Long-Term Effects of Nitrogen (N) Additions on Boreal Forest

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Abstract
The studies presented in this thesis aim to increase our understanding of the long-term effects of anthropogenic nitrogen (N) input via fertilization or atmospheric N deposition on tree growth and forest understory in boreal forest.

Firstly, I studied the effects of low annual N addition simulating atmospheric N deposition on tree growth and variables linked to site fertility in two different forest ecosystems, *i.e.* on one Norway spruce (*Picea abies* [L.] H. Karst.) dominated site and on one Scots pine (*Pinus sylvestris* L.) dominated site (Paper I and II). On the *P. abies* dominated site tree growth and needle N concentration were higher on plots treated with 12.5 and 50 kg N ha$^{-1}$ year$^{-1}$ than on control plots, which only received background N deposition (1 to 2 kg N ha$^{-1}$ year$^{-1}$). *P. abies* growth increased linearly with 1.2% for every kg of N added, which corresponded to a net tree carbon (C) sequestration of approximately 19 kg C per kg N (Paper II). On the *P. sylvestris* dominated site tree growth increased linearly with 1.6% per kg N added.

Secondly, I studied carry-over effects of forest fertilization (150 kg N ha$^{-1}$) between tree generations. Growth of 10 year old trees on sites fertilized during the previous tree generation was measured (Paper III). On sites that were previously fertilized twice (25 and 33 years prior to my study), the young *P. abies* and *P. sylvestris* trees were on average 24% taller than trees on sites that had never been fertilized. Furthermore, the soil N mineralization rates were nearly four times higher and the amount of plant available soil-N two times higher on sites that were previously fertilized twice than on the other sites.

Thirdly, I studied the interaction effects between N form and N dose on tree growth, mycorrhizal fungi and on forest floor vegetation in a c. 50 year old pine forest (Paper IV). Five years after adding 50 and 150 kg N ha$^{-1}$ tree growth responded similarly on plots treated with the amino acid arginine (ARG) and on plots treated with ammonium-nitrate (AN). The basal area growth was c. 40% higher on the N addition plots than on control plots. The abundance of sporocarps (fruit bodies) of mycorrhizal fungi was significantly reduced in AN treated plots compared to ARG treated plots and the control. Both ARG and AN affected the forest floor vegetation, there was rapid increase of wavy hair-grass (*Avenella flexuosa* [L.] Parl.) following N addition, which remained throughout the study period. Also, at the end of the study period there was a decrease in lingonberry shrub (*Vaccinium vitis-idaea* L.) abundance on N treated plots.

In conclusion, I found linear relationships between N addition, up to 50 kg N ha$^{-1}$ year$^{-1}$, and *P. abies* and *P. sylvestris* growth. Also, I present data suggesting that the mechanisms affecting tree growth, on an ecosystem level, can be changed in the long-term by repeated N fertilization with 150 kg N ha$^{-1}$ occasion$^{-1}$. That is, N addition can promote a positive long-term effect on an ecosystem’s nutritional status. In addition, I show that inorganic and organic N additions to a forest ecosystem after five years have rather similar effects on tree growth and understory vegetation, whereas organic N addition has significantly less negative effects on sporocarp production of mycorrhizal fungi than inorganic N addition.

**Keywords:** annual nitrogen (N) addition, N deposition, arginine, carbon (C) sequestration, N fertilization, forest growth, N turnover.

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Dedication

Mätty, Igor, Kerstin, Gilbert, Sören.

"The wheel weaves as the wheel wills"
R.J.
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List of Publications

This thesis is based on the following papers, which will be referred to by Roman numerals in the text:


Paper I is reproduced with kind permission from Canadian Science Publishing and its licensors ©. Paper II is reproduced with kind permission of the publishers (John Wiley and Sons, License Number: 3967830926134 and 3971451504181). Paper III is open access.
The contribution of Fredrik From to the papers included in this thesis was as follows:

I Planned the field work together with the co-authors, performed the field work and analysed the data, and wrote the paper together with the co-authors.

II Planned and performed the field work together with the co-authors, analysed the data and participated in writing the paper together with the co-authors.

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

IV Performed parts of the field work, assisted in data analyses, and participated in writing the paper together with the co-authors.
### Abbreviations

**Table 1.** Abbreviations used in Paper I through IV. Abbreviations are also explained when they first appear in the text.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition or inferring to</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF</td>
<td>Confer or compare</td>
</tr>
<tr>
<td>C.</td>
<td>Circa or approximately</td>
</tr>
<tr>
<td>DBH</td>
<td>Diameter at breast height (i.e. 1.3 m above ground, VanSoest <em>et al.</em> (1959))</td>
</tr>
<tr>
<td>Dg</td>
<td>Basal-area weighted mean diameter. The diameter corresponding to the mean basal-area of the stand (VanSoest <em>et al.</em>, 1959)</td>
</tr>
<tr>
<td>DW</td>
<td>Dry weight</td>
</tr>
<tr>
<td>$H_{100}$</td>
<td>The average height (m) at the age of 100 years for the 100 widest (DBH) trees per hectare (ha) (Hägglund, 1981a; Hägglund, 1981b)</td>
</tr>
<tr>
<td>$N_r$</td>
<td>Reactive nitrogen</td>
</tr>
<tr>
<td>$NO_3$</td>
<td>Nitrate</td>
</tr>
<tr>
<td>$NH_4$</td>
<td>Ammonium</td>
</tr>
<tr>
<td>N1</td>
<td>Fertilization with 150 kg N ha$^{-1}$, in Paper III</td>
</tr>
<tr>
<td>N2</td>
<td>Fertilization with 150 kg N ha$^{-1}$ applied twice, eight years apart, in Paper III</td>
</tr>
<tr>
<td>3N</td>
<td>Addition of 3 kg N ha$^{-1}$ year$^{-1}$, low rate treatment in Paper I and II</td>
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<td>Addition of 6 kg N ha$^{-1}$ year$^{-1}$, low rate treatment in Paper I and II</td>
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<td>Addition of 12.5 kg N ha$^{-1}$ year$^{-1}$, low rate treatment in Paper I and II</td>
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<td>50N</td>
<td>Addition of 50 kg N ha$^{-1}$ year$^{-1}$, high rate treatment in Paper I and II</td>
</tr>
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<td>Addition of 50 kg arginine-N ha$^{-1}$, Paper IV</td>
</tr>
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<td>HARG</td>
<td>Addition of 150 kg arginine-N ha$^{-1}$, Paper IV</td>
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<tr>
<td>LAN</td>
<td>Addition of 50 kg NH$_4$NO$_3$-N ha$^{-1}$, Paper IV</td>
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<tr>
<td>HAN</td>
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1 Introduction

Anthropogenic activities during the last century have considerably increased the amount of biologically accessible N (Nr), e.g. through combustion of fossil fuels, cultivation of N$_2$-fixing crops and the production of fertilizer (Vitousek et al., 2002). Thus, in regard to natural fixation, the amount of reactive N in the global N cycle has more than doubled due to human activity (Galloway et al., 2004; Vitousek et al., 1997), and anthropogenic N input today is as large as the terrestrial biological fixation (Galloway et al., 2004).

This thesis aims to explore long-term effects from anthropogenic N addition on boreal forest growth; particularly in forests dominated by either Norway spruce (Picea abies [L.] H. Karst.) or Scots pine (Pinus sylvestris L.). The thesis focuses on two different categories of N addition: commercial forest fertilization, Paper III, and atmospheric N deposition (Paper I and II). Forest fertilization can, in this respect, be regarded as ‘intentional’ N addition while N deposition on the other hand is ‘unintentional’ (Fig. 1). Both intentional and unintentional anthropogenic N additions affect our forests in many ways not yet fully understood. So far most studies on ecosystem effects of anthropogenic N have focussed on inorganic N. The plant available N pool in boreal forests is, however, dominated by organic N forms and in my thesis I also explore interactive effects of N form and N dose on a number of forest ecosystem traits (Paper IV).
1.1 Nitrogen turnover in boreal forest soils

Boreal forests contain large amounts of complex organic N, but the decomposition rate is relatively slow and the quantity of soluble N is low (Rustad et al., 2001; Tamm, 1991; Vitousek & Howarth, 1991; Nommik & Vahtras, 1982). The low production rate of plant accessible N may be caused by low soil pH (acidic soils) and low soil temperature. Nitrogen leaching from undisturbed boreal forests is rare, but the tight N cycle can be disturbed by abiotic factors such as tree harvest, soil scarification and forest fertilization causing organic N to mineralize into inorganic N (Pietikainen & Fritze, 1995; Priha & Smolander, 1995; Smolander et al., 1995). Figure 2 shows a simplified conceptual model of the natural N turnover in boreal forests, with the addition of artificial fertilizer.

