PH.D. THESIS

The nitrogen cycle in soil

– Climate impact and methodological challenges in natural ecosystems

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ISBN: 978-91-7833-113-0 (Print)
ISBN: 978-91-7833-114-7 (PDF)
Available at: http://hdl.handle.net/2077/56724
Printed by BrandFactory, Gothenburg, Sweden

Distribution: Department of Earth Sciences, University of Gothenburg, Sweden

Cover illustration: Sofia Rybrand
Portrait photo: Ingrid Björsne

”Utan tvivel är man inte klok”

– Tage Danielsson

Abstract

Nitrogen (N) is a fundamental element for life, and limiting in many terrestrial ecosystems. In non-N-fertilized ecosystems, the N inputs can be low, and the nutrient availability for plants is determined by the internal cycling of N. The N availability might alter with different factors, such as climate change, forest management practices, and tree species. Soil N cycling is investigated using stable isotopes, where the activity in the soil can be monitored over time. The overall aim of this thesis is to increase the understanding of the N cycle in natural and semi-natural ecosystems and the environmental factors important for nutrient cycling.

The results show that all sites investigated in this thesis had higher NH_4^+ turnover than NO_3^- turnover. The mineralization rates were highest in the site with the lowest C:N ratio, and the lowest mineralization rates and the highest C:N ratio in the spruce forests, which demonstrate the importance of organic matter quality on gross N transformation rates. The N cycle responses to combined climate treatments were generally lower than responses to single climate treatments. For some processes, we observed opposing responses for eCO_2 as single and main treatment compared to the plots receiving the full treatment. This point to the importance of conducting multifactor climate change experiments, as many feedback controls are yet unknown. Gross nitrification was lowered with fertilization in a northern boreal forest, which is an interesting result in the light of the very low nitrous oxide (N_2O) emissions from the investigated site, despite heavy annual fertilization of 50–70 kg ha^{-1}. Moreover, the results from an experiment with soil of common origin and land history showed generally higher gross mineralization, immobilization and nitrification rates a beech stand compared to a spruce stand. The beech stand had also higher initial concentration of nitrate (NO_3^-) which indicates a more NO_3^- based N cycling. Finally, numerical modeling together with ^{15}N tracing is an improvement for simultaneously determining free amino acid (FAA) mineralization, peptide depolymerization and gross N mineralization rates, compared to analytical solutions.

This thesis confirms that N cycling in natural ecosystems is governed by the properties of the soil, vegetation and climate, but also that the experimental set-up strongly affects the outcome of the experiment. In turn, this affects the potential of doing reliable experiments, especially in ecosystems where the external inputs of N are very low. The thesis also highlights some methodological challenges that lie in the future of N cycling research.

Table of Contents

Preface

List of papers

The thesis is based on the following papers, which are referred to in the text by their Roman numerals:

I. **Björsne, A.-K.**, Rutting, T., and Ambus, P. (2014) Combined climate factors alleviate changes in gross soil nitrogen dynamics in heathlands, Biogeochemistry, 120, 191-201

A-K.B conducted the laboratory experiment, analyzed the data and led the writing of the manuscript. The paper is reprinted by permission from Springer Publications.

II. **Björsne, A-K.**, Weslien, P., Kasimir, Å. Klemedtsson, L., Rütting, T. Low N_2O emissions and gross nitrification in a boreal spruce forest soil, despite heavy nitrogen fertilization (Manuscript)

A-K.B conducted the laboratory experiment, analyzed the data, re-analyzed previous unpublished data and led the writing of the manuscript.

III. **Björsne, A-K.**, Rütting, T. Gundersen, P. Tree species influence on the gross N dynamics in soil (Manuscript)

A-K.B collected the samples in the field, conducted the laboratory experiment and led the writing of the manuscript

IV. Andresen L.C., **Björsne A-K.**, Bodé S, Klemedtsson L, Boeckx P, Rütting T (2016) Simultaneous quantification of depolymerization and mineralization rates by a novel ^{15}N tracing model. SOIL 2(3): 433-442

A-K.B collected the samples in the field, took part in the laboratory experiment and contributed to the writing of the manuscript. This paper is licensed under Creative Commons Attribution 3.0 License. 

Abbreviations

A	Ambient (no treatment)
AA	Amino Acid
AIC	Akaike Information Criterion
CI	Confidence Interval
C_{NH4}	Gross rate of ammonium consumption
D	Drought treatment
D_{NO3}	Gross rate of dissimilatory nitrate reduction to ammonium
DNRA	Dissimilatory Nitrate Reduction to Ammonium
DON	Dissolved Organic Nitrogen
D_{SON}	Gross rate of peptide depolymerization
eCO_2	Elevated carbon dioxide treatment
FAA	Free Amino Acid
FACE	Free Air Carbon dioxide Enrichment
I_{FAA}	Gross rate of free amino acid immobilization
IN	Inorganic Nitrogen
I_{NH4}	Gross rate of ammonium immobilization
I_{NO3}	Gross rate of nitrate immobilization
IRMS	Isotope Ratio Mass Spectrometry
M	Gross rate of mineralization
M_{FAA}	Gross rate of free amino acid mineralization
M_{SON}	Gross rate of organic N mineralization
N_{lab}	Labile organic nitrogen pool
NPP	Net Primary Production
N_r	Reactive nitrogen
N_{rec}	Recalcitrant organic nitrogen pool
O_{NH4}	Gross rate of ammonium oxidation
OM	Organic Matter
PNL	Progressive Nitrogen Limitation
SOM	Soil Organic Matter
SON	Soil Organic Nitrogen
SPINMAS	Sample Preparation unit for Inorganic Nitrogen Mass Spectrometer
T	Temperature treatment

Chemical formulas

C	Carbon
$CaSO_4$	Calcium sulphate
CH_2O	Formaldehyde
CO_2	Carbon dioxide
KCl	Potassium chloride
N	Nitrogen
N_2	Dinitrogen
N_2O	Nitrous oxide
NH_3	Ammonia
NH_4^+	Ammonium
NH_4NO_3	Ammonium nitrate
NO_x	Nitrogen oxides
NO_2^-	Nitrite
NO_3^-	Nitrate

Introduction

Nitrogen (N) is a fundamental element for life. It is an essential component of proteins, present in all enzymes and DNA of living organisms. In plants, N is important for photosynthesis as an essential part of chlorophyll (Brady & Weil 2000). N is also one of the most abundant elements on Earth, but the bulk of the Earth's total N is not readily available for living organisms, including plants (Galloway et al. 2003). The Earth's atmosphere consists of 78% nitrogen gas (or dinitrogen, N_2). The N_2 molecule is very inert; it has a strong triple bond that holds the atoms together. When the triple bond is broken the N atoms become reactive (N_r) and can form several different substances in the soil, water and atmosphere, all parts of the N cycle on Earth (Galloway et al. 2004). The cycling of N is a complex system of different processes driven by microorganisms and spontaneous chemical reactions (Schlesinger & Bernhardt 2013). Naturally, the bond of the N_2 atom can be broken in two ways; by lightning or by specialized bacteria that can fix atmospheric N_2, provided with energy from light or from symbioses with plants. In the modern age, anthropogenic N fixation has played an increasing role in distributing N_r in the ecosystems, translocating N from the atmosphere into the soil with the large-scale production and use of inorganic fertilizers (Fowler et al. 2013). This process requires excessive amounts of energy to facilitate the reaction between hydrogen gas (H_2) and N_2 to form ammonia (NH_3). In addition, the burning of fossil fuels and worldwide agricultural production of N fixing crops like soybean, alfalfa and clover have increased the input of N_r to the soil and atmosphere, making the N cycle unbalanced (Vitousek et al. 1997). Besides an important nutrient, N_r is now a pollutant as well, and contributes to a number of environmental and health problems, including increasing concentrations of tropospheric ozone (via NO_x production), acidification in streams and lakes, eutrophication, smog, atmospheric haze and nitrate pollution of drinking water (Aber et al. 1989; Fowler et al. 2013; Vitousek et al. 1997). Another concern is the emissions of nitrous oxide (N_2O), which is a very strong greenhouse gas, with a global warming potential 265 times higher than CO_2 and an estimated atmospheric lifetime of 130 years (Myhre et al. 2013; Prather et al. 2012). It is today the main stratospheric ozone depletion substance in the atmosphere, and is expected to remain so throughout the 21st century (Ravishankara et al. 2009). Galloway (2003) introduced the term "the nitrogen cascade" to describe the movement of a molecule of N_r through the environment, transforming into

different forms of N_r, with different environmental consequences at each step of the cycle. The effect of the N_r molecule will not cease until it is stored in a long-time reservoir or denitrified back to N_2.

In non-N-fertilized ecosystems, the N inputs consist of deposition and N fixation, rates that naturally can be low. Therefore the nutrient availability for plants is determined by the internal cycling of N (Boudot & Chome 1985; Davidson et al. 1992) as well as bedrock weathering (Houlton et al. 2018). This N availability might be modified with climate change, but the full repercussion of this is not yet understood. The main concern has been that N will become even more limiting in terrestrial ecosystems with increasing levels of carbon dioxide (CO_2) (Luo et al. 2004), since plants are expected to take up more N with increasing levels of CO_2 (Idso & Kimball 1993). A better understanding of the N cycle as a whole and provision of real data that can be used in regional and global climate models is necessary to model ecosystem responses to climate change in the future.

Due to its high environmental impact, agricultural land has mainly been the focus of many investigations of N cycling and N_2O emissions (Mosier et al. 1998; Rees et al. 2013). However, forests soils are also known to release N_2O to the atmosphere (Blais et al. 2005; Kesik et al. 2005), but there are many uncertainties regarding the mechanisms and regulators of the emissions. The area of intensively managed and planted forests is increasing in Europe (FAO 2010). Different forest management practices, such as tree species choice and fertilization, will influence the soil processes. Therefore it is important to know how N cycling in soils responds to different forest management. Forest fertilization can substantially increase the forest production, but is controversial because of the induced changes on the ecosystem level, as well as the risks of N leaching to groundwater and higher N_2O emissions to the atmosphere (Binkley et al. 1999; Bremner & Blackmer 1978).

Objectives

N cycling is a complex mix of processes that involves multiple levels of organism interactions as well as chemical reaction pathways (Robertson & Groffman 2007) and although the N cycle has been studied for decades, many aspects are still poorly understood. Due to technological development and more refined methods, the field of soil science has done some fast advances in recent years, many of them coupled to N cycling (Schimel & Bennett 2004). However, there are still improvements necessary regarding the methodology used to investigate the N cycle.

The overall aim of this thesis is to increase the understanding of the N cycle in natural ecosystems and the environmental factors important for nutrient cycling. The specific aims are:

I. To examine how the N cycle is influenced by a changing climate with higher CO_2 levels, warming and drought (**paper I**)
II. To investigate how the N cycle and N_2O emissions in forests are influenced by climate as well as different forest management practices, such as fertilization and tree species choice (**paper II and III**)
III. To further develop ^{15}N isotope methods, in order to estimate rates of amino acid mineralization with numerical modeling (**paper IV**).

The work, presented in four papers, was conducted by soil sampling in different natural ecosystems, combined with isotope techniques to trace specific N processes in the soil. Samples were taken from four field sites in Denmark and Sweden.

Background

The nitrogen cycle

In natural ecosystems N_r enters the soil via biological or chemical N fixation, wet and dry deposition from atmospheric NO_x and decomposition of plant and animal matter (Fig. 1). Small organic compounds can be taken up directly by plants (Näsholm et al. 1998) or they can be mineralized to NH_4^+. Mineralization is carried out by heterotrophic organisms in the soil that consume organic material as a way of obtaining energy and sustaining growth (Robertson & Groffman 2007). This is a process in several steps: first, larger proteins are depolymerized to smaller peptides, thereafter depolymerized again to free amino acids (FAA) and finally mineralized to inorganic N (Fig. 2). Depolymerization rate has been suggested to be the main limiting step for the terrestrial N cycle in natural ecosystems (Schimel & Bennett 2004), since transformation of N from proteins to NH_4^+ is a slower process than from amino acids to NH_4^+ (Jones & Kielland 2002; 2012)

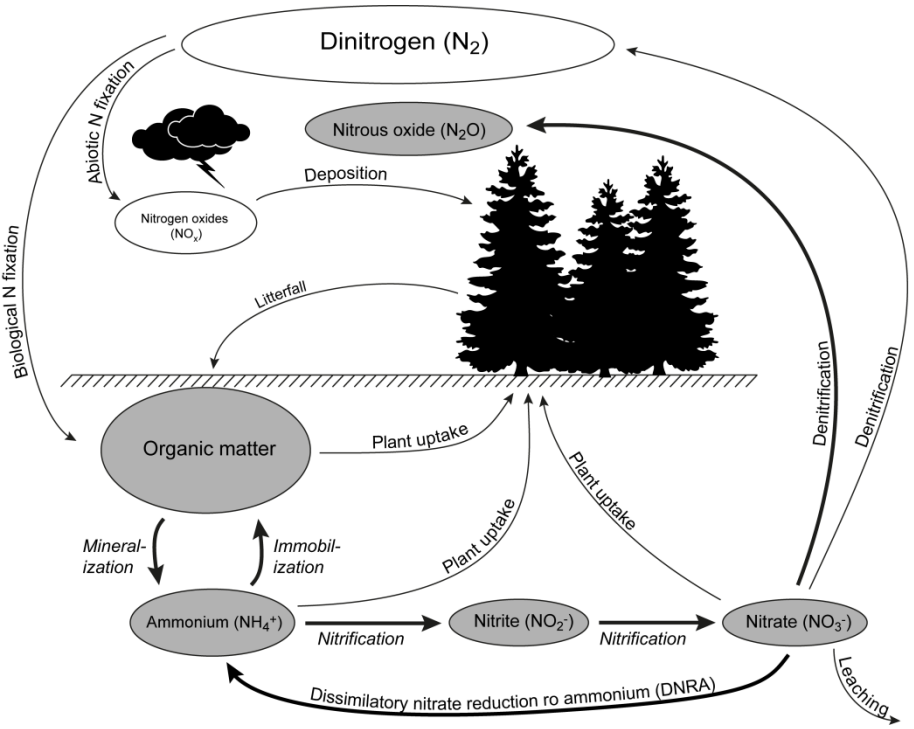


Figure 1. The terrestrial N cycle with the different soil N pools and its processes. The N pools indicated in grey and the processes shown with bold arrows are discussed in this thesis.

As a result of mineralization, more simple forms of N (organic or inorganic) that previously were bound up in larger organic molecules, become available to other organisms. Microbes need N for their growth, and to obtain it they scavenge the soil, incorporating N in their biomass, thereby making it unavailable to plants and other microbes. This process is called immobilization. The ratio between mineralization and immobilization is what determines the nutrient status of the soil. If mineralization is larger than immobilization there is a surplus of N for plants and other organisms, and if immobilization is larger than mineralization the soil is depleted in N (Robertson & Groffman 2007).

