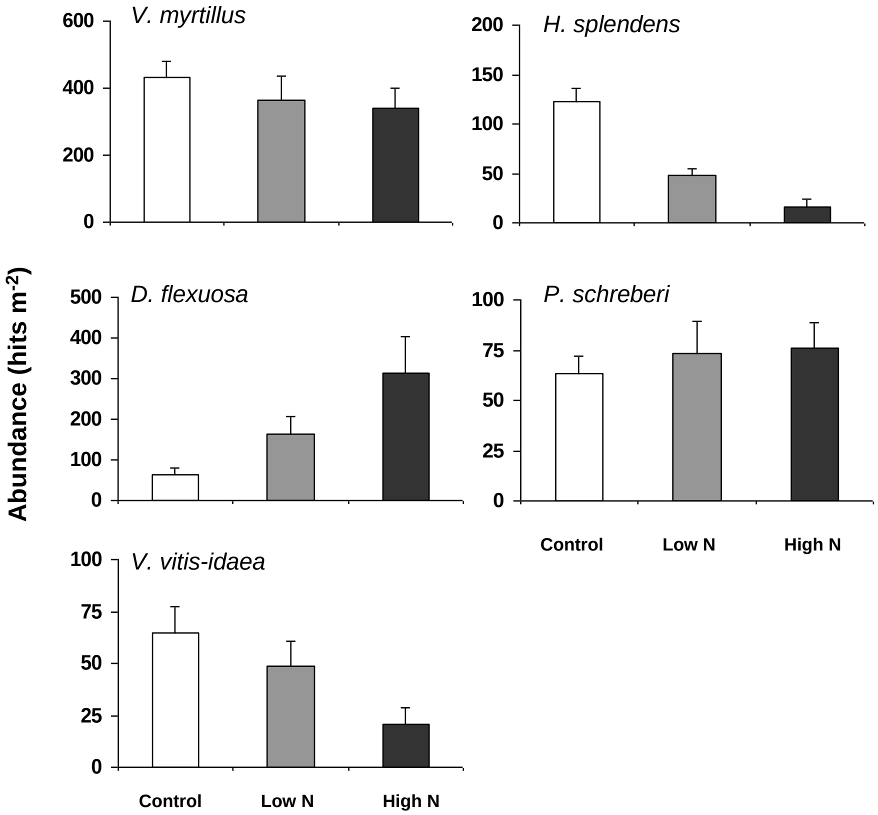

Figure 6. Abundance (hits m^{-2}) of *V. myrtillus, D. flexuosa*, *V. vitis- idaea*, *H. splendens* and *P. schreberi* in three different N treatment plots: control (0 kg N ha^{-1} yr^{-1}), low N addition (12.5 kg N ha^{-1} yr^{-1}) and high N addition (50 kg N ha^{-1} yr^{-1}) in the year 2007 (following 12 years of N addition). Means (n = 6) ±1 S.E. One-way ANOVAs showed a significant effect of N treatment for *D. flexuosa* (p= 0.028), *V. vitis-idaea* (p=0.044) and *H. splendens* (p=0.000). Note the different scales on the Y-axes.

5 Summary of major findings

Monitoring of throughfall precipitation N in a full-grown spruce-dominated forest in north-Sweden showed that rainwater during the vegetation period contributed with c. 2 kg N ha^{-1} yr^{-1} and snowmelt with c. 0.3 kg N ha^{-1} yr^{-1} to the forest vegetation. During the vegetation period the majority of this N was in the form of NH_4^+ while NO_3^- dominated the plant available N pool during snowmelt. Organic N in the form of amino acids made a substantial contribution to plant available throughfall precipitation N (17% of the total N pool in summer and 3 % during snowmelt). Experimental N enrichment of the ecosystem did not alter throughfall precipitation N quantity or quality. This indicates that the magnitude of experimental N enrichment was not enough to cause increased N leaching to intercepted precipitation from the tree canopy. Soil concentrations of inorganic N were, however, elevated during the vegetation period on plots subjected to experimental N enrichment.

^{15}N uptake experiments targeting the ground-living bryophyte *Hylocomium splendens* and the tree-living lichen *Platismatia glauca* on plots treated with 0 kg N ha^{-1} yr^{-1} (Control), 50 kg N ha^{-1} yr^{-1} for eight years (N addition) and 50 kg N ha^{-1} yr^{-1} for five years and no N addition for three years (Recovery) showed that the long-term N enrichment had no significant effect on cryptogam N uptake. On all plots both species were capable of taking up both inorganic (NH_4^+, NO_3^-) and organic (glycine) N to the same extent.

Vegetation analysis revealed that addition of 50 kg N ha^{-1} yr^{-1} for eight years had decreased *H. splendens* abundance with 81%. Biochemical analysis of *H. splendens* collected during the vegetation period from Control, N addition and Recovery plots revealed higher concentrations of amino acids (especially of arginine) in *H. splendens* from N additions plots. Concentrations of soluble carbohydrates and chlorophyll remained unchanged from the N treatments. Interestingly, at the time of snowmelt arginine concentrations were similar in moss from all N treatment plots. Following snowmelt the arginine concentrations in moss from the N addition plots increased rapidly during a three week period suggesting internal reallocation of N during this time of year. During the same time period also sucrose concentrations increased in moss from all N treatment plots.