*Figure 1. A simplified conceptual model of intentional and unintentional anthropogenic N additions to boreal forest.*
1.2 Boreal forest growth is nitrogen limited

Even though boreal forest soils are often rich in organic N, tree growth is usually N limited (Binkley & Högberg, 2016; Jarvis & Linder, 2000; Tamm, 1991) and will respond with increased growth to N addition (Nohrstedt, 2001). When provided with extra N boreal conifer trees expand their photosynthetic apparatus by increasing the amount of foliage and by increasing the amount of N within foliage (Tamm, 1991; Linder & Troeng, 1980), mainly as RuBisCo (presumably the most common enzyme on earth) needed for photosynthesis. Fertilizing with N also shifts tree relative biomass allocation from below to above ground parts (Lim et al., 2015; Axelsson & Axelsson, 1986). During the past century anthropogenic N has mainly been incorporated in Swedish boreal forest ecosystems through two major pathways, as atmospheric N deposition and through commercial fertilization (Figs. 1 and 2). Nitrogen may also be sequestered by N$_2$ fixing, free living or symbiotic. Examples of the latter includes symbiotic relationships e.g. N$_2$-fixing cyanobacteria in association with bryophytes, e.g. red-stemmed feather-moss (*Pleurozium schreberi* [Bird] Mitt.) (Gundale et al., 2011), glittering woodlandmoss (*Hylocomium splendens*...
[Hedw.] Schimp) (Ackermann et al., 2012), or Frankia bacteria in symbiosis with grey alder (Alnus incana [L.] Moench) (Binkley et al., 1992). Nitrogen is also readily converted back to its gaseous form through denitrification and burning of biomass.

1.3 Anthropogenic nitrogen input to boreal forests

Nitrogen deposition has taken place all over Sweden, to a greater extent in the south than in the north. The opposite is true for forest fertilization; it is done locally, under controlled conditions and mainly in the middle and northern parts of the country. The two anthropogenic N input pathways to forests could be called ‘unintentional’ and ‘intentional’ fertilization. Nitrogen deposition is unintentional in the sense that we have no control over where deposition occurs, \( N_r \) travels in the prevailing wind direction, sometimes far from the source. In contrast, today's commercial forest fertilization is a very precise method and applied \( N \) is usually retained in the intended forest stand.

1.3.1 Forest fertilization in practice

In the middle of the 20th century the demand for forest products increased dramatically cf Kardell and Lindkvist (2010) and Lindkvist et al. (2011). Forest managers and academics predicted dwindling stocks and sought ways to increase forest growth to ensure a sustainable supply of the raw material to the industry. With the discovery of the Haber-Bosch process decades earlier, artificial \( N \) fertilizer could be mass produced and was widely used in agriculture. The process presented by Haber in 1909, and later refined by Bosch in 1910, converts gaseous \( N_2 \) into \( NH_3 \) by a reaction with \( H_2 \) and a metal catalyst under high temperature and high atmospheric pressure. With the increments in yield from artificial fertilizers observed in agriculture for ulterior motives, foresters researched the growth enhancing effects of adding inorganic \( N \) to forest stands in early fertilization field trials. They found that forest fertilization was a good method for increasing tree production to meet the growing demand. From these early experiments it was also determined that from low \( N \) application amounts (<50 kg N ha\(^{-1}\)) the forest growth increments were minor or statistically insignificant (Pettersson, F. 2013\(^1\)). In a Norwegian study, Brantseg et al. (1970) concluded that fertilizing with less than 75 kg N ha\(^{-1}\) did seldom increase growth significantly in Norwegian \( P. abies \) and \( P.

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sylvestris forests. Möller (1978) estimated that only about 55% of the possible growth enhancing effect seen in early fertilization experiments was reached in commercial forest fertilization at that time. Possible reasons for the lower effect were presumed to be uneven fertilizer spreading, abiotic inadequacy (i.e. sites less susceptible to N addition, often very low or high productivity forests), disparate stand characteristics and estimation errors in optimal harvest volume. In the modern day this proportion is likely to have increased, since the environment and forest production is treated as equals under Swedish forestry law; and from an economic point of view, more and more studies showed which stands were susceptible to high growth increments from N addition, with accurate spreading being of higher importance today than in the 1970’s.

The area annually fertilized in Sweden rapidly increased from only a few thousand hectares in the early 1960’s to about 190 000 ha (about 0.8% of the productive forest area, i.e. 23 million ha) in the mid-1970’s (Fig. 3) (Anon, 2014a). This was followed by a decrease to 30 000 ha in the beginning of the 1990’s. The swift decrease in commercial fertilization was likely due to the weak economic climate at that time and the public concern for fertilization causing acidification and eutrophication (Lindkvist et al., 2011; Kardell & Lindkvist, 2010). At the beginning of this century the use of N fertilizer increased and in 2010 about 80 000 ha of forest land was fertilized (Anon, 2014a). Following the decline in the global economy in recent years the total area annually fertilized decreased to about 53 000 ha in 2011, a continued to decrease down to 46 000 ha in 2012 and in 2013 it was 24 000 ha (Anon, 2014b).

During the commercial forest fertilization upswing in the 1960’s, 111.5 kg N ha⁻¹, in form of urea, was often applied (Lindkvist et al., 2011; Kardell & Lindkvist, 2010). As field experiments increased foresters’ understanding of the relation between different sources of N and its effects on the forest surrounding the controlled experiments, the applied N form was changed to ammonium-nitrate (NH₄NO₃) and later SkogCAN (ammonium-nitrate, circa 27% N, with dolomite chalk and Boron), which is still commonly used. Simultaneously the N dose applied was increased to 150 kg N ha⁻¹. In more recent years there have been experimental field trials with organic N fertilizer (about 4% N, dry weight [DW]) produced from sewage sludge (Sahlén et al., 2011; Sahlén, 2006). The effect of organic N is expected to extend over 15 years and early experiments have shown an increased production by 15 to 70% after adding 300 to 2000 kg sludge ha⁻¹.

In commercial forestry N fertilization applied eight to nine years before final harvest increases net present value by about 11% and is considered one of the most profitable management practices in established boreal forests.
(Simonsen et al., 2010). It is estimated that about 10% of Sweden’s forest area has been fertilized at least once since this management practice started (Näslund et al., 2013; Lindkvist et al., 2011). In the 1990’s private and corporate forest owners could, in accordance with recommendations from the Swedish Forest Agency, fertilize suitable forest stands in northern Sweden with up to 600 kg N ha⁻¹ rotation⁻¹ but with no more than 200 kg per occasion, cf Näslund et al. (2013). Today, the Swedish Forest Agency recommends that no more than 450 kg N ha⁻¹ can be applied during a rotation period in the northern parts of Sweden. For central Sweden no more than 300 kg is recommended and in southern Sweden a maximum of 150 or no fertilization at all, is advised.

For private forest owners, the cost of fertilizing 1 ha with 150 kg of N (SkogCAN) is about 250 to 300 € (in Sweden) when contracting one of the major forest companies for the procedure, of which about 40% of the cost is for transport and spreading, and 60% for the actual fertilizer².

Figure 3. The forest area in Sweden (in thousands of hectares) annually fertilized with N between 1962 and 2013. From 1986 through 2013 only data from the large forest companies is included. During this time period, small-scale private forest owners are estimated to have fertilized between 1000 and 3000 ha annually. Based on official national data (Skogsstatistisk årsbok) from the Swedish Forest Agency (Anon, 2014a; Anon, 2014b).

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1.3.2 Forest fertilization studies

Intentional forest fertilization has been studied in many ways and there seems to be some consensus within the research community - commercial N fertilizer rarely affects the system for more than 10 years (Nohrstedt, 2001; Saarssalmi & Mälkönen, 2001). Until the present time, most of the previous Swedish forest fertilization studies have, with a few exceptions (Johansson et al., 2013; Sikström, 2005; Högbom et al., 2001), mainly studied the growth enhancing effects within one forest generation. However, the current paradigm has been questioned in recent years. Studies have demonstrated long-term effects (>20 years since the last fertilization) on the composition of forest floor species (Strengbom & Nordin, 2012; Strengbom & Nordin, 2008), where normal commercial fertilization (150 kg N ha\(^{-1}\)) applied in the preceding rotation period increased the proportion of graminoids and decreased the proportion of heather (Ericaceae) species, mainly dwarf shrubs such as lingonberry (Vaccinium vitis-idaea L.) and billberry (Vaccinium myrtillus L.).