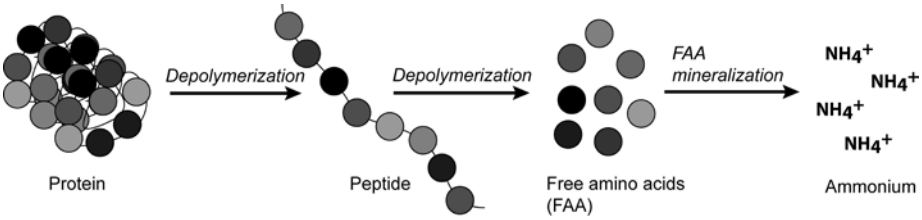


Figure 2. The steps of N mineralization from organic to inorganic N.

If the NH_4^+ molecule is not immobilized by plants or microbes, it can be oxidized in the process of nitrification. This process can be both autotrophic (where the carbon (C) source is CO_2 or carbonates) and heterotrophic (where the C source comes from the organic matter). Autotrophic nitrification is commonly found in most soils, but heterotrophic nitrification has also been shown to be important (De Boer & Kowalchuk 2001). Autotrophic nitrification is commonly a two-step process. The first part is the oxidation of NH_4^+ to NO_2^-, carried out by ammonia oxidizers like *Nitrosomonas* and *Nitrosospira*. The second part is oxidation of NO_2^- to NO_3^- and is carried out by a large group of microorganisms, like *Nitrobacter* and *Nitrospira* (Prosser 2007). Recent studies have also found *Nitrospira* species that can perform complete nitrification, oxidation of NH_3 to NO_3^- in a single organism (comammox, Daims et al. 2015; van Kessel et al. 2015). Heterotrophic nitrification is carried out by fungi and heterotrophic bacteria (Zhang et al. 2015). Nitrification is an acidifying process, and is a major source of soil acidification in many regions (Högberg et al. 2006b; Liu et al. 2010).

NO_3^- can also be used by plants but is, due to its negative charge, a moveable molecule that often is leached to groundwater, or denitrified back to the

atmosphere. The denitrification from NO_3^- to N_2 is an anoxic process and the closing step of the N cycle, where N is lost from the soil. Denitrification is controlled by several different factors, such as oxygen levels, C content, pH, and temperature (Knowles 1982; Robertson & Groffman 2007). If the denitrification is incomplete, the intermediate product N_2O is released instead. The emissions of N_2O have been shown to be related to N input, through deposition (Zechmeister-Boltenstern et al. 2002), soil water content (Butterbach-Bahl et al. 2013) and the mineral N content of soils in general, especially in environments where the dominant form is NO_3^- (Davidson et al. 2000).

A competing process with denitrification is dissimilatory nitrate reduction to ammonium (DNRA). This is an anaerobic process that reduces NO_3^- to NH_4^+, and therefore has an N preserving effect on the ecosystem. In environments with low soil NO_3^-, where the ratio of available C to the electron acceptor NO_3^- is high, DNRA is a more energetically effective pathway than denitrification (Rütting et al. 2011a; Tiedje et al. 1982)

The climate change impact on soil N

The increase in atmospheric CO_2 combined with changes in temperature and precipitation patterns are the most important factors that will affect terrestrial ecosystems in the future (Ciais et al. 2013). Conditions for net primary production (NPP) on Earth are changing with rising atmospheric concentrations of CO_2. Plants often respond quickly to higher concentrations of CO_2, increasing photosynthetic activity as well as water use efficiency (Myhre et al. 2013). This effect is known as CO_2 fertilization and is the reason why forests have been suggested to increase the storage of C in the future, mitigating a part of the ongoing climate change (Idso & Kimball 1993). One way to experimentally test the ecosystem response to elevated CO_2 (eCO_2) is to conduct Free-Air CO_2 Enrichment (FACE) experiments, where CO_2 is released over an experimental surface, elevating the atmospheric concentrations at the site. Most of these experiments have been carried out in grasslands or fields with low growing vegetation (Allard et al. 2004; Dijkstra et al. 2005; Hovenden et al. 2008; Jäger et al. 2003) and only few in forests (Miglietta et al. 2001; Norby et al. 2001; Schlesinger et al. 2006), due to the costs and complexity of such experiments.

Furthermore, many of these experiments have shown that the CO_2 fertilization effect is not persistent with time (Reich et al. 2006), and that

eCO_2 together with warming could result in a negative feedback with increasing respiration from trees (Thompson et al. 2004). These biosphere-atmosphere-interactions and feedbacks are the largest uncertainties in global climate models (Bonan 2008), especially due to the fact that CO_2 uptake is often limited by N (Oren et al. 2001; Rastetter et al. 1997; Reich et al. 2006; van Groenigen et al. 2006). The nutrient constraints in ecosystems were traditionally not accounted for in the dynamic vegetation models used to simulate biosphere interactions with climate, and thereby the size of the terrestrial C sink was overestimated in many models (Hungate et al. 2003). Vegetation and climate models that account for N cycling now exist (Goll et al. 2012; Smith et al. 2014; Zaehle & Friend 2010), but estimations of N cycling in models are very different depending on the model and settings used. There are also limitations in the understanding when it comes to general patterns of N cycling controls on a global scale (Zaehle & Dalmonech 2011). The hypothesis of progressive N limitation (PNL) suggests that N may become gradually more limiting for NPP under eCO_2, due to increased C and N storage in long-term ecosystem pools (Luo et al. 2004). The likelihood that an ecosystem will suffer from PNL is highest in ecosystems where external inputs are low (Hu et al. 2006). In ecosystems that have shown indications of PNL, the N mineralization has decreased and composition of soil organic matter (SOM) has changed (Gill et al. 2002; 2006; Schneider et al. 2004). However, there might be other feedback mechanisms preventing a development of PNL, e.g. increased decomposition with increased C input to the soil (priming effects) that in turn leads to increased mineralization (Rütting & Andresen 2015). There are many unknown feedbacks that regulate N cycling processes in soils (Dijkstra et al. 2010; Pendall et al. 2004). To be able to perform representative ecosystem modeling, more information is needed about what is governing the N cycle in different kinds of ecosystems.

N in natural ecosystems

Forests cover around 70% of the surface of Sweden, whereof the majority is productive forest (Nilsson & Cory 2017). Norway spruce (*Picea abies*) is the most common tree species in Sweden and can be found in the whole country. It is also one of the most important trees for forest production in Sweden, and covers 41% of the forested area (Nilsson & Cory 2017). Even if the majority of the forest in Sweden today is managed, it can be considered more natural than many agricultural systems, in the sense that trees are dependent on

internal nutrient cycling as well as low external inputs of N from deposition and N fixation (Hart & Firestone 1991). Tree species is an important factor influencing nutrient cycling and soil properties in forests (Legout et al. 2016; Staelens et al. 2012), including SOM (Binkley & Giardina 1998), pH (Hansson et al. 2011; Mareschal et al. 2010) and microbial biomass (Buée et al. 2011; Malchair & Carnol 2009; Zhong & Makeschin 2006a). Also physical properties are affected, like porosity and structure (Augusto et al. 2002). Trees affect the soil with both roots and canopies, for example by allocating more N belowground (Hobbie 1992), through symbiotic interactions with N fixing bacteria (Vitousek et al. 1987) or through different susceptibility to N deposition (Lovett & Lindberg 1986). However, the most important control is the chemical composition of the leaf litter (Lovett et al. 2004). Litter from broadleaved and coniferous species differs in structure and composition. The leaves of broadleaved species are more easily decomposed (López et al. 2001), while coniferous soil has a higher SOM and litter content, due to slower decomposition (Vesterdal et al. 2013) and higher concentrations of humic acids and polyphenols (Northup et al. 1998). The presence of soil fauna, especially earthworms, has a large impact on the soil structure and has been seen to be more abundant in soils under deciduous species (De Schrijver et al. 2012; Reich et al. 2005). Norway spruce (*Picea abies*) is the most common tree species in Sweden and can be found in the whole country. It is also one of the most important trees for forest production in Sweden, and covers 41% of the forested area (Nilsson & Cory 2017).

Nitrogen (N) is the main limiting nutrient for growth in many boreal forests (Tamm 1991; Vitousek & Howarth 1991) and fertilization is therefore an effective way to increase the NPP in slow growing ecosystems (Axelsson & Axelsson 1986; Linder 1987), thereby also increasing C storage. In 2011 the Swedish mining company LKAB suggested to fertilize forests in Northern Sweden with N as a way of compensating for their large CO_2 emissions (Unga 2012). In the North of Sweden, fertilization can increase stem wood production by more than 350% (Bergh et al. 2005), which in theory may seem like an appealing way to mitigate climate change. However, in reality, intensive fertilization is accompanied by hidden costs, such as loss of biodiversity (Olsson & Kellner 2006; Strengbom et al. 2011) and CO_2 emissions during the highly energy-demanding production and application of inorganic fertilizer (Schlesinger 2000). The risk of N losses to water and air has also been discussed as a potential problem (Binkley et al. 1999; Bremner

& Blackmer 1978). The cascade effect of any released N_r atom is also worth considering when using commercial fertilizer (Galloway et al. 2003; Vitousek et al. 1997); although the environmental risks might be low in the short time-span, eventually there will be losses of N_r to water and air, when forestry products are later consumed and burned as waste. Natural soils are to a large extent sources of N_2O on global scale (37% of total emissions, Ciais et al. 2013) and forests contribute a significant part of these emissions (Ambus & Christensen 1995; Bowden et al. 1990; Kesik et al. 2005). Soil N_2O emissions are mainly derived from nitrification and denitrification (Ambus et al. 2006), but can also be released from other processes, such as heterotrophic nitrification (Zhang et al. 2015), co-denitrification (Spott & Stange 2011) and DNRA (Rütting et al. 2011a; van den Berg et al. 2016). Leaching of NO_3^- is often related to anthropogenic inputs, either N deposition or fertilization (Sponseller et al. 2014). In non-fertilized boreal forests with low N deposition, the majority of the leaching to streams and waters is in the form of dissolved organic nitrogen (DON, Hedin et al. 1995; Stepanauskas et al. 2000).

Measuring transformation rates

The N cycle has been investigated in many different ecosystems, where rates of specific processes are studied by means of stable isotopes (Di et al. 2000). Isotopes are variants of elements that have different numbers of neutrons in the nuclei. There are two stable N isotopes; ^{14}N with a natural abundance of 99.6% and ^{15}N, with a natural abundance of 0.37%. The relative abundance of ^{15}N and ^{14}N varies in the environment as a result of physical, chemical and biological processes (Högberg 1997). In chemical reactions, the heavy isotope has a tendency to remain in the substrate, while the lighter ends up in the product (Peterson & Fry 1987; Tiwari et al. 2015) and this partitioning of the isotopes is called fractionation. Natural ^{15}N abundance is expressed in delta (δ) units, that denote the deviation from the ratio of ^{15}N to ^{14}N of atmospheric N_2, in parts per thousand (‰, Peterson & Fry 1987). Because the isotopes have different weight, it is possible to separate them with mass spectrometry.

The heavy ^{15}N isotope is naturally scarce in the environment and therefore it is commonly used as a tracer, tracking activity in the soil and flow of N through the ecosystem. The concentrations of different N species in the soil are measureable, while the process rates have to be calculated. The difference

between the inflow and the outflow of an N pool is the net rate of that process, whereas the gross rates refer to the real, unidirectional flux between two pools. In a soil with a constant production and consumption of certain N species, the concentrations will remain constant over time, although the turnover rate of the N pool might be high. Net and gross transformations are not always correlated (Hart et al. 1994), and therefore gross rates provide a more robust measurement of what is going on in the soil.

Gross transformation rates have been estimated since the 1950's when Kirkham and Bartholomew (1954) published a well-cited article about the isotope pool dilution method. In this method, ^{15}N is added to a soil N pool as a form of tracer, and the dilution of the ^{15}N in the pool over a shorter time-span, usually 0–24 hours, is monitored. Total gross production and consumption of the pool is calculated from the changes in ^{15}N label, with help of a set of analytically solved differential equations. Three main assumptions are associated with the isotope pool dilution method:

(I) Isotopic fractionation is negligible during microbial transformations of soil N, due to the fact that the heavy isotope is added in large abundance compared to the light one, i.e. the microbes do not discriminate between heavy and light isotopes.
(II) no remineralization of the added ^{15}N occurs, and
(III) N transformations rates are constant during the incubation period.

Because of the second and third assumptions this method is only applicable to short-term incubations. The rates will not be constant for longer incubation times and the microbes should not have time to mineralize, immobilize, and then re-mineralize the same N again during the experiment. With this method, only gross production and consumption of one specific pool can be calculated, not rates of specific processes (Rütting et al. 2011b). Analytical solutions to calculate rates of other processes in the soil have been developed as well, such as DNRA (Silver et al. 2001) and free amino acid (FAA) mineralization (Wanek et al. 2010). The pool dilution method by Kirkham and Bartholomew (1954) is still widely used, but more advanced numerical models have also been developed for ^{15}N tracing. Using these methods, the possibility has emerged of analyzing several specific processes in the N cycle simultaneously (Müller et al. 2007; Myrold & Tiedje 1986).

By using ^{15}N labels of different N-species, the flow of ^{15}N can be followed through different compartments of the ecosystem. It is also possible to investigate other rate kinetics than zero-order and to consider longer incubation time spans to allow re-mineralization.

One problem with the commonly used ^{15}N tracing techniques for calculating gross transformation rates is that they require the addition of substrate to the soil, which stimulates consumption processes of mineral N (Di et al. 2000; Schimel 1996). This stimulated consumption may result in a higher ^{15}N label disappearance which gives the impression of a dilution of the mineral ^{15}N pool, and therefore is calculated as higher mineralization, thus overestimating the actual rate (Barraclough 1991).

To induce as little change as possible to the system when doing ^{15}N tracing experiments, it is important to add low but highly enriched amounts of N, to get a clear ^{15}N signal. Recommendations of a maximum addition of 5 – 10% of the initial soil mineral N concentration have been made (Di et al. 2000). If ^{15}N labeled amino acids are to be used, the recommendations are to add a maximum of 25% of the background concentration (Wanek et al. 2010). When working in soils with very low mineral N content, the recommended 5–10% addition of N cannot always be followed, since the initial concentrations can be very small, or even undetectable. To add more N to ensure detectable concentrations for the mass spectrometer is one solution (Davidson et al. 1991), but this addition could then be many times higher than the natural amount, especially with regards to NO_3^- which is low in many natural forest soils.

There is an additional concern regarding the recovery of labeled ^{15}N. To be able to calculate accurate gross transformation rates, a sufficient amount of the added ^{15}N label must be extracted from the soil. If ^{15}N recovery is found to be low it could be a result of direct microbial uptake (Jones et al. 2013), abiotic fixation to clay particles (Nieder et al. 2011) or organic matter (Whitehead et al. 1990), or losses to air (Bremner & Blackmer 1978).