A ^{15}N experiment targeting N competition between *Vaccinium myrtillus* and *Deschampsia flexuosa* on plots treated with 0 and 12.5 kg N ha^{-1} yr^{-1} for nine years showed no significant effect of the long-term N treatments on the plant uptake of NH_4^+, NO_3^-, glycine or peptides. The results revealed that *V. myrtillus* and *D. flexuosa* were both capable of acquiring N from NH_4^+, NO_3^- and glycine, but not substantially from peptides. When N uptake of the two species was related to the plant biomass, *D. flexuosa* was more efficient than *V. myrtillus* in acquiring all the N forms, although the difference between the species was greatest for NO_3^-.

The long-term (12 years) monitoring of the understory vegetation on plots treated with 0, 12.5 and 50.0 kg N ha^{-1} yr^{-1} demonstrated that two organisms functioning as natural enemies to the dominant plant, *V. myrtillus*, the fungal pathogen *Valdensia heterodoxa* and herbivorous *Operophtera* spp. larvae, exerted strong control over the vegetation dynamics.

6 Conclusion

The work described in this thesis demonstrates that N enrichment of a boreal forest ecosystem has profound implications for several ecophysiological processes that influence understory species composition. The studies focused particularly on plant N uptake in relation to qualitative and quantitative aspects of N supply. It was found that for the studied species (including both vascular plants and a common bryophyte in boreal forests) N enrichment did not influence either the quality or quantity of N taken up by the plants, i.e. neither their capacity to take up different forms of N, or the magnitude of this uptake. For the studied bryophyte its apparent inability to downregulate N uptake when high levels of N were available, resulted in N accumulation in the form of free amino acids (mainly arginine) in its tissues and a decline in its abundance.

The relative amounts of different N forms taken up by the studied vascular species (the ericaceous shrub *Vaccinium myrtillus* and the graminoid *Deschampsia flexuosa*) on control plots and N-addition plots appeared to be very similar, indicating that neither of these species has the capacity to change their N form preferences in response to N enrichment. However, when N uptake per unit biomass of these competing plant species was compared, *D. flexuosa* was found to acquire N, in all available forms (but especially NO_3), more efficiently than *V. myrtillus*. In addition, *D. flexuosa* efficiently allocated NO_3^- to aboveground growth. Thus, the well documented proliferation of *D. flexuosa* under conditions of N enrichment appears to be related to a relatively high capacity of this species to acquire N, especially as NO_3^-, and efficiently use it.

Further ecophysiological factors that influence competition between *V. myrtillus* and *D. flexuosa* under conditions of N enrichment are the effects of increases in N concentrations in their tissues, and consequent increases in the nutritional value of the plants, which in turn may result in greater damage to them by their natural enemies. The results from long-term (12 years) vegetation monitoring showed that the competition between *V. myrtillus* and *D. flexuosa* was influenced by two natural enemies, both targeting *V. myrtillus*. The pathogenic fungi *Valdensia heterodoxa* and herbivorous *Operophtera* spp. larvae both affected the abundance of *V. myrtillus*, but the relative strength of their effects alternated over time. N enrichment had a significant positive effect on the abundance of the organisms, resulting in greater damage to *V. myrtillus* on N-enriched plots.

In summary, the studies included in this thesis conceptualize the intricate interactions between biotic and abiotic factors that determine the plant community structure of boreal forests. The results have several implications for boreal forest management strategies, especially the use of N fertilizers in forestry to increase forest yields. The thesis displays that N supply is a forceful factor interfering with many central mechanisms directing plant community structure of boreal forests.

7 Future challenges

Bryophytes have a central role controlling nutrient fluxes in the boreal ecosystem. They are the first receiver of throughfall and are well capable of acquiring N from both precipitation and snowmelt (I, II). They also contribute to ecosystem N retention by being associated with N_2-fixing bacteria (DeLuca *et al.* 2002). Moreover, reductions in moss mat density have been shown to increase soil moisture (Gornall *et al.* 2007) and temperatures (Van der Wal & Brooker 2004, Gornall *et al.* 2007), which promote increased decomposition rates (Hobbie *et al.* 2000) and consequently, increased mineralization rates (Schmidt *et al.* 2002, Gornall *et al.* 2007). Bryophytes thus seem to have large impact on N cycling in ecosystems and hence the ecological consequences of reductions in the moss layer in areas of high N deposition merits further investigations.

A related specific question concerns the fate of N originating from snowmelt not retained by ground-living bryophytes. It remains to be examined if, and to what extent, boreal vascular plants are capable of taking up N during snowmelt and if the N is incorporated into the soil microbial biomass or ends up in streams and rivers. Another question related to throughfall precipitation is if there are differences in throughfall precipitation chemistry depending on surrounding vegetation and background N deposition.

It is clear that N enrichment have direct as well as indirect effects on plant specie composition of naturally N limited ecosystems The results from Paper III indicate that species differ in their uptake efficiency of different N forms. The question remains wether if the effects on understory vegetation after for example a forest fertilization event can be reduced by altering the composition of chemical N fertilizers.

I would also like to point out the need for more knowledge of how N is retained in the ecosystem after N input is stopped, for example as N deposition decreases, or after a commercial forest fertilization event. Specifically more data is needed on whether N induced vegetation changes are reversible or not. Although existing data indicates that the soil seems to approach similar values of N availability as before N addition within a reasonable time period (Quist *et al.* 1999, Högberg *et al.* 2007b) changes in the vascular vegetation persists for much longer. For example, Strengbom and Nordin (2008) found higher N concentrations in the vascular vegetation 20 years after commercial forest fertilization events. Increased infections by *Valdensia heterodoxa* on *Vaccinium myrtillus* up to 47 years after terminated N input has also been recorded (Strengbom *et al.* 2001). Thus addition of N affects an N limited ecosystem in complex longlasting manner that are not fully understood.

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