Much of the added N when fertilizing forest is retained in the soil layer (Nohrstedt, 1990; Nommik & Larsson, 1989; Melin & Nommik, 1988; Melin et al., 1983) and a lesser proportion in the trees. Tops and branches are relatively rich in nutrients (Harrington et al., 2013; Olsson et al., 2000; Smith et al., 1994; Mann et al., 1988), and are generally left after thinning and sometimes after clear-felling. The remaining harvest residues and needles that have been previously enriched by N fertilization may therefore be transferred to the next tree generation after clear-felling. Furthermore, soil N mineralization rates and the amount of available soil nutrients generally increase after final felling and soil scarification (Lundmark-Thelin & Johansson, 1997; Rosén et al., 1996; Vitousek & Matson, 1985). Previously immobilized N may therefore become accessible to the subsequent tree generation when the mineralization rates increases after physical disturbances (e.g. soil scarification).

1.3.3 Chemical form of forest fertilizers

SkogCAN with N in the form of ammonium-nitrate is the most commonly used N fertilizer in forestry in the Scandinavian boreal region, even though boreal conifer trees often prefer ammonium over nitrate (Kronzucker et al., 1997; Buchmann et al., 1995) and nitrate is often assimilated in forest floor vegetation, or even leached. For over a hundred years it has been known that higher plants can also assimilate organic N (Hutchinson & Miller, 1912), however, it is only during the last couple of decades that this research area has expanded (Näsholm et al., 2009; Näsholm & Persson, 2001; Näsholm et al., 1998). Particularly the amino acid arginine (ARG) has been in focus of
Researchers’ attention in exploring alternatives to conventional fertilizers in nurseries. Due to its chemical structure (Fig. 4), ARG is positively charged under normal boreal forest soil pH and is easily retained within the system. For example, after switching from conventional fertilizers to ARG in conifer seedling nurseries the N leakage to surrounding areas has been shown to decrease (Öhlund & Näsholm, 2002). Arginine has a high N to C ratio (4:6) and greenhouse experiments have shown that it works well for cultivating seedlings (Persson & Näsholm, 2002).

Arginine is also a plant N storage compound that is easily stored away during times of high N availability or before dormancy (Nordin & Näsholm, 1997; Edfast et al., 1996). Organic N is the predominant soluble soil N source in nutrient poor boreal coniferous forests (Inselsbacher & Näsholm, 2012) that is readily available for vegetation uptake (McFarland et al., 2010; Kielland et al., 2007; Jones & Kielland, 2002; Nordin et al., 2001).

To my knowledge, Paper IV is the first large stand-scale experiment that studies interactive effects between N dose and N form including an organic N form in boreal forest.

![Figure 4. Chemical structure of L-arginine at pH 7 in its ionized form.](image)

1.3.4 Past and present atmospheric nitrogen deposition

During the last century global emission rates of reduced (NH₃) and oxidized N (NOₓ) increased rapidly due to human activity which resulted in increased atmospheric N deposition rates (Galloway et al., 2008; Reay et al., 2008; Lamarque et al., 2005). As a response to the increasing emission rates the global N deposition rates are predicted to increase further worldwide (Evans, 2001). Anthropogenic N deposition over Europe (Fig. 5) has long been a widespread phenomenon and according to the European Monitoring and Evaluation Programme (EMEP) model MSC-W the estimated deposition in 2013
(the latest available data set) ranged from 1 to 25 kg N ha\(^{-1}\) (UNECE, 2013). In Sweden the total deposition of \(\text{NH}_x\) and \(\text{NO}_y\) is about 1 to 12 kg N ha\(^{-1}\) year\(^{-1}\) (Fig. 6). Much of the N deposition over southern Sweden followed with prevailing winds mainly from the UK and Europe's northwest coast; although the emissions have decreased in northern Europe no general significant decrease in deposition rates has yet been observed in Sweden (Akselsson, C. 2013\(^3\)), but long-term studies of watershed export has shown a steady decline in inorganic N since 1985 (Lucas \textit{et al.}, 2016), and it is often speculated that the N deposition over Sweden is in decline.

Some N deposition is natural, \textit{e.g.} formed by lightning in combination with gaseous N (mainly NO\(_x\)), this natural formation and following deposition cannot easily be separated from the anthropogenic source, but it can probably be assumed that the proportion of natural deposition is a small fraction of the total deposition, especially in southern Sweden.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure5.png}
\caption{The total amount of reduced (\(\text{NH}_x\)) and oxidized (\(\text{NO}_y\)) N deposited over Europe in 2013. This figure is produced with the European Monitoring and Evaluation Programme (EMEP) model MSC-W (UNECE, 2013). 100 mg m\(^{-2}\) equals 1 kg ha\(^{-1}\).}
\end{figure}

\textbf{3.} Akselsson, C., personal communication April 22\textsuperscript{nd}, 2013. Swedish Environmental Research Institute.
The effect of nitrogen deposition on tree growth

The effect of deposited N on boreal forest growth has not been studied to the same extent as the effect of forest fertilization. There are many indications that the on-going deposition of N has increased tree production but natural N deposition is in essence hard to study. To exclude natural N deposition, experimental sites have to be enclosed in transparent material, e.g. roofing studies (Corre & Lamersdorf, 2004; Koopmans et al., 1996). The roofing method also excludes natural rainfall which must be artificially simulated through expensive irrigation systems, however, this approach offsets the local environment and cannot in a perfectly good way substitute natural conditions. Because of these technical challenges, most of the previous studies on the effects of N deposition on tree growth in Europe have been correlative, e.g. Solberg et al. (2004) and Solberg et al. (2009).

Another approach is to locate the experiments to an area with low atmospheric N deposition. Under these circumstances, the effects of different N addition rates on tree growth can be studied with minimal disturbance to the
natural environment. Most long-term stand-scale empirical studies of annual N addition on tree growth have, however, applied N at much higher rates (≥30 kg N ha\(^{-1}\)) than is realistic for boreal and temperate forests (de Vries et al., 2009).

1.4 Aim

The purpose of the studies upon which this thesis is based was to increase our understanding of long-term effects of N addition on tree growth in boreal forest ecosystems. To accomplish this, several variables associated with site fertility and tree growth have been measured in the field in areas with low background N deposition. Nitrogen was either applied annually at low rates (≤12.5 kg N ha\(^{-1}\) year\(^{-1}\)), simulating N deposition in boreal forests and at a higher rate (50 kg N ha\(^{-1}\) year\(^{-1}\)) similar to previous long-term N addition studies (Paper I and II), or as normal commercial forest fertilization (150 kg N \(-1\) occasion\(^{-1}\)) in the stand preceding the one studied (Paper III). Also interactive effects between N form and N dose were investigated (Paper IV). Specifically I have explored the following questions:

1. What are the tree growth responses to long-term annual N addition at relatively low rates in mature forests dominated by \textit{P. sylvestris} or \textit{P. abies}?
   (Paper I and II)

2. Are there long-term carry-over effects between forest stands, stretching over tree generations, of commercial N fertilization on tree growth, plant available soil N and soil N mineralization rates?
   (Paper III)

3. What are the interactive effects between N form (inorganic and organic) and N dose on various ecosystem variables?
   (Paper IV)
2 Materials and methods

The method section herein is a description of the materials and methods in Papers I through IV. I refer to the respective Paper and references cited therein for more detailed descriptions.

This thesis focuses on two economically vital tree species for Swedish forestry, *P. abies* and *P. sylvestris*. The conifer forest stands included in the studies I through IV were all representative of Sweden’s boreal forests in terms of soil type, standing stock and abiotic conditions.