Study area

The field sampling for ^{15}N tracing experiments in this thesis was carried out at four study sites, two in Denmark (Vallø and Brandbjerg) and two in Sweden (Skogaryd and Flakaliden, see Fig. 3 and Table 1, both sites are part of the SITES network, see http://www.fieldsites.se). Three of them, Vallø, Skogaryd and Flakaliden are forested with Norway spruce and located along a climatic gradient with annual average temperatures ranging between 2-8°C. Brandbjerg is a semi-natural heathland and was chosen for the ongoing FACE at the site. Soil sampling took place on several occasions during the years 2010–2016. The soil was sampled with an auger and five samples were taken at each plot. In Brandbjerg, Vallø and Skogaryd the mineral horizon 0–10 cm was sampled. In Flakaliden the organic layer was sampled (except the litter layer), which was 3–6 cm.

Figure 3. Map of Sweden and Denmark and the locations of the study sites

Table 1. Specifics of the sites investigated in this thesis.

Site	Country	Coordinates	Mean annual temp-erature (°C)	Mean annual precip-itation (mm)	Texture	pH (KCl)	Horizon sampled	Vege-tation type	Sampling date	Details described in paper
Flakaliden	Sweden	64º07'N 19º27'E	2.0	580	Sandy loam	3	Organic 0 – 6 cm	Spruce forest	September 2016	II
Skogaryd	Sweden	58°23'N 12°09'E	6.2	709	Sandy loam /Podzol	4	Mineral 0 – 10 cm	Spruce forest	April and May 2014	IV
Brandbjerg	Denmark	55°53'N 11°58'E	8.0	613	Sandy	4 – 5	Mineral 0 – 10 cm	Heathland	November 2010	I
Vallø	Denmark	55º25'N 12º03'E	7.7	625	Loamy	3 (spruce) 4 (beech)	Mineral 0 – 10 cm	Spruce and beech forest	September 2014	III

Flakaliden (paper II)

Flakaliden is a spruce forest, situated in Vindeln municipality, Northeastern Sweden. The altitude is 310–320 m above sea level. The climate is boreal, with long days and moderate temperatures in the short summers, while the long winters are characterized by short days and cold temperatures. More than one-third of the annual precipitation falls as snow (Bergh et al. 1999). The soil is a Podzol with sandy texture with an organic layer between 3 and 6 cm thick. In 1963, the site was planted with seedlings of Norway spruce of local descent. In 1986, an experiment with different types of fertilization treatments was started (Linder 1995). The fertilized plots were annually given a solid fertilized mix (NH_4NO_3), with about 70 kg N ha^{-1} (see Fig. 4). The field layer in the fertilized plots is very sparse (see Fig. 5), while in control plots it is dominated by dwarf-shrubs (*Vaccinium myrtillus, Vaccinium vitis-idaea*, Strengbom et al. 2011).

Skogaryd (paper IV)

Skogaryd is a site situated in the Southwest of Sweden where two different soil types were sampled. The first was an Umbrisol with a sandy loam texture that was planted with Norway spruce in the 1950s. The vegetation was classified as a spruce forest of the low-herb type based on the classification system by Påhlsson (1998), with sparse ground vegetation dominated by mosses (*Mnium hornum, Polytrichum formosum*, and *Pleurozium schreberi*, see Fig. 6). The second soil type was a Podzol, where the vegetation has been classified as a spruce forest of the bilberry type (Påhlsson 1998). The tree stand is 55–130 years old and 23–30 m in height. The ground vegetation is dominated by dwarf-shrubs (*Vaccinium myrtillus*) and mosses (*Hylocomium splendens, Pleurozium schreberi, Polytrichum sp., Dicranum sp.*).

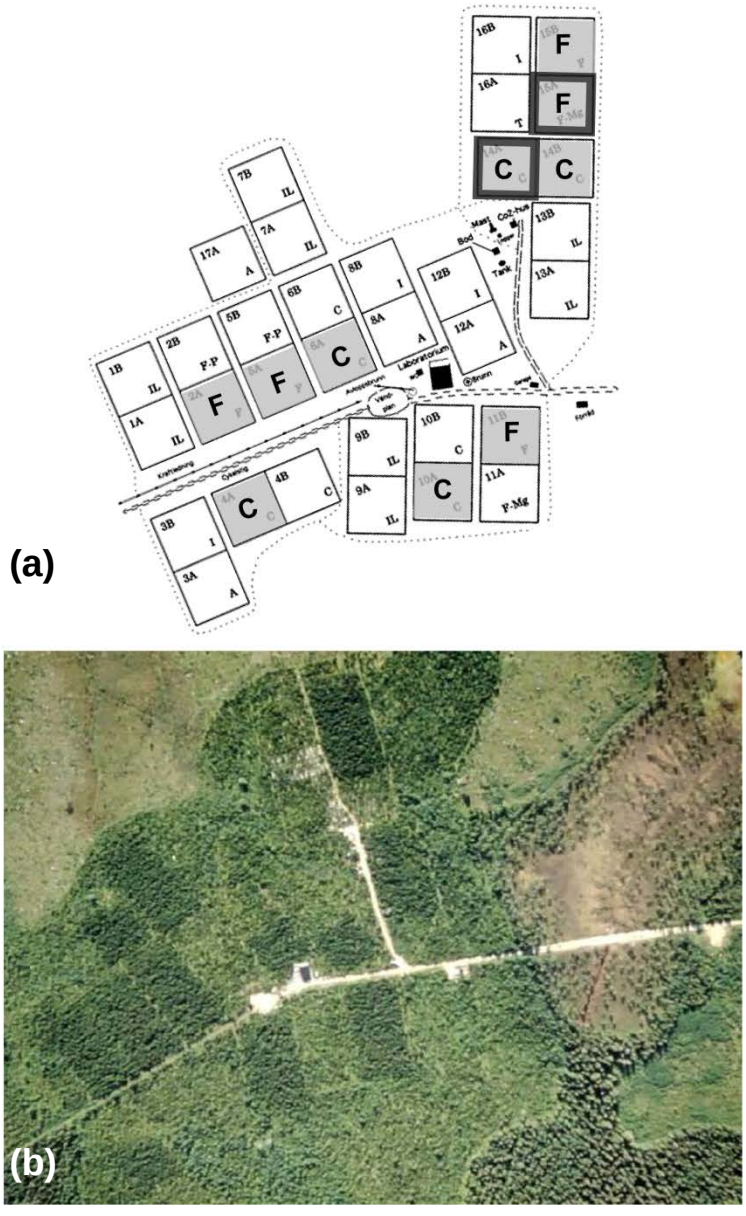


Figure 4. (a) Plot overview of the fertilization experiment in Flakaliden. Grey marked plots are the plots sampled in this thesis. F = fertilized with a solid fertilizer mix, C = untreated control. The framed plots indicate where the field chamber measurements of N_2O were made (b) Aerial photo of the Flakaliden experiment. The fertilized plots are seen as darker squares. Photo: Sune Linder

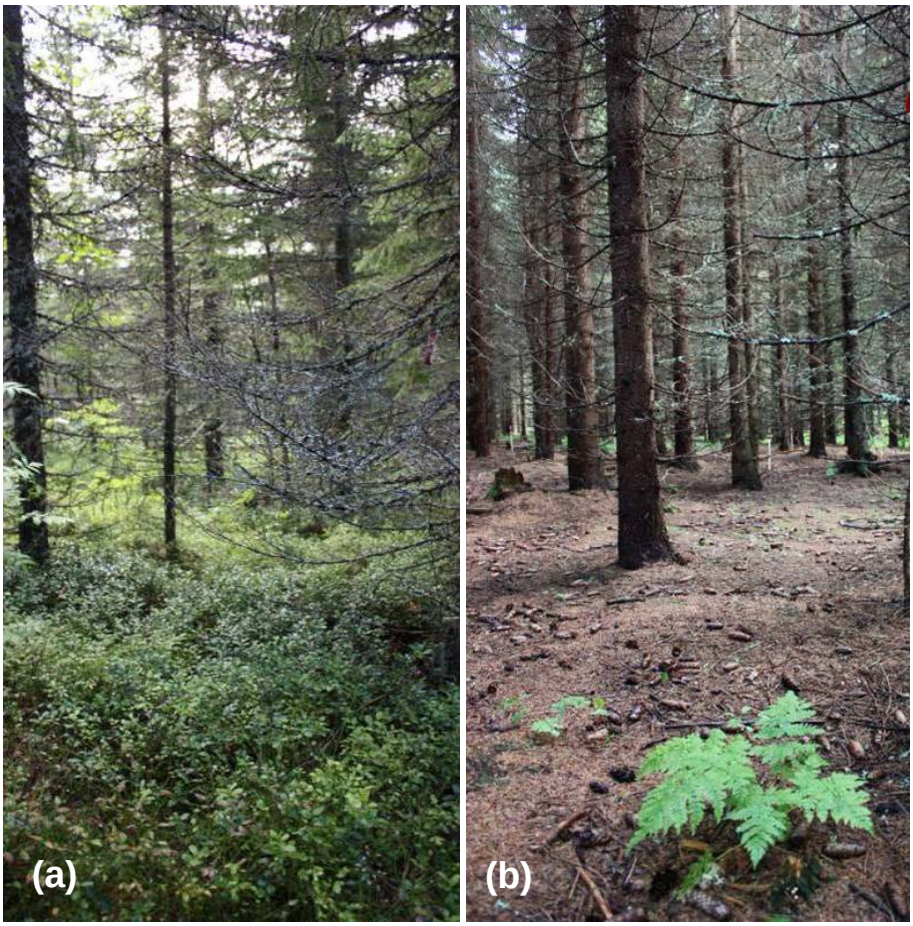


Figure 5. Differences in understory vegetation between a control plot (a) and a fertilized plot (b) in Flakaliden. Photos: Joachim Strengbom

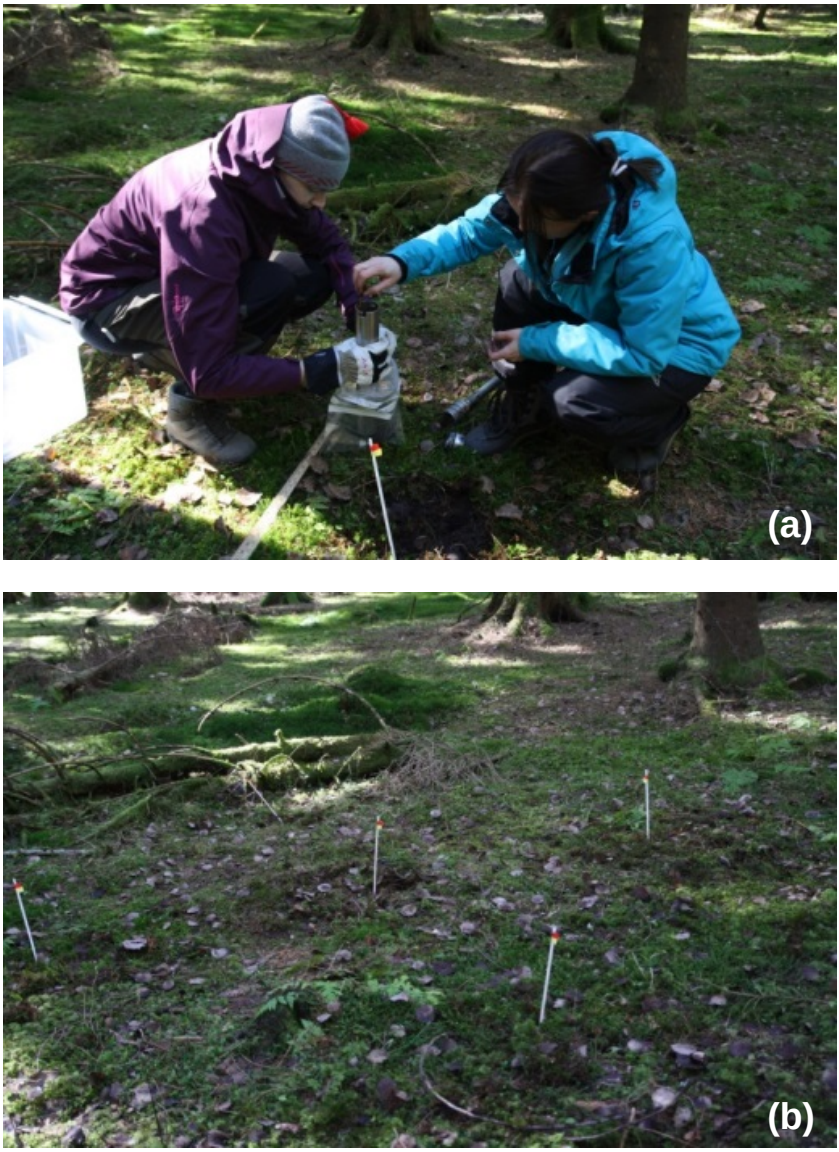


Figure 6. Skogaryd Umbrisol soil (a) soil sampling (b) the ground vegetation is dominated by mosses. Photos: Freia Kutchinsky

Vallø (paper III)

The common garden experiment of Vallø is situated in Zealand in Denmark where different tree species (e.g. *Picea abies*, *Fagus sylvatica*, *Quercus robur*, *Acer pseudoplatanus*, *Abies grandis*, *Tilia cordata*) have been planted in plots of 0.25 ha. The area was a beech (*Fagus sylvatica*) forest until year 1973 when the experiment was established. The plots have been thinned every fourth year since 1987. The soil classification is Hapludalf (Vesterdal et al. 2008). The ground vegetation in the sampled plots with Norway spruce and beech was very sparse due to the dense canopy cover (see Fig. 9).

Brandbjerg (paper I)

The site is located in Zealand, about 50 km northwest of Copenhagen in Denmark and is a temperate heathland, dominated by heather (*Calluna vulgaris*) and wavy hair-grass (*Deschampsia flexuosa*, see Fig. 8b). The soil is well-drained, sandy and nutrient poor. The climate FACE experiment was initiated in 2005 with the aim of simulating the expected major environmental changes at the site in the year 2075. It consists of twelve octagons, each 7 m in diameter, distributed pairwise in six blocks (see Fig. 7). Within each block, one octagon is exposed to ambient CO_2 concentration and one to elevated CO_2 (eCO_2 = 510 ppm). Each octagon was also divided into four subplots that received additional treatments; an untreated control (A), elevated air temperature (T) of 1–2 ºC in the form of passive night-time warming (see Fig. 8a), increased summer drought (D) by exclusion of rainwater (5–8 % of annual precipitation received) and one with combined warming and increased drought. In total there were eight treatments within each block: A, T, D, TD, eCO_2, $TeCO_2$, $DeCO_2$ and $TDeCO_2$. The complete experimental set-up is described in detail by Mikkelsen et al. (2008).

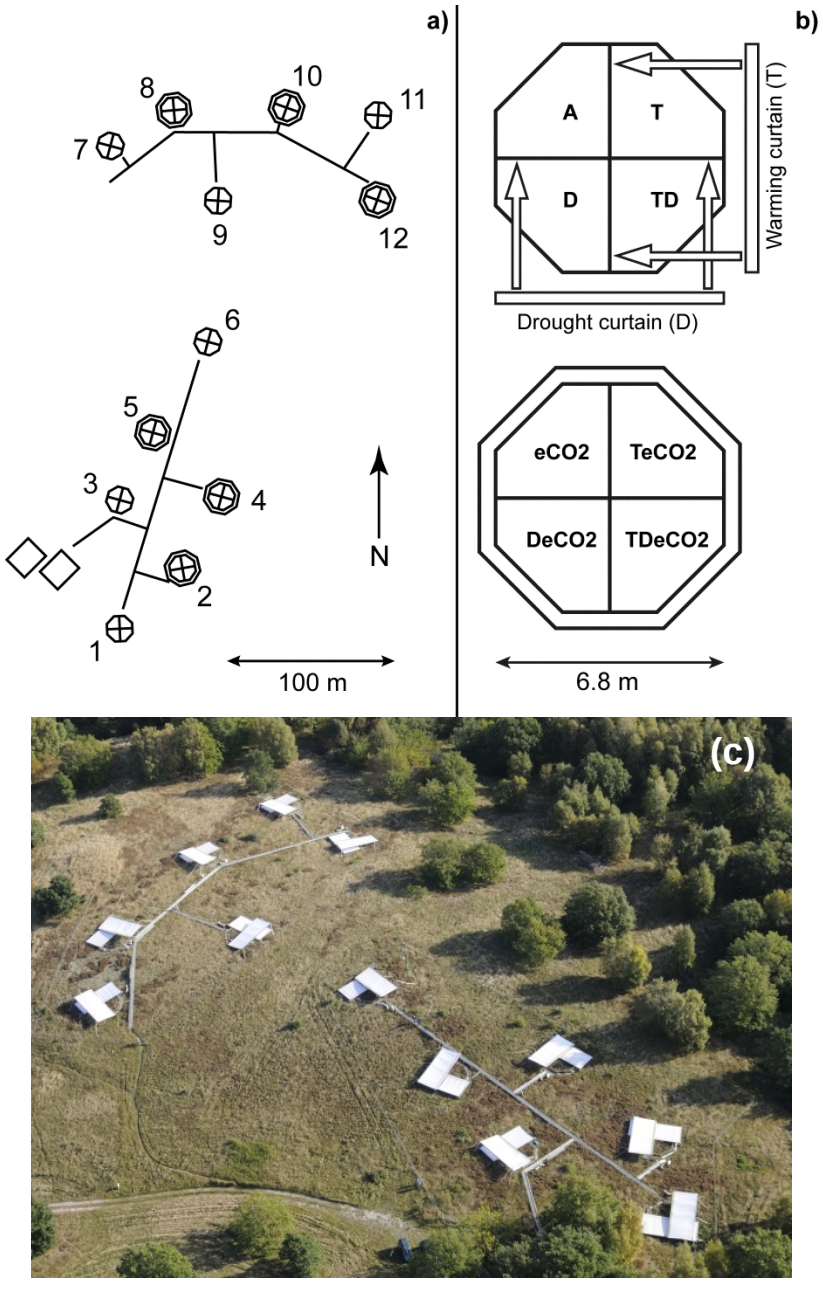


Figure 7. (a) Schematic overview of the experiment area (b), detail of the octagons and (c) aerial photo of Brandbjerg, with both rain and night curtains out. Photo: Kim Pilegaard

Figure 8. (a) The result of the night warming curtains. Photo: Poul T. Sørensen (b) the dominant vegetation, consisting of heather (*Calluna vulgaris*) and wavy hair grass (*Deschampsia flexuosa*). Photo: Tobias Rütting

Figure 9. (a) Soil sampling from beech plot in Vallø. (b) Ground litter at spruce plot in Vallø. Photos: Anna-Karin Björsne

Methods

Isotope tracing studies

Tracing experiments with ^{15}N were performed with soil from all the sampled sites to determine gross rates of mineralization, immobilization, nitrification, and DNRA. Figure 10 shows a flowchart of the experiments conducted in this thesis. After the field sampling the soil was taken into the laboratory where roots, stones and other larger distinguishable materials were hand-picked from the samples. Soil properties like gravimetric soil water content, SOM content, total C and N content, pH, and mineral concentrations of NH_4^+ and NO_3 were determined. The soil was weighed and put into glass bottles and pre-incubated for one week at room temperature before start of the incubation experiment with ^{15}N. The soil was then labelled with $^{15}NH_4^+$ and $^{15}NO_3^-$ and extracted with KCl at different points in time during the incubation period. After extractions, the samples were filtered and prepared for analysis of ^{15}N contents.

In Skogaryd (**paper IV**), an additional tracing experiment with ^{15}N labeled amino acids was performed to estimate gross rates of FAA mineralization. The soil was labeled with a ^{15}N amino acid mixture (20 AA, ^{15}N 96-99%) and extracted with calcium sulphate ($CaSO_4$) and formaldehyde (CH_2O) at 13 min, 30 min, 1 h, 2 h and 6 h after labelling.

In the Flakaliden study (**paper II**), N_2O samples were taken from the headspace of the incubation bottles at each time step, except time zero. The bottles were sealed with an airtight lid with a septum for 1 h, and samples were then taken with a syringe and transferred to pre-evacuated gas vials (Labco Exetainer, 12 mL).

Methods for ^{15}N determination

Three different methods have been used to analyze the mineral ^{15}N content of the samples, which was the result of a collaboration with different laboratories. In the Brandbjerg study (**paper I**) the samples were prepared according to the micro diffusion technique described by Sørensen and Jensen (1991) and were analyzed by elemental flash combustion analysis in combination with IRMS. In the Vallø (**paper III**) and Skogaryd (**paper IV**) studies the ^{15}N in the samples was converted to N_2O according to Stevens and Laughlin (1994) and measured on a trace gas unit interfaced with an IRMS (ANCA-TGII 20-20 IRMS, SerCon, UK).

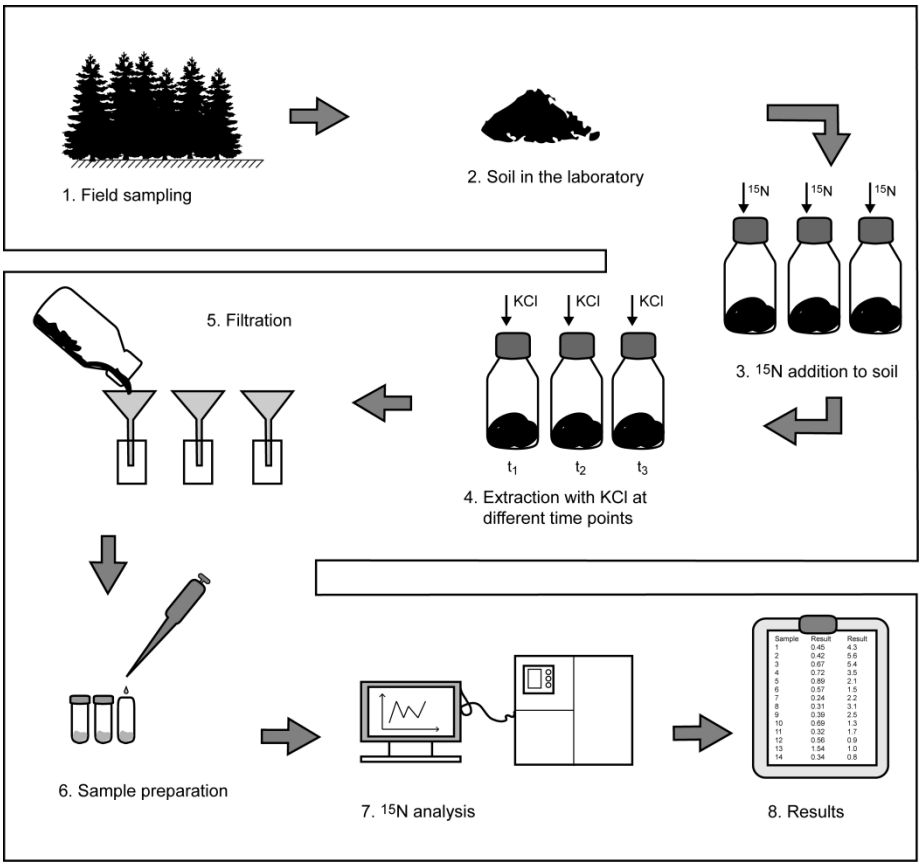


Figure 10. Flowchart of ^{15}N experiments conducted in this thesis. Soil samples were taken in the field (1), brought to the laboratory for pre-incubation (2) before ^{15}N was added to the bottles (3). The samples were then extracted with KCl at different time points (4), filtered (5) and prepared for isotope ratio mass spectrometry (IRMS) (6). The samples were analyzed with IRMS (7) and results were received (8)

The $CaSO_4$ extracts for ^{15}N-AA analysis in **paper IV** were purified using cation-exchange cartridges (Hušek 1991; Wanek et al. 2010) and after purification and derivatization the individual FAAs in the samples were measured by gas chromatography-mass spectrometry (GC-MS, Trace GC-DSQ, Thermo Fisher). All sample preparation for **paper IV** was carried out by the ISOFYS laboratory in Ghent, Belgium. In the Flakaliden (**paper II**) study the ^{15}N content of the samples was analyzed automatically directly in the soil extracts with an automated sample preparation unit for inorganic nitrogen (SPIN), coupled to a mass spectrometer (MAS). The SPINMAS samples were analyzed at the University of Gothenburg and the technique is

described by Stange et al. (2007). N_2O gas samples were sent to UC Davis Stable Isotope Facility for analysis using IRMS.

Calculation of recovery

The ^{15}N recovery is defined as the ratio between the extracted ^{15}N and the added ^{15}N, i.e. how much ^{15}N can be extracted with the method.

$$^{15}N\ recovery = \frac{n_{mea} \times x_{mea}}{n_{add} \times x_{add}} \times 100$$

n_{mea} = amount of N measured in the extracts (μmol g^{-1} dry soil)
n_{add} = amount of N added to the soil (μmol g^{-1} dry soil)
x_{mea} = ^{15}N / ^{14}N excess ratio, measured samples
x_{add} = ^{15}N / ^{14}N excess ratio, added samples

^{15}N tracing with numerical modelling

Quantifications of specific gross rates of the various N transformations were done with the numerical ^{15}N tracing model *Ntrace* (Müller et al. 2007; Rütting & Müller 2007). In the model, a Markov Chain Monte Carlo sampling scheme is performed, based on 10,000 iterations. The aim of the model is to find the best fit between the modelled and measured values of concentrations and ^{15}N enrichments of mineral N. From the original model, presented in Figure 11, several model set-ups were used. The model was configured according to the best fit of the data sets respectively, with a different number of processes and kinetic settings included for each individual soil investigated. The model gives a value of the Akaike Information Criterion (AIC) for each model run, which helps to determine the best model out of a range of models from the same data set. The AIC takes into account the number of parameters in the model, as well as the squared differences between the modeled and the measured data. If the AIC is smaller, the model has a better fit to the measured data (Motulsky & Christopoulos 2003; Staelens et al. 2012). In **paper IV,** we developed a model for estimating gross rates of mineralization and peptide depolymerization (Fig. 12). See the papers for details of the model set-up in each individual study. The initial pool sizes and ^{15}N enrichments for NH_4^+ and NO_3^- were estimated by extrapolating the measurements from the first two soil extractions back to the time point zero. As a proxy for the organic N pool N_{rec} total soil N was used. In **paper I**, DON data from Larsen et al. (2011) was used as a proxy for N_{lab}. The outcome of the model is a

probability density function for each model parameter. The average value of the probability density function is the kinetic factor (*k*) and standard errors of means are calculated based on autocorrelation, described by Harmon and Challenor (1997). For substrate dependent transformations following first order kinetics, average rates were determined by multiplying the modelled parameters with the average concentration of the substrate pool over the whole incubation period.

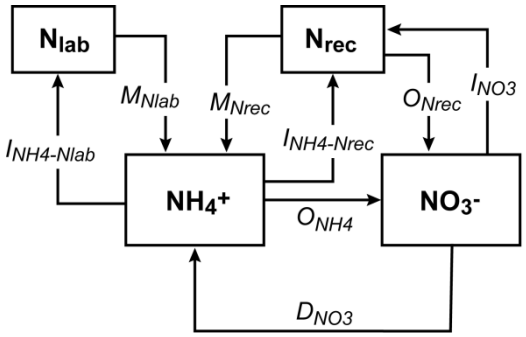


Figure 11. Conceptual N tracing model used to quantify gross N transformations. Different variations of this model set-up have been used in all the experiments in this thesis. The model considers pools for labile organic N (N_{lab}), recalcitrant organic N (N_{rec}), ammonium (NH_4^+) and nitrate (NO_3^-). The gross transformation processes considered are mineralization of labile organic N (M_{Nlab}), mineralization of recalcitrant organic N (M_{Nrec}), immobilization of NH_4^+ to Nlab ($I_{NH4\text{-}Nlab}$), immobilization of NH_4^+ to N_{rec} ($I_{NH4\text{-}Nrec}$), oxidation of NH_4^+ to NO_3^- (O_{NH4}), oxidation of N_{rec} to NO_3^- (O_{Nrec}), immobilization of NO_3^- to N_{rec} (I_{NO3}) and dissimilatory NO_3^- reduction to NH_4^+ (D_{NO3})

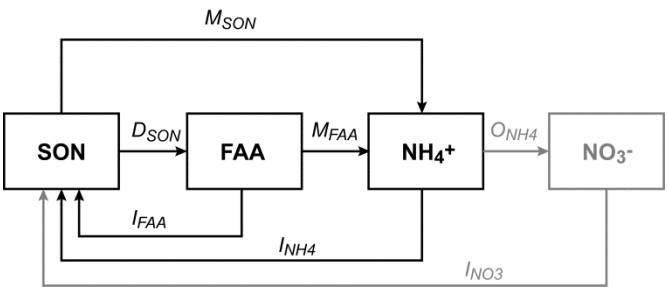


Figure 12. Conceptual N tracing model used to quantify mineralization and peptide depolymerization in **paper IV**. The model considers pools for soil organic nitrogen (SON), free amino acids (FAA), ammonium (NH_4^+) and nitrate (NO_3^-). Gross transformation rates considered are peptide depolymerization (D_{SON}), FAA mineralization (M_{FAA}), FAA immobilization (I_{FAA}), mineralization of organic nitrogen (M_{SON}), immobilization of NH_4^+ (I_{NH4}), NH_4^+ oxidation (O_{NH4}) and NO_3^- immobilization (I_{NO3}). Grey pools and fluxes could not be investigated in **paper IV** due to too low a NO_3^- content.

In **paper III**, the recovery of the ^{15}N label was low and differed between the two investigated tree stands. In order to compare between them, we wanted to correct for this. There is no standard way of correcting for low ^{15}N recovery in calculations of gross N transformation rates, however, we have identified two different methods in the literature. Davidson et al. (1991) calculated initial pool sizes by summarizing the measured initial concentrations and the added ^{15}N tracer, from which they estimated gross transformation rates. However, when they found that the recovery was low they corrected the initial pool size with the fraction of the extractable ^{15}N and calculated new, corrected, gross rates. Contrastingly, Münchmeyer (2001) corrected the rates in the other direction, by measuring the extractable mineral N and the ^{15}N, calculating the recovery and then correcting the initial pool, by assuming 100% recovery in the calculations.