Paper I, II and IV are all situated close to each other in the vicinity of Vindeln municipality (Fig. 7). As such, they share many of the same basic environmental conditions and have similar forest floor vegetation, see Table 2 for descriptive site data in studies I through IV. The weather conditions (monthly mean temperature and precipitation) in Vindeln are shown in figure 8.
<table>
<thead>
<tr>
<th>Study</th>
<th>I, II</th>
<th>I</th>
<th>III</th>
<th>IV</th>
</tr>
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<tbody>
<tr>
<td>Location</td>
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<td>Åheden</td>
<td>Bispgården</td>
<td>Holmyrbränna</td>
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<tr>
<td>Coordinates (WGS 84)</td>
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<td>64°14′N, 19°46′E</td>
<td>63°00′ N, 16°40′ E</td>
<td>64°11′N, 19°33′E</td>
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<td>Background N deposition kg N ha⁻¹ year⁻¹</td>
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<td>Precipitation (mm year⁻¹)</td>
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<td>During growing season (mm year⁻¹)</td>
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<td>Chronology</td>
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<td>Once or twice rotation¹</td>
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<tr>
<td></td>
<td>V. vitis-idaea</td>
<td>C. vulgaris</td>
<td>V. vitis-idaea</td>
<td>V. vitis-idaea</td>
</tr>
<tr>
<td></td>
<td>A. flexuosa</td>
<td>P. schreberi</td>
<td>A. flexuosa</td>
<td>A. flexuosa</td>
</tr>
<tr>
<td></td>
<td>P. schreberi</td>
<td>Dicranum sp.</td>
<td>P. schreberi</td>
<td>P. schreberi</td>
</tr>
<tr>
<td></td>
<td>H. splendens</td>
<td>Cladonia sp.</td>
<td>H. splendens</td>
<td>H. splendens</td>
</tr>
</tbody>
</table>

¹
Figure 7. A map showing the locations of Studies I through IV. Bispgården is c. 250 km from Vindeln. In the lower right corner is a map of Sweden, the arrow shows the location of the Swedish University of Agricultural Sciences in Umeå.

Figure 8. The monthly average temperature and precipitation (±SE) in Vindeln, 1994 through 2011. Studies I, II and IV were situated in Vindeln municipality (Ottosson-Löfvenius, 2013).
I analysed the effect of continuous low N addition on tree growth in two different forest types, one *P. abies* dominated mesic site and one *P. sylvestris* dominated heath site. The studies were conducted at Svartberget and Åheden in Vindeln, Vindeln Experimental Forest (Unit for field based research, SLU) where N deposition was simulated by manually spreading ammonium-nitrate (NH$_4$NO$_3$) by hand each spring (early May) before the commencing growth season in amounts corresponding to past and present deposition rates over large parts of Europe (Schulz *et al.*, 2013; Wenche, 2012; Phil-Karlsson *et al.*, 2011) and at a higher rate commonly included in previous annual N addition experiments in the boreal region (Hyvönen *et al.*, 2008; Högberg *et al.*, 2006).

The two forest ecosystems have been fertilized since 1996 in the *P. abies* stand (plots sized 0.1 ha) and since 2005 in the *P. sylvestris* stand (plots sized 0.1 ha). The treatments in the *P. abies* site were 0, 12.5 and 50 kg N ha$^{-1}$ year$^{-1}$ (k=1, L=3, n=12), and 0, 3, 6, 12.5 and 50 kg N ha$^{-1}$ year$^{-1}$ in the *P. sylvestris* site (k=1, L=5, n=6). See Table 2 for descriptive site data. For simplicity the treatments are represented as the amount N applied in kg and the letter N (i.e. 3N, 6N, 12.5N and 50N for 3, 6, 12.5 and 50 kg N ha$^{-1}$ year$^{-1}$), or as low N addition rates ($\leq$12.5kg N ha$^{-1}$ year$^{-1}$) and high N addition rate (50 kg N ha$^{-1}$ year$^{-1}$). For a full description of the *P. abies* site refer to Strengbom *et al.* (2002) and to Gundale *et al.* (2011) for the *P. sylvestris* site. Figure 9 depicts a 3D model of one of the studied *P. abies* plots.

*Figure 9. A 3D model of one of the studied *P. abies* plots produced with the software programme Heureka with actual measurement data from the plot (The Heureka Forestry Decision Support System [DSS], Standwise, version 2.2.0.9).*
I acknowledge that manually spreading N fertilizer in the beginning of each growing season is not interchangeable with natural and anthropogenic N deposition because of e.g. temporal differences and canopy uptake. Nitrogen interception in tree crowns in boreal vegetation has, however, been shown to be of minor importance (Adriaenssens et al., 2012; Boyce et al., 1996). An exact replication of natural N deposition is difficult to practically achieve without expensive irrigation systems.

2.1.1 Sample trees, increment cores and WinDendro
Sample trees in Paper I were selected with a hand-held field computer and 43% of all trees were selected in the P. abies stand and 48% in the P. sylvestris stand. Sample trees were selected by a custom built software (HandheldClient, developer Magnus Mossberg, SLU, Alnarp) to accurately represent all trees in each plot. Current year needles were collected from the sample trees from branches facing south. The needles were oven dried (65°C for 24 hours), grinded with a bead mill to a fine powder and analysed for C and N concentrations. Increment cores were extracted at DBH from sample trees, these increment cores were analysed in WinDendro 2012 (Regent Instruments, Quebec, Canada). WinDendro is a commonly used software program for dendrochronology, in which annual year tree ring widths can be measured from scanned images of increment cores. Examples of drill cores analysed in WinDendro are shown in figures 10 and 11. The basal-area growth and size corrected basal area growth rate (RGR) reported in Paper I were calculated with the annual year ring widths obtained in WinDendro.

This non-destructive growth measurement method was chosen over cutting down the trees since the experiment is still on-going. The increment cores were taken from the same cardinal direction and misshapes, e.g. from wind exposure, should thus be approximately evenly distributed over plots and treatments.

The first years after N fertilization the tree growth response varies between stands (Nohrstedt, 2001; Sikström et al., 1998), and the diameter growth response to N addition increases progressively during the first years following the fertilization event (Valinger et al., 2000). Hence, when calculating the relative basal area growth response to N addition the first five years of the fertilization was excluded to allow for the initial adaption phase for trees to express a full growth response to N input to pass.
2.2 Paper II

The effect of low annual N additions on whole ecosystem biomass production and N allocation was studied on the same *P. abies* plots as used in Paper I. See Table 2 for descriptive site data. To estimate the forest floor biomass allometric equations were established from analyzing harvested above and below ground samples of bilberry (*Vaccinium myrtillus* L.) and wavy hair-grass (*Avenella flexuosa* [L.] Parl., previously known as *Deschampsia flexuosa* [L.] Trin.). The pinpoint intercept method, described in Strengbom *et al.* (2002), was used to estimate *Vaccinium* sp. and *A. flexuosa* abundance and with the allometric equations this was converted to biomass per unit area. The biomasses ha$^{-1}$ of feather moss *P. schreberi* and glittering wood-moss (*Hylocomium splendens* [Hedw.] B.S.G.) were estimated by multiplying the mean shoot density (shoots per unit area) with the mean shoot mass (of collected samples) and again multiplying with the visually-determined coverage (in per cent, converted to a per ha basis). The density was measured in five 10 cm circles within each plot where the species altogether covered 100% of the bottom layer. The shoot mass was estimated from 25 shoot samples from all species from five locations within each plot. Feather moss coverage was determined by visually estimating...
percent coverage of ground vegetation in 15 randomly placed 0.25 m² rectangles within each plot.

The tree growth was estimated with backward chronological analyses of the annual year ring widths. Observe that this study is based on data acquired earlier than the data in Paper I. *P. abies* biomass was estimated with the mean plot tree diameter using Marklund’s (1988; 1987) allometric equations G3, G12, G15, G16 and G23 for stem, branches, needles, dead branches, stump and the root system. The carbon (C) contents of the studied compartments were estimated to be 50% of the total biomass (DW) and the total tree C ha⁻¹ was estimated by multiplying the tree C content with the average number of trees ha⁻¹ on the site. The annual increase in C biomass per year was estimated by dividing the total increase in tree C ha⁻¹ with the length of the treatment period, i.e. 14 years.

N allocation within different ecological compartments was identified in a field tracer study, in the spring of 2011, 99 atom% ¹⁵N-labeled ¹⁵NO₃¹⁵NH₄ was applied in 15.5 m² circular sections (Fig. 12) surrounding six pre-selected sample trees. The labeled isotope was applied with a standard watering can on the 16th of June, and samples from the studied compartments, *P. abies* roots and needles, and forest floor above and below ground vegetation, were acquired on September 5th 2011. Three samples from each plant part were collected from different locations in the 15.5 m² plots. Foliar tissue samples from feather mosses, *A. flexuosa*, *V. myrtillus* and *P. abies* were collected from each plot where no label had been applied to assess natural abundance of δ¹⁵N and per cent N for each species. Before analyses, all samples were dried in an oven (65°C) for 24 hours and grinded to a fine powder in a ball mill.