The difference between the two methods is the assumptions associated with the causes of low recovery. According to Münchmeyer, the non-extractable ^{15}N is assumed to be adsorbed to soil particles, and thereby possibly available for soil microbes if released to the soil solution again. The mineralization during the incubation period is assumed to dilute the total N pool, not only the extractable one, and that is why the total N pool should be in the calculations instead of only the extractable N pool. Davidson et al. (1991), on the other hand argue that the non-extractable ^{15}N is not available for microbes (i.e. it could have been immobilized), and therefore the initial pool size must be corrected with the actual ^{15}N recovery. We cannot determine which method is most accurate for our study, since we do not know the processes behind the low recovery in this particular soil. The fast disappearance of the ^{15}N label could also be a combination of abiotic fixation and immobilization. We therefore present the results from **paper III** as two *Ntrace* model set-ups, estimating gross rates according to both Münchmeyer (2001) and Davidson et al. (1991).

The measured initial ^{15}N abundance was estimated from the measured data by extrapolating the enrichments from the first two soil extractions back to the time point zero. The calculated initial ^{15}N abundance was estimated by multiplying the fraction of ^{15}N in the label solution (0.99) with the concentration of the label solution, divided by the initial pool size (measured data). For beech data, both $^{15}NH_4^+$ and $^{15}NO_3^-$ abundances were corrected this

way, while for spruce data, the $^{15}NO_3^-$ recovery was 100% and therefore only the $^{15}NH_4^+$ was corrected.

In **paper II**, previous unpublished field N_2O flux data is presented from measurements in Flakaliden during two years, in 2009–2011. Three different methods were used: manual chambers in June–December 2009, automatic chambers June–December 2010 and with snow probes during the winters, March–April 2009, March–May 2010 and January–April 2011. The data was re-analyzed and used as additional data to interpret the soil N processes at the site.

Statistics

In **paper I,** the statistical analysis of soil parameters was carried out in R version 2.2.1. All parameters were characterized by homogenous variances (Levene's Test of Equality of Variance). Effects of climate treatments on soil parameters were tested using a Fit Mixed-Effect model (lmer) in the package lme4 (Bates et al. 2015). The model structure included fixed effects which corresponded to the climatic treatments (T, D, eCO2) and random effects, where the experimental split plot design could be described appropriately. In **paper II** and **III** and in the thesis summary, soil properties data were analyzed with two-tailed t-tests and regression analysis in SPSS (IBM SPSS Statistics 25). The results from the ^{15}N tracing model are not appropriate for statistical tests, due to the high number of iterations in the model. We therefore present data in figures as means and 85 % confidence intervals (CI). The 85 % CI can be used analogous to statistical tests with a 5 % significance level (Payton et al. 2000; Rütting et al. 2011b). Rates of ^{15}N recovery and correlations between soil variables in this synthesis were tested using linear regression in SPSS (IBM SPSS Statistics 25).

Results

Soil properties and ^{15}N recovery

All investigated soils were acidic, the forested soils (**papers II**, **III** and **IV**) had a pH between 3.0 and 3.7 (Table 2), and the heathland site (**paper I**) had a pH between 3.9 and 4.1. pH was negatively correlated to soil C content (fig. 14a). Except for the fertilized soil in **paper II**, the mineral N content increased from north to south, both for NH_4^+ and NO_3^- following the temperature gradient. The C:N ratio also followed the gradient, with the highest values in the north and lower in the south (Table 2). Soil NH_4^+ content (expressed per dry weight) was correlated with both soil C (Fig. 13a) and N content (Fig. 13b), in the forested sites. Soil NO_3^- content showed no correlation with neither soil C (Fig. 13c) nor N content (Fig 13d).

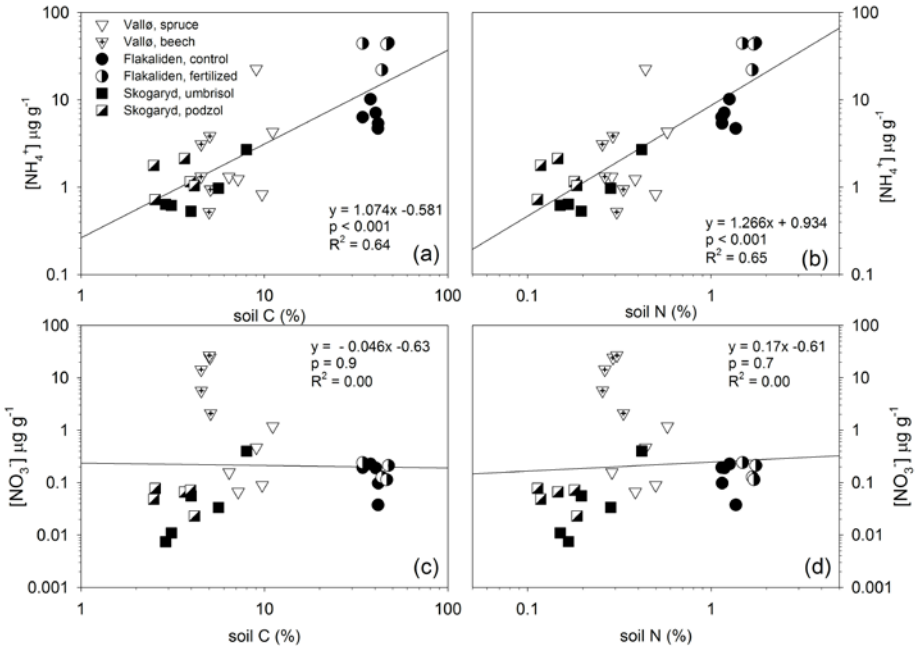


Figure 13. Relationships between NH_4^+ content before label addition expressed per dry weight and (a) soil C and (b) soil N, as well as NO_3^- content before label addition and (c) soil C and (d) soil N. The data are from the forested sites (n = 29, **papers II**, **III** and **IV**). Data from Brandbjerg (**paper I**) is missing due to lack of mineral N data.

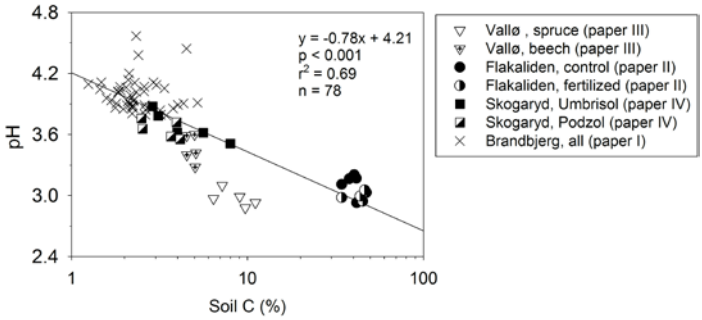


Figure 14. Relationship between soil C and pH, all investigated sites.

The heathland soil (**paper I**) was the only soil investigated in this thesis with a ^{15}N recovery of 100 % for both NH_4^+ and NO_3^-. In the forest experiments (**papers II**, **III** and **IV**), no samples had full NH_4^+ recovery (18 – 77% in the first extraction points, see Fig. 15). In **paper II**, there was a strong correlation between initial NH_4^+ concentrations and recovery after 1 h of incubation (Fig. 17). The NO_3^- recovery was 50 – 126% (Fig. 16) with the highest numbers in Brandbjerg (**paper I**). The NO_3^- recovery increased after 24 h, both in Brandbjerg and Vallø soil (**paper III**). In Vallø, the mean $^{15}NO_3^-$ recovery for spruce soil was 101%, but lower for beech (56 %).

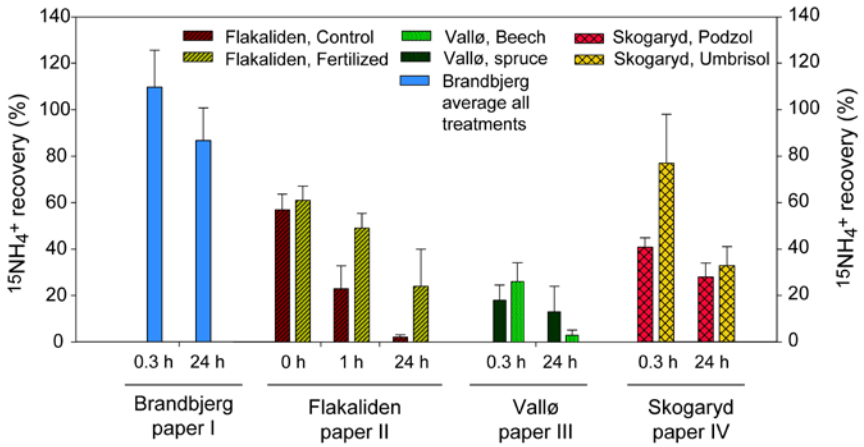


Figure 15. Recovery of $^{15}NH_4^+$ in the soil at the first extraction times (0 h, 0.3 h, 1 h and 24 h) for the different sites. Bars show means and error bars indicate one standard deviation. For Flakaliden and Skogaryd data, n = 10, and for Vallø, n = 5. The Brandbjerg bar shows an average of all the treatments (n = 48).

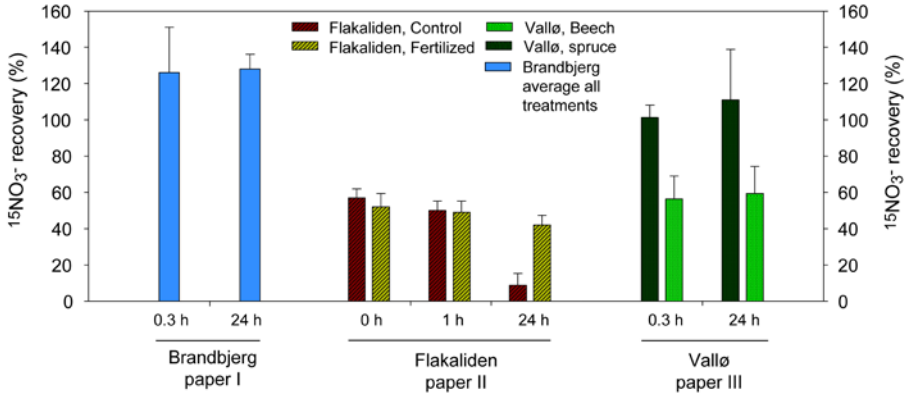


Figure 16. Recovery of $^{15}NO_3^-$ in the soil at the first extraction times (0 h, 0.3 h, 1 h and 24 h) for the different sites. Bars show means and error bars indicate one standard deviation. For Flakaliden data, n = 10, for Vallø, n = 5 and the Brandbjerg bar is showing an average of all the treatments (n = 48). Skogaryd data is missing due to non-detectable amounts of $^{15}NO_3^-$ in the samples.

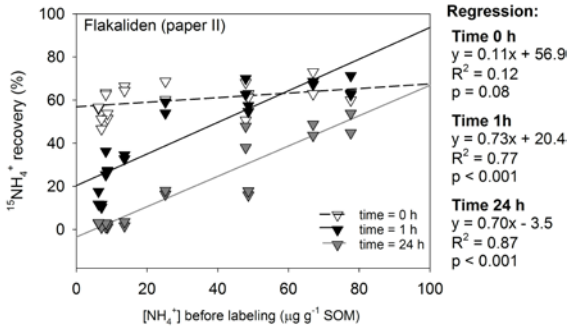


Figure 17. Initial concentrations of NH_4^+ in the Flakaliden samples, plotted against the $^{15}NH_4^+$ recovery, at three different time points with regression lines (n = 20). White symbols and dashed line shows data from immediate extraction (t = 0 h), black symbols and black solid line shows data from extraction after one hour (t = 1 h) and grey symbols and grey solid line shows data from 24 hours (t = 24 h).

Table 2. Gross transformation rates and soil properties from the all four sites. Data are shown normalized to SOM content. Values shown are means with one standard deviation within parenthesis, except for Vallø gross transformation data, where numbers are shown as ranges, depending on initial pool size estimation.

		Mineralization	NH_4^+ immobilization	Nitrification	Nitrate immobilization	DNRA	C/N	NH_4^+ content	NO_3^- content	SOM	pH
		($\mu g\ g^{-1}$ OM day^{-1})						($\mu g\ g^{-1}$ OM)		(%)	
Flakaliden	Control	342* (29.2)	336* (29.2)	11.3 (0.35)	6.43 (0.39)	4.89 (0.32)	32.3 (2.9)	8.83 (2.86)	0.19 (0.10)	76.7 (10.6)	3.1 (0.1)
	Fertilized	34.0 (1.4)	36.8 (1.5)	0	1.42 (0.05)	0	26.4 (2.1)	53.3 (20.1)	0.22 (0.11)	84.5 (8.5)	3.0 (0.04)
Skogaryd	Podzol	25.3 (2.4)	20.0 (1.95)	n.d.	n.d.	n.d.	22.7 (1.4)	20.5 (9.85)	1.24 (0.36)	6.9 (1.4)	3.7 (0.1)
	Umbrisol	34.2 (3.3)	33.4 (5.08)	n.d.	n.d.	n.d.	19.4 (1.3)	10.8 (4.94)	0.83 (1.13)	9.3 (3.1)	3.7 (0.2)
Vallø	Spruce	6.10 – 36.8	5.80 – 36.7	0.96 – 1.05	0	0.17 – 0.46	20.0 (1.4)	42.2 (65.4)	2.47 (2.49)	13.9 (3.1)	3.0 (0.1)
	Beech	84.9 – 91.8	33.1 – 80.3	6.73 – 11.4	0	0	16.8 (1.0)	25.3 (16.0)	205.9 (158.0)	7.3 (1.0)	3.5 (0.1)
Brandbjerg	A	45.8 (8.1)	41.1 (6.0)	5.9 (1.3)	0	0.61 (0.07)	21.3 (4.0)	n.d.	n.d.	4.3 (0.02)	3.9 (0.02)
	T	69.4 (3.4)	49.6 (3.3)	13.8 (1.0)	0	0	19.4 (2.1)	n.d.	n.d.	3.5 (0.09)	4.0 (0.04)
	D	48.2 (11.0)	34.3 (4.7)	13.5 (1.4)	0	0.52 (0.10)	20.9 (2.5)	n.d.	n.d.	3.9 (0.07)	4.0 (0.02)
	TD	63.5 (10.7)	44.0 (7.0)	16.1 (1.1)	0	0.97 (0.14)	19.4 (2.2)	n.d.	n.d.	3.7 (0.08)	4.1 (0.03)
	CO2	61.6 (15.9)	55.8 (10.9)	5.8 (1.1)	0	0.46 (0.16)	21.1 (3.9)	n.d.	n.d.	4.0 (0.08)	4.0 (0.05)
	TCO2	62.5 (10.7)	53.3 (9.2)	9.5 (0.9)	0	0	19.1 (2.2)	n.d.	n.d.	3.6 (0.13)	4.0 (0.02)
	DCO2	53.1 (12.7)	47.2 (6.4)	5.0 (0.9)	0	0.31 (0.13)	19.9 (3.5)	n.d.	n.d.	3.5 (0.11)	4.0 (0.02)
	TDCO2	52.3 (11.1)	37.1 (6.1)	8.1 (0.4)	0	0	19.9 (2.8)	n.d.	n.d.	3.7 (0.17)	3.9 (0.02)

*Overestimated rates due to apparent priming (Jenkinson et al. 1985; Kuzyakov et al. 2000)

Paper I (Brandbjerg)

In **paper I**, climate treatment effects on the N cycling processes were discussed as single and main effects. Single treatment effects were defined as the differences between the ambient (A), not receiving any treatment, and the single treatment plots (eCO2, T and D, respectively, n = 6, see Fig. 18). The main treatment effects describe the overall effects of eCO_2, warming and drought observed in single treatment as well as combined treatment plots, i.e. the average of all plots receiving a specific climate change treatment versus the average of all plots not receiving that treatment (n = 24, see Fig. 19). The term "full treatment" refers to the plots receiving all three treatments combined ($TDeCO_2$). Generally the responses were lower when looking at main treatment effects compared to single treatments effects, and the responses in the $TDeCO_2$ treatment were very similar to the responses in A plots.

Table 3. Direction of responses of gross N transformation rates to three climate change factors. Responses in parentheses are for main treatment effects (see text for explanation).

	Mineral-ization	Immobilization	Nitrification	Total NH_4^+ consumption
Temp (T)	↑ (↔)	↔ (↓)	↑ (↑)	↑ (↔)
Drought (D)	↔ (↔)	↓ (↓)	↑ (↑)	↓ (↓)
eCO_2	↑ (↔)	↑ (↑)	↓ (↓)	↑ (↔)
Future ($TDeCO_2$)	↔	↓	↑	↓

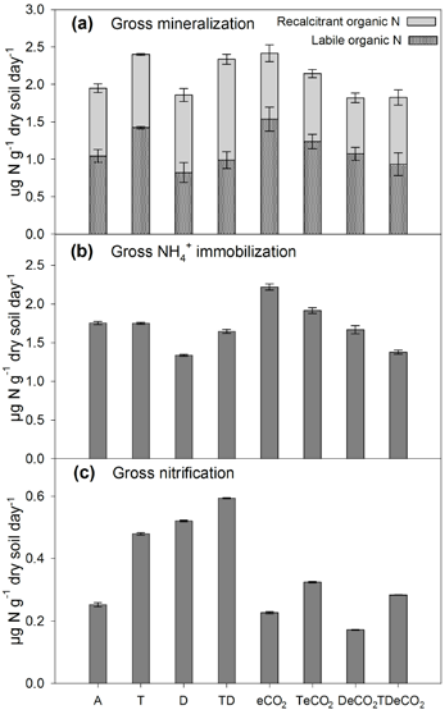


Figure 18. Gross rates of (a) mineralization of labile and recalcitrant N, (b) NH_4^+ immobilization and (c) nitrification in each treatment combination. Treatments are control (A), elevated temperature (T), prolonged summer drought (D), elevated CO_2 (eCO_2) and all combinations. Error bars show 85% confidence intervals.

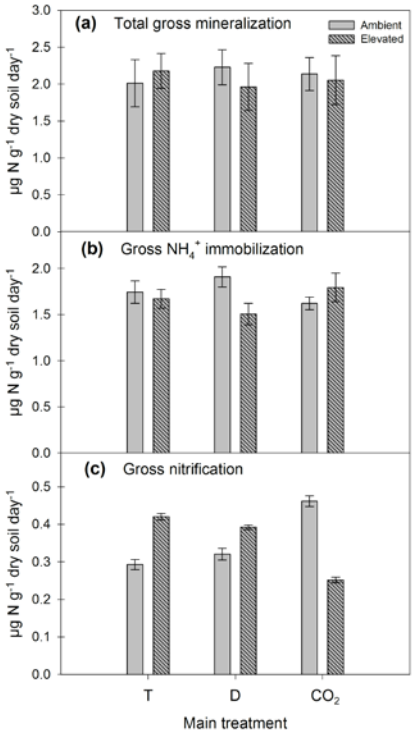


Figure 19. Main effects of temperature (T), prolonged summer drought (D) and elevated CO_2 (eCO_2) on (a) total gross mineralization (sum of labile and recalcitrant N mineralization) (b) NH_4^+ immobilization and (c) nitrification. Error bars show 85% confidence intervals calculated from error propagation.

Gross mineralization rates were between 1.8–2.4 µg g^{-1} day^{-1} (Fig. 18a) and not significantly different in ambient compared to the full combination of climate factors. There was a significant increase in mineralization rates with temperature as a single treatment, but as main treatment there was no significant effect (Fig. 19a). Immobilization was 1.3–2.2 µg g^{-1} day^{-1} (Fig. 18b) and significantly decreased in full combination compared to ambient. Nitrification significantly decreased by eCO_2 as a main treatment (Fig. 19c) but in $TDeCO_2$ there was instead a slight increase of nitrification (Fig 18c). We observed opposing responses for eCO_2 as single and main treatment compared to the plots receiving the full treatment ($TDeCO_2$, Table 3), especially for NH_4^+ consumption processes (both nitrification and NH_4^+ immobilization).

Paper II (Flakaliden)

Gross nitrification and DNRA were zero in the fertilized samples, while in control samples, DNRA and NO_3^- immobilization rates added together were in the same range as nitrification (Table 4). The gross immobilization rate was slightly higher than the mineralization rate in the fertilized samples. In the control samples, the very fast disappearance of the $^{15}NH_4^+$ label resulted in overestimated gross mineralization and immobilization rates ten-fold higher than the control (Table 4).

Despite mostly negative net N_2O fluxes in the incubation bottles during the experiment (Fig. 20a–b), the $\delta^{15}N$ values in the gas samples showed gross emissions of N_2O derived from the ^{15}N labelling. The $\delta^{15}N$ values in the samples deriving from bottles with $^{15}NH_4^+$ labeled soil were low, and no different from the unlabeled soil (Fig. 20c–d). However, in the incubation bottles with $^{15}NO_3^-$ labeled soil there was a clear difference in the $\delta^{15}N$ pattern between control samples and fertilized samples during the first 96 hours of the incubation. In control samples, the $\delta^{15}N$ average for both $^{15}NO_3^-$ treatments was high in the beginning (1 h) of the incubation, then decreased to background levels after 216 h (Fig. 20c). In the fertilized treatment, the $\delta^{15}N$ values from $^{15}NO_3^-$ labeled samples were slightly higher than the samples from $^{15}NH_4^+$ labeled and unlabeled soil, but constant throughout the whole incubation period (Fig. 20d).

Table 4. Gross N transformation rates from **paper II** presented in μg g^{-1} OM day^{-1} and standard deviation within parenthesis.

	Control	Fertilized
Gross mineralization (M_{Norg})	342* (29.2)	34.0 (1.4)
Gross NH_4^+ immobilization (I_{NH4})	336* (29.2)	36.8 (1.5)
Gross nitrification (O_{NH4})	11.3 (0.35)	0
Gross NO_3^- immobilization (I_{NO3})	6.43 (0.39)	1.42 (0.05)
DNRA (D_{NO3})	4.89 (0.32)	0

*overestimated rates, due to apparent priming (Jenkinson et al. 1985; Kuzyakov et al. 2000)

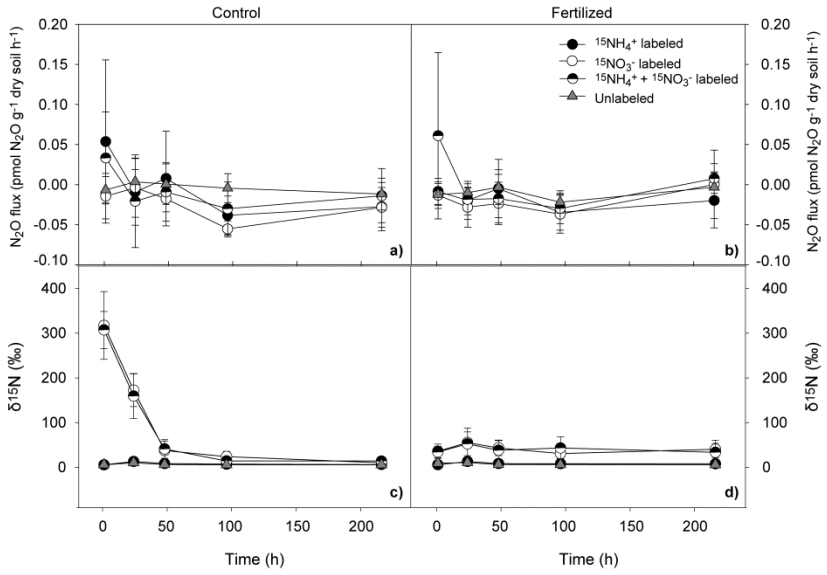


Figure 20. Fluxes of N_2O from the laboratory bottles during the incubation period for (a) control samples and (b) fertilized samples. Bottom panels show $\delta^{15}N$ values of the N_2O samples, for (c) control samples and d) fertilized samples. Symbols show means (n =5) and error bars indicate standard deviation.

Paper III (Vallø)

The largest differences in soil properties between the two stands were seen in the C/N ratio, SOM content, pH and most prominently, the NO_3^- concentration, which was significantly higher in beech soil (Table 5). This was also reflected in the gross N rates, with gross nitrification in the beech soil six to eleven times higher than in the spruce soil, regardless of initial pool size estimation (6.73 – 11.4 μg g^{-1} OM day^{-1} compared to 0.96 – 1.05 μg g^{-1} OM day^{-1} for beech and spruce respectively, see Fig. 21)

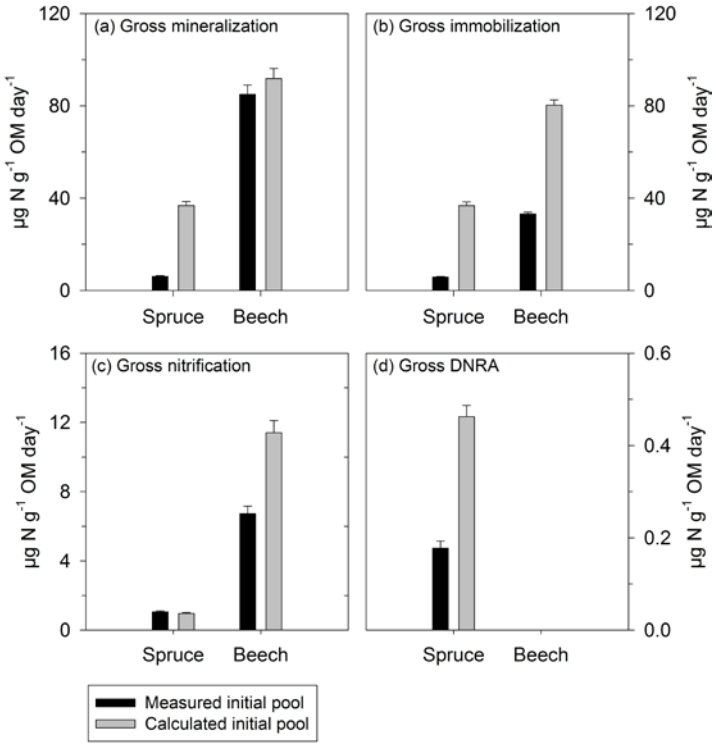


Figure 21. Gross rates of (a) mineralization, (b) immobilization, (c) nitrification and (d) DNRA from spruce and beech stand. Black bars represent the rates estimated from measured initial ^{15}N abundance, while grey bars represent rates estimated when the initial ^{15}N abundances were calculated. Error bars show 85% confidence intervals.

Table 5. Soil properties for both tree stands in **paper III**, mean (n =5) and standard deviation within parenthesis.

	Spruce	**Beech**	
pH (in 1M KCl)	2.97 (0.08)	3.46 (0.14)	***
GWC (%)	21.1 (3.24)	26.7 (4.44)	
SOM (%)	13.9 (3.13)	7.26 (1.01)	**
C/N	20.0 (1.45)	16.8 (1.03)	**
NH_4^+ (µg N/g OM)	42.2 (65.4)	25.3 (16.0)	
NO_3^- (µg N/g OM)	2.47 (2.49)	206 (158)	*

*P < 0.05, **P < 0.01 ***P < 0.001, two tailed t-test

Table 6. N dynamics rates from analytical equations and *Ntrace* numerical model, average in nmol g^{-1} h^{-1} and one standard deviation within parenthesis. For D_{SON} and C_{FAA} the time step 30 to 360 min is presented. M_{FAA} and I_{FAA} are presented for all 20 amino acids over 240 h. C_{FAA} and M from *Ntrace* are calculated sums ($M = M_{SON} + M_{FAA}$ and $C_{FAA} = I_{FAA} + M_{FAA}$).

	Podzol		**Umbrisol**	
	Analytical	**Ntrace**	**Analytical**	**Ntrace**
Peptide depolymerization (D_{SON})	58.8 (53.2)	18.2 (12.6)	316.6 (151.3)	288.5 (40.6)
FAA immobilization (I_{FAA})	–	16.8 (1.4)	–	172.3 (18.2)
FAA mineralization (M_{FAA})	–	11.2 (1.4)	–	131.7 (12.6)
Amino acid consumption (C_{FAA})	313.8 (40.6)	28.0 (2.8)	851.6 (191.9)	303.9 (30.8)
Mineralization of organic N (M_{SON})	–	61.6 (5.6)	–	0.0
Gross N mineralization (M)	100.8 (35.0)	72.8 (7.0)	239.5 (135.9)	131.7 (12.6)
NH_4^+ consumption (C_{NH4})	50.4 (11.2)	57.4 (5.6)	166.7 (198.9)	128.9 (19.6)

Paper IV (Skogaryd)

The total gross N mineralization (M) in the Umbrisol estimated from *Ntrace* was entirely assigned to FAA mineralization (M_{FAA}), while the Podzol showed mineralization from other organic compounds (M_{SON}) as well (Table 6). Generally, the gross transformation rates were lower when calculated with the numerical method, due to the rates being integrated over a longer period of time and that the stimulation of rates are largest in the beginning, right after label addition. The gross rates were generally higher in the Umbrisol than the Podzol, regardless of the method used. A good fit of the model to the experimental data was achieved (Fig. 22).