Soil nutrient availability was assessed with five ion-exchange resin capsules per plot in 2010. The capsules were buried 5 cm below the humus layer (the decomposed vegetation layer just under the living species) within one week after snowmelt (early May) and collected in November (2010). The capsules were extracted in 1M KCL and analysed for NH₄⁺ and NO₃⁻ using an Autoanalyzer (Omni Process, Solna, Sweden).
2.3 Paper III

I revisited previously studied sites where changes in field vegetation as an effect of previous commercial fertilization had been documented Strengbom and Nordin (2012); (2008). The correlation between the previous fertilization and the compositional changes in forest floor vegetation prompted me to further investigate whether long-term fertilizer-induced changes in tree growth could be found in the young (10 years) conifer trees. I analysed tree growth and stand biogeochemical variables in second rotation stands where normal commercial N fertilizer had been applied once (as 150 kg N ha⁻¹, 25 years before our study) or twice (2 x 150 kg N ha⁻¹, 33 and 25 years before our study) with the last N addition 10 years before final harvest. The fertilization was made from aircraft or by tractor. The experiment was treated as a completely randomized design with the number of factors (k)=1, number of levels (L)=3, and number of replicates (n)=7. The fertilized stands are hereafter noted as sites with treatment N1 (150 kg N ha⁻¹) and N2 (2 x 150 kg N ha⁻¹). See Table 2 for descriptive site data). Final felling, soil scarification (disc-trenching) and regeneration (planting) were performed ten years before field measurements (2010), with the exception for the forest floor vegetation which was measured in 2008 and 2009. There was no difference in time since clear-cutting between
fertilized and unfertilized sites, for a full description of the studied sites see Strengbom and Nordin (2008).

Tree growth was measured as annual height growth increments, i.e. distance between branch nodes, from first visible node through full tree height, and DBH was measured with a caliper. I decided to measure tree height growth as annual increments as a similar study from North America showed that a growth difference occurred first after a few years after seedling establishment (Footen et al., 2009). This design was chosen in order to visualize any growth enhancements caused by the N addition in the previous stand rotation period.

In addition to apparent indicators of tree productivity, such as height and diameter, needle N concentration was also measured. Needle samples were collected from the sample trees top branches, dried at 70°C for 24 h, grinded to a fine powder in a bead mill (5 min per sample), the ground material was then analysed for C and N concentrations.

The forest floor vegetation was inventoried with the pin-point intercept method, described in Strengbom et al. (2002), at 200 random points along a 45 m transect in each forest stand in August 2007 and in August 2008. All contacts with each individual species along a plastic pin were counted as it passed through the vegetation; see Strengbom and Nordin (2012; 2008) for details. Also, soil N mineralization rates and amount of mobile soil ammonium (NH₄⁺) and nitrate (NO₃⁻) ions were measured. The soil N availability was measured with resin (ion-exchange) capsules, buried just under the mor-layer (above the mineral layer) in the beginning of the growing season (1st through 3rd of June) and retrieved between the 28th and 30th September. The gathered samples were kept in plastic bags and transported to a lab for analyses.

Soil mineralization rates of NH₄⁺ and NO₃⁻ were measured with the buried bag technique. Humus samples were collected at the same time as the capsules were buried, half of the samples were sent to a lab for analysis and the other half were put in plastic bags and deposited at the same locations as the resin capsules.

2.4 Paper IV

At Holmyrbränna, at a P. sylvestris dominated site, ecosystem interactive effects between N form and N dose were investigated; 0, 50 and 150 kg N ha⁻¹ of ammonium-nitrate (AN) and of the amino acid arginine (ARG).

The experiment was a completely randomized block design with plots sized 25 · 25 m, with a 10 m buffer zone around them. See Table 2 for descriptive site data. Treatments were low 50 (LARG) and high 150 kg (HARG) of powdery ARG per ha, and low 50 (LAN) and high 150 kg (HAN) of granulated
AN per ha, and the control (n=6). Nitrogen was spread by hand in June 2008. Basal area was measured for all trees before N addition and after the growing season in 2013, and the basal area growth was used to estimate treatments effects on tree growth.

To examine mobile soil N four to five ion exchange capsules per plot were inserted between the organic field layer and the mineral soil before N fertilization in 2008. The first set of capsules was collected in late October 2008 where after new capsules were positioned, and this procedure was repeated so that old capsules were replaced with new ones in October 2009, 2010 and 2013. After each retrieval, four to five new capsules were inserted in each plot.

The presence and species of fungal sporocarps was counted six times per year (at two week intervals) between August and October in 2008, 2009 and 2013 by two persons, seven times per plot each. Destructive sampling was used to avoid multiple counting. The number of sporocarps from the four most common species and the total number of sporocarps were used as response variables.

The pin-point intercept method was used to estimate changes in forest floor vegetation in seven sub plots (0.2 · 0.6 m) within each plot. A plastic stick (4 mm diameter) was inserted at 30 random points within the 0.2 · 0.6 m grid and individual species and number of contacts with each species were counted. The pin-point intercept was done in August 2007, 2008, 2009 and 2013 and the number of hits per m² was calculated for each plot and year. The five most common species or species groups were used as response variables in statistical analyses.

2.5 Statistical analyses

The software programs Minitab (v.16), MYSTAT (v.13), JMP (v. 12), R (v. 3.1.1) and SPSS (v.20) was used for the statistical analyses in Paper I through IV. For a detailed description of the linear regressions, analyses of variance (ANOVA), analysis of covariance (ANCOVA), Generalized Linear Mixed Models (GLMM) and the repeated measure ANOVA see the statistical paragraphs in each Paper. All variables were checked for normal distribution and homoscedasticity, and when these requirements were not met, data was transformed to meet these requirements. Mean values (untransformed) ±1 standard error (SE) are depicted in figures and tables except where otherwise stated.
3 Results

3.1 Paper I

Paper I focused on tree growth responses to low rates of N addition in *P. abies* and *P. sylvestris*.

For the N addition rates reflecting the current N deposition levels over parts of Europe (12.5 kg N ha\(^{-1}\) year\(^{-1}\)), I found a significant increase in tree growth only in *P. abies*, which had been subjected to 19 years of consecutive N addition treatments (Paper I, Tables 1 and 2). The annual growth rates in the 12.5N treatment were, however, lower during the period before N addition started than during the N addition period in both *P. abies* and *P. sylvestris* (Fig. 13; Paper I, Fig. 3). *P. sylvestris* was repeatedly subjected to N addition for 10 years. For the lowest N addition rates (≤6 kg N ha\(^{-1}\) year\(^{-1}\)), tested only in *P. sylvestris* plots, I could not find any significant effects on tree growth (Paper I, Table 2), but there were clearly visible upward growth trends towards the end of the studied period (Fig. 13b).

In the high N addition treatment (50 kg N ha\(^{-1}\) year\(^{-1}\)), *P. abies* and *P. sylvestris* showed similar growth response patterns; during the first six years there were continued increments in relative growth, but from there on the growth rate levelled off at about double the relative growth rate of the controls (Fig. 13). During the last five years of treatment, the *P. abies* relative growth rate declined compared to the control, it was however, always higher than that of the control. I did not observe a similar decline in *P. sylvestris*. On the *P. abies* and *P. sylvestris* sites there were also significant increments in absolute growth (m\(^2\) ha\(^{-1}\)) in response to the 50N treatment, the total basal area increased almost twice as much as the controls did (Paper I, Table 1).

*P. abies* and *P. sylvestris* height growth did not differ between treatments during N addition (Paper I, Table 2). For *P. abies*, the length of the needle carrying tree crown was, however, significantly smaller on the 50N plots.
where the tree crown covered about 75% of the tree height) than on the control plots (about 80% coverage). I did not find a similar pattern in *P. sylvestris* where tree crowns were of similar size regardless of treatment (covering about 50% of the tree stem). I did not observe any changes in stem shape (*i.e.* the height to DBH ratio) in response to the N addition treatments.

Needle N concentrations on both sites increased significantly from the N treatments. In *P. abies* the concentration increased by 28% from the 50N treatment and by 7% from the 12.5N treatment, and in *P. sylvestris* the concentration increased by 21% (Paper I, Table 1).

In linear regression analyses I found that for every kg of N added the relative basal area growth increased by 1.2% in *P. abies* and by 1.6% in *P. sylvestris* (Figs. 14 and 15; Paper I, Fig. 4). The regression relationship was, however, weaker on the *P. abies* site ($R^2 = 0.26$, *P*-value $= 0.002$) than on the *P. sylvestris* site ($R^2 = 0.64$, *P*-value $< 0.001$).
Figure 13. Reproduced from From et al. (2016), Canadian Journal of Forest research, 2016, 46:1396-1403, with permission from the publisher, Canadian Science Publishing and its licensors ©.

a) Picea abies annual basal area growth rates (RGR) during a 19-year N addition period in relation to the average annual RGR during a five year period before N addition started (1990 through 1995). An annual relative basal area growth of 1.0 (red dashed line) denotes equal growth between that year’s growth rate and the average growth rate over the five-year period before N addition started.

b) Pinus sylvestris annual size-corrected basal area growth rate (RGR) during a 10-year N addition period in relation to the average annual RGR during a five year period before N addition started (1999 through 2004). An annual relative basal area growth of 1.0 (red dashed line) denotes equal growth between that year’s growth rate and the average growth rate over the five-year period before N addition started.
Figure 14. *Picea abies* average annual size-corrected relative basal area growth rate (RGR) during the period of N addition in relation to the average annual RGR during a five year period before N addition started, with the average increase of the control between the two time periods as base. A size corrected annual relative basal area growth of 1.0 denotes no difference from the average growth of the control. Plots had been treated with 0, 12.5 and 50 kg N ha$^{-1}$ year$^{-1}$ for 10 years. Overlaid is a simple regression line. Reproduced from From *et al.* (2016), Canadian Journal of Forest research, 2016, 46:1396-1403, with permission from the publisher, Canadian Science Publishing and its licensors ©.

Figure 15. *Pinus sylvestris* average annual size-corrected relative basal area growth rate (RGR) during the period of N addition in relation to the average annual RGR during a five year period before N addition started, with the average increase of the control between the two time periods as base. A size corrected annual relative basal area growth of 1.0 denotes no difference in growth from the average growth of the control. Plots had been treated with 0, 3, 6, 12.5 and 50 kg N ha$^{-1}$ year$^{-1}$ for 10 years. Overlaid is a simple regression line. Reproduced from From *et al.* (2016), Canadian Journal of Forest research, 2016, 46:1396-1403, with permission from the publisher, Canadian Science Publishing and its licensors ©.
3.2 Paper II

Paper II focuses on C assimilation in *P. abies*, shrubs and mosses caused by low rates of annual N addition.

Nitrogen concentrations in the measured plant tissues were consistently higher in plots with the high N addition rate (50 kg N ha\(^{-1}\) year\(^{-1}\)) than in the control plots (Paper II, Table 1), whereas the low N addition rate (12.5 kg N ha\(^{-1}\) year\(^{-1}\)) did not alter the N concentrations significantly. In plots with the high N addition rate the understory biomass was lower than in the control plots (Paper II, Table 2), primarily due to a significant decrease in feather mosses, although, the abundance of *A. flexuosa* increased in response to the high N addition.

Analysis of the resin capsules showed that the amount of mobile soil NH\(_4^+\) and NO\(_3^-\) increased in the high N addition plots compared to the control and low N addition plots (Fig. 16; Paper II Fig. 1).

The \(^{15}\)N labeling experiment showed that it was only in the above ground parts of *V. myrtillus* that the N addition treatments affected \(^{15}\)N allocation, for the other fractions no significant changes occurred across treatments. More \(^{15}\)N was allocated to new and old parts of *V. myrtillus* in plots with treatment 50N than in control plots (Paper II, Table 2). Approximately one-half of the applied label was detected in the measured plant pools and about as much was found in the humus layer (Fig. 17; Paper II Fig. 2). The largest proportion of \(^{15}\)N was located in the feather mosses, where a larger proportion was sequestered in the low N and control plots compared to the high N plots. *A. flexuosa* only sequestered a small proportion of the added label and the amount was higher in N addition plots than in control plots. *V. myrtillus* sequestered about 10 to 13% and *P. abies* sequestered about 7 to 9% of the applied label, and the amounts did not differ across the N addition treatments in any of the two pools.

Biomass C sequestration of *P. abies* increased linearly to increasing N addition rates (Fig. 18a; Paper II Fig. 3a), and the total biomass C sequestration of all vegetation pools measured increased with about 16 kg C per kg N added (\(R^2_{(adj.)}=0.563, P<0.001\), Fig. 18b; Paper II Fig. 3b). The net C sequestration in *P. abies* was about 19 kg per kg N added when not compensating for the negative changes in the understory biomass.
Figure 16. The mean (±SE) quantity of NO$_3^-$ (a) and NH$_4^+$ (b) sequestered to ionic resin capsules in replicated forest plots treated with 0, 12.5, or 50 kg N ha$^{-1}$ year$^{-1}$. The capsules were placed at a 5 cm depth in the humus layer between May and October. Different letters above means (a or b) indicate significant differences determined using S-N-K post hoc analyses. Reproduced from Gundale et al. (2014), Global Change Biology, 20(1), pp. 276-286, with permission from the publisher.
Figure 17. The percent of the total $^{15}$N label recovered in the biomass of feather mosses, *V. myrtillus* (V.m.), *D. flexulosa* (D.f. [now known as *A. flexuosa*]), and *P. abies* (P.a.) four months following application of 1 kg $^{15}$NH$_4^{15}$NO$_3$ ha$^{-1}$. The label was applied to a 15.5 m$^2$ portion of each 0.25 ha plot, with plots treated for the previous 14 years with one of three nitrogen addition treatment (0, 12.5, or 50 kg N ha$^{-1}$ year$^{-1}$) simulating atmospheric N deposition. Within each group of bars, different letters (a or b) indicate significant pairwise difference determined through S-N-K post hoc tests. Reproduced from Gundale *et al.* (2014), *Global Change Biology*, 20(1), pp. 276-286, with permission from the publisher.
Figure 18. a) The mean (±SE) change in biomass C accumulation (kg C ha⁻¹ year⁻¹) of *P. abies* (solid circles) and understory biomass (open circles) after 14 years of simulated N deposition. Data are derived from plots treated with three different simulated Nr deposition treatments (0, 12.5, or 50 kg N ha⁻¹ year⁻¹) since 1996. Different letters above or below means (a or b; y or z) indicate significant pairwise differences determined through S-N-K post hoc tests. b) The change in total biomass C accumulated per kg N added (kg C kg⁻¹ N). Reproduced from Gundale et al. (2014), Global Change Biology, 20(1), pp. 276-286, with permission from the publisher.
3.3 Paper III

Paper III focuses on the long-term effects of common forest fertilization on young tree growth in the tree generation following the one fertilized, *i.e.* following clear-cutting and regeneration.

The annual shoot height growth over time (Fig. 19; Paper III Fig. 1) was higher in the young trees (about 10 years old) grown on sites that had been fertilized twice in the preceding rotation period than trees in control stands. On sites where two previous fertilizations had been applied the trees were about 24% taller than trees on unfertilized control sites. Trees on previously fertilized sites (N1 and N2) also had about 15% more N in their current year needles compared to unfertilized controls. There were no differences in needle C concentration between sites with the different N treatments. Also, tree diameter (DBH) was not affected by the previous fertilization treatments (Paper III, Table 3).

It took two previous fertilizations for effects on soil mineralization and the amount of mobile soil N to remain evident 25 years later (Paper III, Table 2). Analyses of resin ion exchange capsules showed that the previously fertilized stands (N2) had about twice the amount of available NO$_3^-$ and NH$_4^+$ in the soil organic layer than in control stands. The mineralization rates measured with the buried bags showed that the rate on sites with two previous fertilizations was about 4 times the average of the controls, whereas there were no differences in N mineralization rates between the control and the N1 sites.

A stepwise backward elimination regression analysis of the relation between tree height as response and the abundance of *A. flexuosa*, *V. myrtillus*, the needle N concentration, soil mineralization rates and the amount of mobile soil N, showed that the needle N concentration and the amount of mobile soil N was relevant as explaining factors for mean tree height ($R^2_{(adj.)}=0.45$, $F=9.14$, $P<0.001$).
3.4 Paper IV

Paper IV investigated if there were any differences in how organic and inorganic N applied in two different doses (50 and 150 kg N ha\(^{-1}\)) affected the forest ecosystem, focusing on tree growth, the forest floor vegetation, ectomycorrhizal fungi and soil N turnover during five years following the N addition event. The organic N form was the amino acid arginine (ARG) and the inorganic N form was ammonium-nitrate (AN).