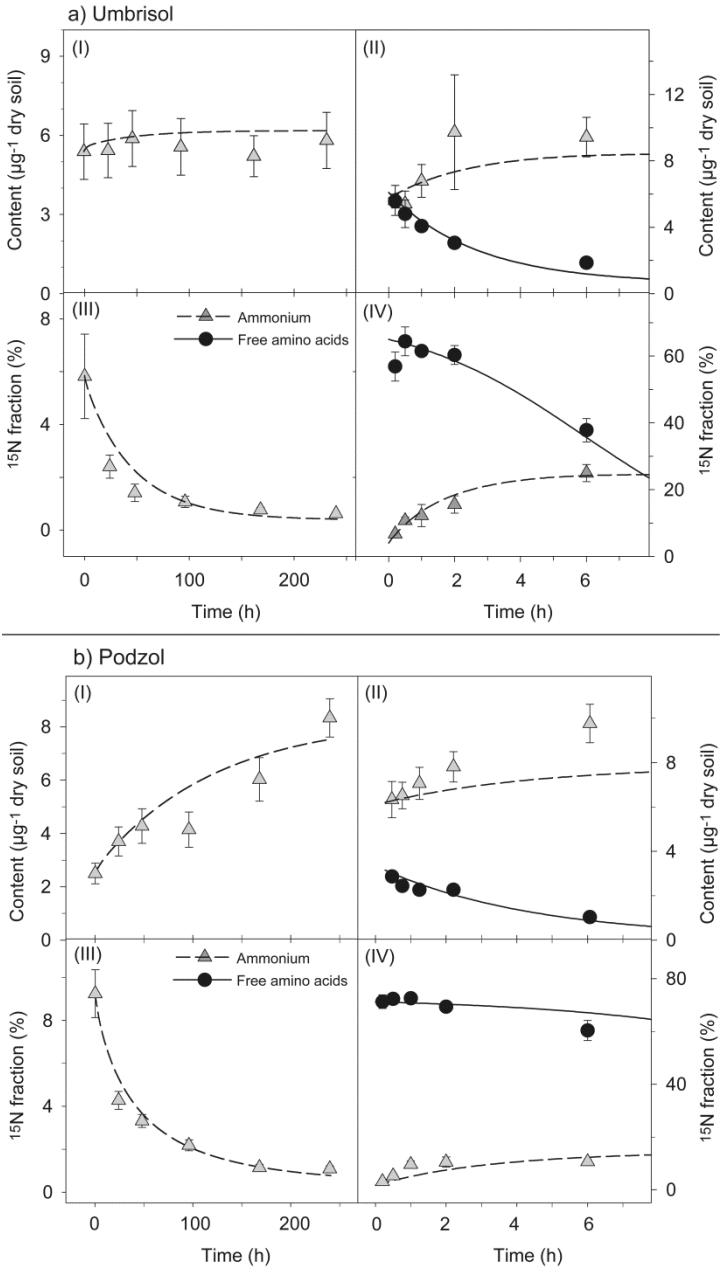


Figure 22. Time flow of the two labelling experiments (a) Umbrisol and (b) Podzol. The $^{15}NH_4^+$ labelling is shown to the left (I and III) and the ^{15}N–FAA labelling to the right (II and IV); symbols indicate data observation with standard deviation (n = 5, except ^{15}N fraction of FAA: n = 4 at 13 min), and lines indicate the two AA-pool models. Triangles and dashed lines are ammonium and circles and solid lines are FAAs.

Discussion

The work in this thesis confirm that the quality of SOM is an important factor regulating mineralization rates, manifested by the total content of C and N, and the ratio between them (Booth et al. 2005; Hart et al. 1994). In an overall comparison between the sites (Table 2, excluding the control plot in **paper II**, due to overestimated rates, see explanation in p. 53), we saw that the mineralization rates were highest in the beech site (85–92 µg g^{-1} OM day^{-1}, **paper III**), moderately high in the heathland site (46–69 µg g^{-1} OM day^{-1} , **paper I**) and lowest in the spruce forests (6.1–37 µg g^{-1} OM day^{-1}, **papers II**, **III** and **IV).** The C:N ratio followed the same pattern, with highest values in the spruce soils and lowest in the beech soil. Temperature is also an important factor regulating mineralization (Melillo et al. 2002), as seen in **paper I**, confirmed also by the higher mineralization rates in the southern spruce forests compared to the northern.

The negative correlation between soil pH and C content (Fig. 15) has been observed in other studies (Booth et al. 2005) and was consistent when the forested sites were analyzed alone (data not shown). Soil pH is seasonally variable and is decreases during the growing season with cation exchange from roots, as well as reliant on the general erosion potential of the soil (Eriksson et al. 2011). The higher soil pH found in the soils in **paper IV** might reflect the sampling date as the sites were sampled in April and May, and the other two forested sites (**papers II** and **III**) were sampled in September. The heathland site (**paper I**) was sampled in November.

Soil N and climate change

To predict how different climate factors affect the N cycle at the global scale is essential as the capacity for C storage in the terrestrial biosphere is dependent on the N cycle (Zaehle et al. 2010). The most important finding from **paper I** is the different responses observed in single and combined treatments, and that some of the processes, such as NH_4^+ immobilization and nitrification, respond differently to eCO_2 depending on the number of treatments involved (Table 3)

Nitrification was significantly decreased by eCO_2, both as single and main treatment, but in the full treatment ($TDeCO_2$), the effect was instead slightly positive. Autotrophic nitrifiers are usually poor competitors for NH_4^+ (Verhagen & Laanbroek 1991) and could be sensitive to eCO_2 due to

increased C input to the soil, which favors heterotrophic processes. In the TDeCO_2 treatment there were apparently feedbacks hampering this response.

Another interactive effect observed was the response of gross mineralization to warming, which was dependent on the CO_2 concentration. This change in gross mineralization under eCO_2 and warming could be related to changes in microbial community size, seen in a previous study from the same site. Microbial biomass was seen to increase both in the eCO_2 and warming treatments, but not when the two treatments were combined (Andresen et al. 2010). At this site, the non-significant increase in plant biomass under eCO_2 (Kongstad et al. 2012) supports the idea that C storage might only change moderately in the future. If mineralization rates decrease under eCO_2 it could be an indication of PNL, which could be driven by the increased C:N ratio of SOM (due to more C input to the soil in eCO_2, Luo et al. 2004). In **paper I** we saw no indication of that, however there were significant changes in total N, where the plots treated with eCO_2 had lower N than the plots with ambient CO_2. However, combined with warming this change was not significant.

As seen in this study, multifactor responses are often non-additive (Dieleman et al. 2012), and more often antagonistic than synergistic, i.e. the combinations of treatments often lead to a reduction rather than an amplification of effects (Larsen et al. 2011). Leuzinger et al. (2011) showed that the effect size of an experiment is decreased with the number of variables tested, which means that the overall response to combined treatments of an ecosystem is lower than responses to single treatments. That is also what we observed in **paper I**. The response of an ecosystem to climate change will be a function of the combined effect of CO_2 and temperature (Norby & Luo 2004) as well as precipitation patterns (Ciais et al. 2013). The results from this study, together with other studies on ecosystem responses to climate change (Albert et al. 2011; Dijkstra et al. 2010; Gray et al. 2011; Horz et al. 2004; Jamieson et al. 1998) highlight the importance of multifactorial experiments to investigate interactive effects between climatic treatments.

N dynamics in forests

With the exception of the overestimated rates in the control plots in **paper II**, the gross mineralization and immobilization rates in **papers II**, **III** and **IV** were in the same range when normalized to SOM content and comparable to other studies in forested ecosystems (Chen & Högberg 2006; Davidson et al. 1991; Gundersen et al. 1998; Högberg et al. 2006a; Tietema 1998).

All forested sites in this thesis had higher NH_4^+ turnover than NO_3^- turnover, a common pattern for coniferous soils. The NH_4^+ concentration was positively correlated to both soil C and N (Fig. 13a–b), indicating that NH_4^+ turnover is more important in soils where C accumulates in soil. NH_4^+ has been seen to be preferred both as a substrate by microbes (Jackson et al. 1989; Jansson et al. 1955) and as a mineral N source by spruce roots (Marschner et al. 1991). The soil NO_3^- concentration showed no correlation with neither soil C, nor N, in contrast to the review by Booth et al. (2005). The soil mineral N content increased from north to south (the fertilized soil in **paper II** not included) both for NH_4^+ and NO_3^- following the temperature gradient.

Mineralization and immobilization

The mineralization dynamics differed between the two soils in **paper IV**. Firstly, the Podzol had a lower peptide depolymerization (D_{SON}) rate compared to the Umbrisol as well as previously studied soils from forests and grasslands (Wild et al. 2015). This might explain the lowered FAA mineralization rate as depolymerization is limiting the substrate for that process. The gross N mineralization in the Umbrisol was estimated as entirely derived from the FAA pool, while in the Podzol, mineralization of FAA contributed only 15% of the total gross N mineralization. Consequently, the Umbrisol strongly depended on FAAs as a source in inorganic N (IN) production, while in the Podzol the mineralization of other organic N forms (aminosugars, such as chitin; Bai et al. 2013) dominated in mineralization. When normalized to the SOM content, Podzol had a higher content of NH_4^+ compared to Umbrisol, while the initial soil FAA content was lower in the Podzol, indicating that the Umbrisol is a system where the N-cycling is more dependent on labile organic-N. The difference in gross transformation rates between the two soils were much smaller when expressed per OM content, conforming the relevance of expressing rates this way, instead of per soil weight.

In a wide range of ecosystems, N mineralization is connected to C and N concentrations, according to a review by Booth et al. (2005). This agrees with the finding that the mineralization rates were lower in the spruce stands compared to the beech stand, as the beech soil had a higher mineral N content and the lowest C:N ratio of all the investigated sites. In **paper III**, this difference in gross mineralization rates between tree stands was regardless of initial pool size estimations (see Fig. 21a). This result is in contrast to a study

by Brüggemann et al. (2005), who found higher rates in spruce forest compared to beech forest. However, other studies found higher mineralization for deciduous stands compared to coniferous (Staelens et al. 2012), both in the forest floor and the mineral soil (Cote et al. 2000), despite similar content of soil C (Booth et al. 2005). The gross NH_4^+ immobilization (Fig. 21b) was higher in the beech stand compared to the spruce stand and consequently, there was net mineralization in both stands though it was lower in the spruce stand. The net mineralization in the beech stand was dependent on initial pool size estimations; it was higher when the initial pool size was measured instead of calculated (Fig 21a–b).

Nitrification

The gross nitrification rate in the beech stand (**paper III**, Fig. 21c) was considerable higher compared to the spruce stand regardless of initial pool size estimation, which was reflected in the larger NO_3^- pool in the beech stand (Table 5). This finding conforms to other studies comparing tree species influence on soil, which have found that N dynamics in beech forests are more NO_3^- dominated in comparison to spruce forests (Jussy et al. 2004; Wedraogo et al. 1993; Zhong & Makeschin 2004; 2006b). Moreover, Christiansen et al. (2010) found high NO_3^- concentrations in soil water under a beech stand compared to other tree stands at the same site. The spruce stand showed low nitrification rates at this site (0.96 – 1.05 µg g^{-1} OM day^{-1}), much lower than the spruce forest in as the control plot in **paper II** (11.3 µg g^{-1} OM day^{-1}). The high nitrification rates in this soil was not expected, since the NO_3^- pool in this Northern spruce forest was small. However, soil NO_3^- turnover, both nitrification and NO_3^- immobilization, has been found to be high even when the NO_3^- pool is very small (Davidson et al. 1992; Stark & Hart 1997).

The absent nitrification in the fertilized plots compared to control plots in **paper II** indicates that there is a fertilization effect on nitrification. Autotrophic nitrification is controlled by the access to NH_4^+ and mineralization rates (Booth et al. 2005; Hart et al. 1997; Robertson & Groffman 2007) and is expected to increase in soils where NH_4^+ accumulates (Aber et al. 1989). We can only speculate in the causes for the decrease in nitrification, since an investigation of specific contribution of different organisms to gross N transformations would have required a more detailed experimental setup, for example by using inhibitors or gene abundance

studies. In concurrence with our study, Gao et al. (2016) observed a decline in nitrification in the mineral layer of soil from fertilized boreal forest, which they concluded derived from lowered activities of ammonia oxidizing archaea (AOA) with increasing soil NH_4^+ content.

Nitrification can also be carried out by heterotrophs, a pathway not directly controlled by NH_4^+ availability, but rather total C and (Killham 1990; Sitaula & Bakken 1993) and fungal heterotrophic nitrification has been concluded to be common in acid coniferous soils (Killham 1987). Generally, fungi drive a large part of the nutrient cycling in low nutrient environments (Thorn & Lynch 2007), and have higher C/N ratios than bacteria (Mouginot et al. 2014); therefore they can scavenge for nutrients more effectively than bacteria in low quality SOM (Herrmann et al. 2014). Fungal biomass has also been shown to decrease with nutrient availability (Demoling et al. 2008), and fungi are often more affected by fertilization compared to other microorganisms (Frey et al. 2004; Högberg 2006; Högberg et al. 2007; Nilsson & Wallander 2003). The handling of the soil in the laboratory incubations could also have affected the gross process rates, as it distorts the structure of the soil as well as microbial community activities (Schimel et al. 1989). This affects especially fungi, which extend through the soil and are negatively affected by the disruption of their hyphae (Johnson et al. 2005). All samples were treated the same in the laboratory, but it is possible that the extra disturbance caused by the laboratory preparation affected the fertilized samples more than the controls, thereby causing lower activity in those samples.

The N_2O measurements from the incubation bottles showed mostly negative fluxes during the incubation period (Fig. 20) which implies that N_2O in the bottle headspaces was consumed. N_2O uptake has frequently been observed in field measurements (Chapuis-Lardy et al. 2007; Wen et al. 2017; Yang & Silver 2016) and can be coupled to the lack of electron acceptor NO_3^- in denitrification, leading to a reduction of N_2O instead (Butterbach-Bahl et al. 1998). The field measurements from Flakaliden also presented in **paper II** showed generally low N_2O emissions from this soil, even in the fertilized plots, and occasionally N_2O uptake. The $\delta^{15}N$ values of the N_2O samples indicate that some of the added $^{15}NO_3^-$ was lost as N_2O during the first 96 h, prominently in the control samples (Fig. 20a–b). That we saw signs of N_2O emissions from control plots rather than fertilized plots could be a result of

the low nitrification rates found in the fertilized samples. Sitaula and Bakken (1993) found that sites with low net nitrification rates also had low emissions of N_2O in a laboratory incubation experiment with soil from a spruce forest in Norway. There was also a difference in water content between the two treatments, as a result of the denser forest and higher evapotranspiration of the trees (see Fig. 5). This difference could have been important for denitrification rates, which are mainly limited by the presence of oxygen (Knowles 1982), and N_2O emissions are sensitive to small variations in temperature and moisture (Sitaula & Bakken 1993).

DNRA

Gross DNRA was found in two of the spruce forested sites, the control plot in **paper II** and the spruce stand in **paper III**. Out of these two sites, DNRA was much higher in the Northern site (**paper II**) compared to the Southern site (**paper III**) which could be expected as the soil in **paper III** had both higher NO_3^- concentration and C:N ratio, and the pathway of DNRA is more energetically effective compared to denitrification when the ratio of available C to the electron acceptor NO_3^- is high (Rütting et al. 2011a; Tiedje et al. 1982). Possibly for the same reason, no DNRA was found in the fertilized plot in **paper II**. Our results are consistent with those of with Bengtsson and Bergwall (2000), who investigated DNRA rates in a fertilized spruce forest in Sweden and found that the DNRA rate was higher in control plots compared to fertilized plots. Some DNRA (0.31–0.97 μg g^{-1} OM day^{-1}) was also found in some of the climate treated plots in **paper I** (Table 2), which was unexpected for this sandy and quite dry heathland soil.