Tree diameter growth increased significantly compared to the control from high ARG addition and from both low and high AN addition (Paper IV, Fig. 1). The low ARG treatment, however, did not significantly increase growth above the average growth of the control.

The N concentrations in September 2008, about 20 weeks after N addition, were significantly higher in leaves of *V. myrtillus*, *A. flexuosa* and *P. schreberi* in all of the N addition treatments, whereas only the high N addition treatments increased N concentration in current year needles of *P. sylvestris* (Paper IV, Table 1).

Both organic and inorganic N addition increased the amounts of mobile NH\(_4^+\) captured by ion-exchange capsules in September during the first year of N addition (Paper IV, Fig 2). The low ARG treatment, however, caused a lesser
amount of ammonium ions from being absorbed compared to the other N addition treatments. The high AN addition resulted in 90 times as much ammonium ions captured compared to the control, which is about 6 times the increase caused by the low ARG addition. After the winter in 2008/2009 all values returned to normal, i.e. similar to that of the control value. In contrast to ammonium, the amount of nitrate captured by ion-exchange capsules was significantly higher in both of the AN treatments than in the ARG treatments and the control. In fact, both ARG treatments did not have a significantly higher amount of captured nitrate ions than the control had.

The year after N addition total number of sporocarps (fungal fruiting bodies) was significantly lower in plots with AN treatment compared to the control and ARG treated plots. Within the two separate N sources, however, there was no significant effect of the amount of added N, i.e. when comparing low vs. high addition rates (Paper IV, Fig. 3). Both low and high AN treatments caused a significant shift in the composition of fungal species compared to the control. For ARG, however, it was only the high addition rate that significantly changed the composition.

The forest floor species composition was also affected by the N addition; in late summer following N addition the abundance of *A. flexuosa* had increased in all N treated plots and this effect remained throughout the whole studied period, although slowly diminishing by each year gone by (Paper IV, Fig. 4). The N additions did not affect the abundance of *V. myrtillus* or forbes but did affect *V. vitis-idea*, whose abundance decreased in all N addition treatments towards the end of the study period (2013). The abundance of the moss *P. schreberi* was also decreased by N addition and was lower in all N treatments than in the control plots in 2013. The high ARG treatment caused a larger decrease in abundance than the AN treatments did.
4 Discussion

4.1 Long-term effects of N addition on tree growth

4.1.1 Effects from simulated N deposition

Boreal forests are generally thought of as a net carbon (C) sink (Kurz et al., 2013), and it has been suggested that N deposition is important for the C sequestration in boreal forests (Gruber & Galloway, 2008; de Vries et al., 2006). Nitrogen deposition in combination with increased levels of atmospheric CO2 and climate change were advocated as explanatory factors for the increased tree growth in British Columbia during the latter part of the 20th century (Wu et al., 2014). Although the long-term effects of N deposition on the ability of forests to function as C sinks have been questioned (Bala et al., 2013; Hyvönen et al., 2008), it has been proposed that climate change, for example, will eventually overshadow the effects from N deposition. In the 1990’s Binkley and Högberg (1997) investigated whether N deposition might actually threaten forest growth, which the authors found no clear evidence of. The authors came to the same conclusion after reviewing 20 years’ of N studies (Binkley & Högberg, 2016). There is a consensus that N deposition usually increases the growth of boreal and temperate forest and scientific discussions, inventory data and models predict that it will likely continue to do so in the foreseeable future (de Vries & Posch, 2011; Bedison & McNeil, 2009; de Vries et al., 2009; Laubhann et al., 2009; Solberg et al., 2009; Pregitzer et al., 2008; Sutton et al., 2008; Högberg, 2007; Solberg et al., 2004).

To my knowledge, no long-term stand-scale experiments, other than the one described herein, with N addition rates close to N deposition rates over boreal forests have been reported, cf de Vries et al. (2009). Previous studies relating tree growth to N deposition have mostly been indirect, correlating current deposition to growth (de Vries & Posch, 2011; Bedison & McNeil, 2009; de Vries et al., 2009; Laubhann et al., 2009; Solberg et al., 2009; Pregitzer et al., 2008; Sutton et al., 2008; Högberg, 2007; Solberg et al., 2004).
2008; Sutton et al., 2008; Högberg, 2007; Solberg et al., 2004). Paper I and II present experimental data that confirm these previously correlative studies on the relationship between boreal coniferous tree growth and low annual N additions. Annual N additions increased the amount of mobile soil NH₄-N and NO₃-N in the P. abies high N addition plots compared to the low N addition and control plots (Paper II). A close relationship between available soil N and tree needle N concentration is often observed, e.g. Binkley and Reid (1985), in accordance, the needle N concentration was higher in the N addition plots than in control plots for P. abies. Moreover, in Paper II the N concentrations were higher in all vegetation pools studied (P. abies, V. myrtillus, A. flexuosa and feather mosses) in the high N addition plots compared to the low N and control plots.

Wu et al. (2014), found positive growth responses in over 3000 permanent forest inventory plots with a large span of boreal and maritime tree species to climate change, increased atmospheric CO₂ and N addition. In accordance with Wu et al. (2014), our data (Paper I and II) show that long-term annual N addition at low rates can increase tree growth, and consequently the C sequestration in P. abies and P. sylvestris. For P. abies the rate at which C was sequestered was around 19 kg per kg of N added, although when compensating for the reduced C sequestration in the forest floor vegetation the total biomass accumulation rate was about 16 kg C kg⁻¹ N. The relationship in Paper II is lower than those reported in previous studies in boreal forests, i.e. around ≥25 kg C per kg of N added (Hyvönen et al., 2008; Högberg et al., 2006). However, the C sequestration per unit N is likely to vary between soil types, tree species and stand ages (Thomas et al., 2010). For example, Thomas et al. (2010) found that, on average, temperate forests in US sequestered c. 60 kg N ha⁻¹ year⁻¹, where N deposition reduced growth in 3 species and elevated growth in 11 species.

Nitrogen uptake by above ground vegetation is often nonlinear (Templer et al., 2012), with N uptake in plants being higher at higher N addition rates (Nadelhoffer et al., 2004). A nonlinear N uptake would presumably cause a nonlinear growth response. In contrast, I found that in P. abies and in P. sylvestris there was no evidence of a nonlinear uptake or growth response to N addition as relative basal-area growth appeared to increase linearly by about 1.2% and 1.6% per kg N added (Paper I). The field tracer experiment also showed that the amount of ^15N taken up by P. abies did not differ between the N addition treatments as an equal part (7 - 9%) was sequestered independent of the N addition rate (Paper II). This suggests that the N uptake by P. abies can be linear, otherwise a larger proportion of the added ^15N would have been sequestered by trees on high N than on low N or control plots. Although the
treatment time differs between the two contrasting sites, tree growth was affected in a fairly similar fashion. There was a 6 to 7 years acclimation period in both *P. abies* and *P. sylvestris* before any growth increments were visible in the low N addition rates, whereas growth in the high N addition rate increased already after the first application.

Anthropogenic emissions of N compounds are expected to increase during the 21st century (Dentener *et al.*, 2006; Lamarque *et al.*, 2005; Galloway *et al.*, 2004), hence the N deposition rates will continue to increase worldwide (Evans, 2001). The N emissions rates have, however, decreased in most parts of northern Europe, but no general significant decrease in N deposition rates has yet been observed in Sweden (Akselsson, C. 2013). However, long-term studies of watershed hydrologic export in northern Sweden have shown a steady decline in inorganic N, about 0.01 kg N ha⁻¹ year⁻¹, since 1985 (Lucas *et al.*, 2016), suggesting that the N deposition could be decreasing. During the same period forest growth has increased and is thus likely to assimilate a larger quantity of the deposited N (Binkley & Högborg, 2016; Lucas *et al.*, 2016). Consequently, it appears difficult to definitely determine whether N deposition over Sweden is currently declining.

Further studies are needed to elucidate where in the two ecosystems that the added N is retained and whether the linear relationship between tree growth and N addition will continue.

### 4.1.2 Effects from forest fertilization

Most of the previous Swedish forest fertilization studies have, with a few exceptions (Johansson *et al.*, 2013; Sikström, 2005; Högbom *et al.*, 2001), mainly studied the growth enhancing effects within one forest generation (Nohrstedt, 2001). Significant increments in several variables associated with site productivity were found in the subsequent stands 25 years after normal commercial forest fertilization of the preceding stands (Paper III). In general it took two fertilizations with 150 kg N ha⁻¹ (N2) of the preceding stand for site alterations to be detectable in the second tree rotation in our study.

A relatively large proportion of added N when fertilizing forests is retained in the soil layer and a lesser part is sequestered in the trees (Nohrstedt, 1990; Nommik & Larsson, 1989; Melin & Nommik, 1988; Melin *et al.*, 1983). It is common that the soil mineralization rates and the amount of mobile soil N increases after physical disturbances such as soil scarification and clear-

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cutting, generally due to increased activity of soil microbes (Lundmark-Thelin & Johansson, 1997; Vitousek & Matson, 1985), clear-cutting especially has been shown to mobilize large amounts of formerly immobilized N (Rosén et al., 1996). Previously added N retained in the soil stratum may therefore be available to regenerated trees after soil preparation following final harvest as mineralization rates increases. In accordance with these previous studies on the interactions between soil disturbance and N mobility, the soil mineralization rates were increased on sites that were fertilized twice in the preceding tree rotation than on unfertilized control sites (Paper III). In addition to the increased mineralization rates, the amount of mobile soil N absorbed by ion-exchange capsules was also higher on sites previously fertilized two times than in unfertilized control sites. The size of the plant available N pool has been correlated to the amount of N in tree needles (Binkley & Reid, 1985), and a close positive relationship between a high needle N concentration and tree growth has been documented (Iivonen et al., 2006; Bauer et al., 1997). The data in Paper III confirms this positive relationship in a multiple regression analysis between tree growth and the amount of mobile soil N and the needle N concentration. In addition to the changes in variables associated with stand productivity, a fertilizer induced shift in the composition of the forest floor vegetation has been documented on the sites studied in Paper III (Strengbom & Nordin, 2012; Strengbom & Nordin, 2008), and it appears that the commercial fertilization of the preceding tree stands has enhanced the site productivity in the long term (>25 years).

Currently there are only a few other studies on this particular subject, one from North America (Footen et al., 2009) and three from southern Sweden (Johansson et al., 2013; Sikström, 2005; Högbom et al., 2001), and there seems to be some discrepancy regarding long-term carry-over effects from forest fertilization. Footen et al. (2009) found that the fertilization of the previous tree stand did increase the growth of second rotation Douglas fir (Pseudotsuga menziesii [Mirb.] Franco), whereas Johansson et al. (2013) in a study of scarification and pre-harvest fertilization did not detect a higher growth in P. sylvestris seedlings that grew on sites with previous N fertilization than seedlings on unfertilized sites. Neither did Sikström (2005) find any growth effect from pre-fertilization (3 · 200 kg N ha⁻¹) in P. abies seedling, planted in summer, a few months after the clear-felling, and planted with replacement seedlings in autumn the same year, with additional replacement seedlings 2 years later. Also, Högbom et al. (2001) found no growth enhancing effect in regenerating P. sylvestris after 5 and 9 years (planting was performed at two occasions, 4 years apart) from adding up to 1800 kg of N ha⁻¹ in the previous
stand. Our study highlights that it may take more than a few years before a growth-enhancing effect of previous fertilization can be discerned.

In Sweden about 10% of the managed forest land area has been subjected to fertilization at least once in the period from 1960 to 2010 (Näslund et al., 2013; Lindkvist et al., 2011). In order to make informed forest management decisions it is important to elucidate which forest stands that are likely to be more susceptible to long-term alterations due to N addition in the preceding tree rotation period since, for example, present growth models for predicting yield might need adjustment due to residual growth effects of previous fertilization.

4.2 Different ecosystem responses to interactive effects between N form and N dose

In the past decade the number of research articles on organic fertilizers has increased rapidly, however, much of the previous studies focus on seedling growth in nurseries (Gruffman et al., 2012; Öhlund & Näsholm, 2002), or on amendments of organic waste material (Sahlén et al., 2011; Sahlén, 2006).

In Paper IV we present the first large scale experiment studying the effects of an amino acid, arginine (ARG), in the field. More precisely we have investigated the effects of N form (ARG and ammonium-nitrate - AN) and N dose (50 and 150 kg N ha⁻¹) on a number of ecosystem variables.

Under greenhouse conditions Gruffman et al. (2013) showed that pine root uptake rates of ARG is considerably higher than uptake rates of ammonium or nitrate. Gruffman et al. (2012) also showed that seedlings pre-treated with ARG grew faster than seedlings pre-treated with conventional fertilizers when planted in the field. These results cannot be directly translated to field conditions, at least not in the short-term since Paper IV shows that basal area growth was not significantly different following five years after adding 150 kg ha⁻¹ of ARG-N and 150 kg ha⁻¹ of AN-N. However, the full effect of N addition on tree growth generally develops over a 10 year period, and the effect after only five years following N addition may vary substantially in relation to the effect after 10 years (Nohrstedt, 2001; Valinger et al., 2000; Sikström et al., 1998). Additionally, below ground tree growth was not measured, possibly masking differences between N form and dose.

The effects of N addition on forest floor vegetation were mostly similar between the two N forms. For example, the abundance of A. flexuosa increased quite rapidly during the first years of N addition and remained elevated in comparison to control plots also five years following the N additions, regardless of the N form applied. In A. flexuosa leaves, however, the high ARG addition caused a higher N concentration than the high AN addition did. The
similar increase in abundance of *A. flexuosa* in ARG and AN plots was somewhat surprising since N uptake studies have shown that *A. flexuosa* does not readily take up ARG whilst it has a large capacity to take up and proliferate on particularly nitrate (Nordin *et al.*, 2006; Persson *et al.*, 2003). There was, however, an increase in mobile ammonium ions in plots with ARG addition and *A. flexuosa* may have utilized this N source to thrive. Effects on *V. myrtillus*, *V. vitis-idaea* and *P. schreberi* were also similar between plots with AN and ARG addition. The abundance of *V. myrtillus*, however, remained similar to that on control plots whereas there was a decrease in abundance of *V. vitis-idaea* and *P. schreberi* over the five year period.

Negative effects of inorganic N addition on forest floor vegetation and soil microbes have previously been reported from experiments with annually repeated N additions (Högberg *et al.*, 2014), and may last for as much as 50 years after ending the N additions (Strengbom *et al.*, 2001). In a meta-analysis of 82 N addition studies, Treseder (2008) showed that adding inorganic N decreased the soil microbial biomass by 15%. On the other hand, *P. abies* and *P. sylvestris* seedlings grown in a greenhouse with ARG as a N source has a higher root biomass, a larger proportion of fine roots and a higher proportion of mycorrhizal fungal mycelia on root tips than seedlings grown on AN (Gruffman *et al.*, 2012). The sporocarp production in our study system was severely decreased in AN treated plots but not in ARG plots, which indicates that ARG and AN affects the soil microbial system in very different ways. However, at this stage, without further studies, we cannot point to the mechanisms responsible for the observed difference.
5 Conclusions and recommendations for future research

In this thesis I have made three major findings, all having implications for our understanding of N addition effects on particularly tree growth in boreal forests;

1) that annual N additions simulating N deposition cause a positive linear growth response in *P. abies* and *P. sylvestris* (Paper I, II) supporting previous results from European modelling studies;

2) that commercial forest N fertilization according to normal practice can have residual effects on site fertility and tree growth that stretches across tree generations (Paper III); and

3) that there are both differences and similarities in boreal forest ecosystem responses to addition of organic (arginine) and inorganic (ammonium-nitrate) N (Paper IV).

Although my research has produced answers to important questions it has also resulted in a number of new questions for future research. In particular, I recommend that the tree growth response to annual N addition at low rates receives further attention by researchers as most of the boreal biome is subjected to very low N deposition rates and it will be important to understand how this may affect the climate change mitigation capacity of boreal forests.

Further studies are also needed to determine whether the increased growth in young forest stands, growing on sites that were fertilized during the prior stand before clear-cutting, will persist over the whole tree rotation period or in time level off. This is important as we need to know the full long-term effect of forest fertilization to make informed decisions regarding this forestry practice.
Another area that needs further investigation is long term studies of both above and below ground responses to organic N addition in the field. Adding organic N with the purpose to fertilize forest is today not an option due to the high production costs of amino acids like arginine. However, considering the interesting and positive effects of arginine observed on tree seedling growth in nurseries it appears important to increase our basic understanding of how arginine addition affects important soil microbial functions of forest ecosystems, in both the long and short-term.
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