Methodological concerns

In **paper IV**, we developed and evaluated a new numerical tracing model as a way of estimating gross rates of depolymerization and mineralization rates in the soil. We compared it with an analytical isotope pool dilution model. The main difference between the two approaches is that the numerical approach estimates the rate for the entire 240 h of incubation, while the analytical approach considers a limited time span of 6 h maximum. In numerical tracing, all data points from the two isotope label experiments and all observed time steps are included, while in analytical models only two time steps can be included. Differences between gross rates derived from analytical and numerical models were greatest for FAA consumption, while smaller differences were found for gross rates of depolymerization and total

mineralization. This points to an overstimulation of the processes by the addition of FAAs and demonstrates the advantage of a longer incubation time with numerical data analysis to estimate more realistic gross rates, as the stimulation will be greatest immediately after ^{15}N labelling (Rütting et al. 2011b). We did indeed find that the numerically derived gross rates for FAA mineralization and FAA immobilization when integrated over 6 h, were several fold higher than rates integrated over the entire experimental duration (240 h). However, the similarity of depolymerization rates quantified with *Ntrace* and by the analytical approach confirms the validity of the numerical tracing model.

Throughout this thesis work, the low initial concentrations of soil mineral N has made it difficult to perform experiments. Several experiments where ^{15}N values could not be detected were conducted (e.g. $^{15}NO_3^-$ in **paper IV**), eventually leading to higher additions of ^{15}N to ensure detection limits. In the experiment in **paper IV**, the FAA label addition was 10 to 20 times larger than the initial FAA soil content. According to Wanek et al. (2010), a maximum of 25% of the background AA content should be added, not to cause a "hot-spot" effect in the soil (Kuzyakov & Blagodatskaya 2015) which stimulates depolymerization via priming (Schimel 1996). Although the advantage of numerical modeling, it is not certain that the method can compensate for the problem of overstimulation. In **paper II** we saw a stimulation of gross rates with ^{15}N addition despite using the numerical model. The ^{15}N label was in this experiment added in amounts large enough to ensure detection on the SPINMAS. However, since the control plots in the experiment had less mineral N than the fertilized plots, the addition caused a fast immobilization of the added label in the control plots, resulting in very high mineralization and immobilization rates estimated by the model. Jenkinson et al. (1985) describes this phenomenon as "pool substitution", since the added ^{15}N is immobilized instead of the native unlabeled N that is already in the soil. According to Jenkinson et al. (1985) this is a form of priming process, but since it in fact does not alter the decomposition rates of organic matter, it is called "apparent" priming (Kuzyakov et al. 2000). This effect is larger with lower initial IN concentrations of the soil, and is also influenced by the amount of added ^{15}N label, which agrees well with the lower initial NH_4^+ concentration of the control plots compared to the fertilized plots in our study. This addition of substrate to the investigated N pool is a particular weakness of the ^{15}N labeling experiments. Di et al. (2000)

recommend spiking of the sample before analyzing in mass spectrometer, instead of adding higher amounts to the soil. However, it is not certain that spiking can solve the problem of too low mineral N concentrations. The ^{15}N chemical conversions used to convert mineral N to N_2O in **paper III** and **IV** (Stevens & Laughlin 1994), require a certain amount of each ion moiety in the sample extracts. In **paper IV**, the NO_3^- concentration in the samples was too low to make the conversions, and spiking could not be performed with more than 1/10 of the sample concentration (Katja van Nieuland, lab engineer, Ghent Uni, pers.comm. Oct 13, 2014).

Recovery of ^{15}N

In the beginning of the incubation period, the ^{15}N recovery is expected to be 90–100%, but is often lower (Davidson et al. 1991; Morier et al. 2008). As seen in **paper III**, the gross rates will be lower with low recovery, as ^{15}N recovery strongly influences the sizes of the initial N pools and thereby the gross rates estimations. The transformation rates could thereby be directly dependent on the recovery instead of the tested experimental parameters (e.g. field treatments), leading to erroneous interpretations of the results. Moreover, there is a risk that the rapidly removed ^{15}N is re-mineralized during the incubation period, thereby influencing the rates later in the experiment.

Both $^{15}NH_4^+$ and $^{15}NO_3^-$ recovery was high in **paper I** probably was probably due to the sandy soil at Brandbjerg, the sand content of the topsoil (0–10 cm) is 90%, while clay content is only 2% (Klaus Steenberg Larsen, pers. comm. June 21, 2018). The soil in **paper III** had a clay content of 17% (Vesterdal et al. 2012) which could have been responsible for a quick abiotic fixation of NH_4^+ at this site (Fig. 15). In **paper II** the organic horizon was sampled, in contrast to the other sites, which may explain the low recovery, as NH_4^+ can be adsorbed to organic molecules as well. The organic horizon is the most active layer in this type of soil (Alexander & Fairley 1983; Schimel & Firestone 1989). The recovery of $^{15}NH_4^+$ has previously been seen to be positively related to the NH_4^+ content of the soil (Braun et al. 2018; Davidson et al. 1991; Högberg et al. 2006a).

NO_3^- immobilization has been seen to be important even in acidic forest soil (Davidson et al. 2003; Davidson et al. 1992; Stark & Hart 1997), and could influence the recovery of $^{15}NO_3^-$. The NO_3^- recovery increased at 24 h, both in Brandbjerg and Vallø soil. An increasing $^{15}NO_3^-$ label at the second

extraction point indicates that some of the added $^{15}NH_4^+$ is nitrified into the NO_3^- pool quickly. However, if the recovery at the first extraction point is lower than 100%, the increased NO_3^- content could also derive from quickly immobilized NO_3^- that were released back into the soil.

The strong correlation between initial NH_4^+ concentrations and ^{15}N recovery (Fig. 17) show that the time of extraction is very important and that immobilization of the ^{15}N label can occur exceedingly quickly. The deferred time of the first extraction point, 20 minutes instead of immediately, might have influenced the low recovery in **paper II** and **III**. In some experiments found in literature, the first extraction was conducted after 2–4 hours (Liimatainen et al. 2018; Marushchak et al. 2011; Pörtl et al. 2007), which for soils with low initial concentrations of mineral N could implicate that most of the added label has been immobilized already at the first extraction point. The subsequent calculations of gross transformation rates will thus involve a small part of the label. Therefore it is very important that the first extraction point is done immediately after label addition, to get a baseline for the maximum extractable ^{15}N label from the soil. In a ^{15}N tracing experiment with several extraction points it is important to schedule the extractions more frequent in the beginning of the experiment, to catch the change in ^{15}N directly after labeling. In order investigate whether fast immobilization is a result of a biotic or an abiotic process, a parallel test can be performed, where ^{15}N label is added to sterilized soil (Braun et al. 2018; Davidson et al. 1991).

Conclusions

The overall objective of this thesis was to increase the understanding of the N cycle in natural and semi-natural ecosystems and the environmental factors important for nutrient cycling. The results show that all sites in this thesis had higher NH_4^+ turnover than NO_3^- turnover. The mineralization rates were highest in the beech site (**paper III)**, moderately high in the heathland site (**paper I**) and lowest in the spruce forests (**papers II**, **III** and **IV).** The C:N ratios followed the opposite pattern, with lowest C:N ratio for the beech site and the highest for the spruce forests, which demonstrate the importance of organic matter quality on gross N transformation rates.

The first specific aim was to examine how the N cycle is influenced by a changing climate with higher CO_2 levels, warming and drought. The results show that responses to combined climate treatments were lower than to single climate treatments, highlighting the necessity of conducting multifactor experiments.

The second aim was to investigate how the N cycle and N_2O emissions in forests are influenced by climate as well as different forest management practices, such as fertilization and tree species choice. We saw a negative effect of fertilization on nitrification rates. In the same experiment we also saw that N_2O release from control soil directly after labeling, but not from fertilized soil (**paper II**). We also observed that gross N dynamics differed to a great extent between two stands of tree species, one spruce and one beech, in a common garden experiment (**paper III**). The beech stand had higher gross mineralization, immobilization and nitrification rates as well as more pronounced NO_3^- dynamics compared to spruce soil, in these specific stands. In a comparison between two different forests we saw that the source of organic N in mineralization differed between a Podzol and an Umbrisol with higher SOM content. In the Umbrisol the mineralization derived totally from FAA's, while in the Podzol the mineralization of other organic N forms dominated the IN production (**paper IV**).

The third aim was to further develop ^{15}N isotope methods, in order to estimate rates of amino acid mineralization with numerical modeling. We show that numerical modeling together with ^{15}N tracing is an improvement for simultaneously determining FAA mineralization, peptide depolymerization and gross N mineralization rates, compared to analytical

solutions (**paper IV**). The results from this thesis also highlight some of the problems connected to ^{15}N tracing experiments, e.g. overstimulation of rates from the addition of ^{15}N label (**paper II** and **IV**) as well as correction for low ^{15}N recovery in experiments (**paper III**). These methodological problems seem to be more prominent in ecosystems where the external inputs of N are very low.

Outlook

The area of soil science comprises the interactions between chemical, biological and physical processes in the soil environment. The soil is a heterogeneous medium, where the processes crucial for ecosystem nutrient status and environmental impact are regulated on microscale (Schimel & Bennett 2004). This thesis has investigated the impact of climate change as well as management practices on gross N cycling in natural and semi-natural ecosystems and highlighted some methodological challenges connected to this field. In this section some examples of further development of the area of soil science are presented, from the findings in this thesis.

Human land use has great impact on the nitrogen cycle, as it is closely related to our food production, as well as forest management. Still, there are large uncertainties regarding the N constraints in global modelling, and more real data is needed to understand the feedback controls of N cycling. As shown in this thesis, large multifactorial ecosystem experiments are important to understand the interactive effects between different climate variables. In addition to that, the existing ecosystem data comes from a limited number of FACE experiments conducted in grasslands in temperate regions (Ciais et al. 2013). Forests cover 31% of the land area on Earth (FAO 2010) and even though forest FACE experiments are both costly and time consuming to operate, climate response data from boreal forest ecosystems would be desirable in the future.

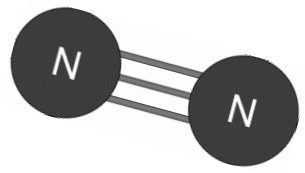
The branch of soil microbiology has made several important advances in later years, e.g. the discovery of ammonia oxidizing archaea (Könneke et al. 2005; Sterngren et al. 2015) and its role in N_2O emissions (Hink et al. 2017; 2018), as well as the process of complete nitrification, where both NH_4^+ oxidation and NO_2^- oxidation takes place within one organism (Daims et al. 2015; van Kessel et al. 2015). However, isolation of microbes in pure cultures is a requisite for detailed studies of processes, and the soil contains many microbial species that cannot be isolated or cultivated in laboratory media (Janssen et al. 2002). Soil microbial activity therefore needs to be studied with a wide array of techniques to connect processes to specific organisms. This can be done with inhibitors (Zhang et al. 2013) exclusion of mycorrhiza (Holz et al. 2016), biomarkers or gene abundance techniques (Angela & Alastair 2010) in combination with ^{15}N tracing studies.

Although fungi are known to have an important roles in both denitrification (Hayatsu et al. 2008; Shoun et al. 1992) and nitrification (Zhang et al. 2015), the function in N cycling needs to be further investigated. Fungal activity may have been underestimated in laboratory ^{15}N pool dilution experiments, due to that the structure and microsites are destroyed as the soil is mixed or sieved before incubations. For this purpose, intact soil cores or *in situ* incubations can be used, which preserve the field conditions of the soil, including plants and roots as well as mycorrhizal interactions. However, this method brings in greater uncertainties into the experiments, especially regarding losses of ^{15}N label and ^{15}N label distribution in soil.

The results from this thesis points to some methodological challenges to overcome, especially regarding investigating gross transformation rates in soils with low mineral N content. With the current methods that require substrate addition to the soil, it is questionable if it is even possible to investigate N cycling in some environments with very low N content. The issue of different ^{15}N recovery and how to correct for that in experiments is highly relevant as the recovery is influencing the gross transformation estimations. Future research on method development would include a wider investigation of the regulating factors of ^{15}N recovery in different kinds of soils, organic as well as mineral, with different additions of ^{15}N to see which environmental and methodological factors are influencing recovery in different systems. This would also include a closer look at the non-extractable N, to examine if the ^{15}N label is immobilized by microbes or abiotically fixed to soil particles.

Populärvetenskaplig sammanfattning

Bakgrund

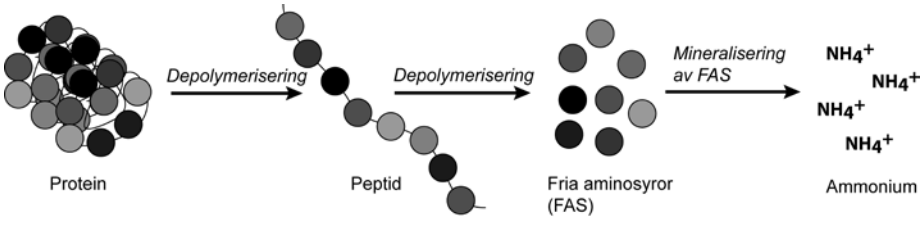


Figur 23. En dikvävemolekyl, N_2

Kväve är ett viktigt ämne för liv. Det finns i alla proteiner, som är en av byggstenarna i våra kroppar och i vårt DNA. För växter är kväve en viktig del av klorofyll, det ämne som gör att bladen får sin gröna färg och kan få sin energi från solen i fotosyntesen. Kväve är det grundämne som det finns mest av på jorden, nästan 80% av atmosfären består av kvävgas (N_2, Figur 23). Men så länge kväve är i form av N_2 kan inte växter och djur använda det, eftersom kvävemolekylen har en stark trippelbindning. Men om N_2-molekylen utsätts för hög energi, som t.ex. i en förbränningsmotor eller ett blixtnedslag, kan den brytas sönder och kvävet omvandlas till s.k. reaktivt kväve (N_r). Det reaktiva kvävet är tillgängligt för livet på jorden och kan användas av växter och mikroorganismer (t.ex. bakterier och svampar), och i förlängningen av djur och människor. Det finns även bakterier som kan använda kväve direkt från luften, i s.k. kvävefixering. En del kan leva i rötterna på baljväxter, och då får de energi av växten de lever på, men det finns också arter som använder fotosyntes och lever i havet (de stora algblomningarna i Östersjön är såna här bakterier som växer till). När en kväveatom blivit reaktiv, kan den ta många olika former och genomgå en rad omvandlingar i miljön innan den slutligen kommer tillbaka till atmosfären igen. Alla dessa omvandlingar kan sammanfattas i kvävekretsloppet (se Figur 1, s. 12). Innan den industriella revo-lutionen var kvävekretsloppet någorlunda balanserat, d.v.s. ungefär lika mycket kväve som kom in till ekosystemen kom även tillbaka till atmosfären igen. Men idag är inflödet mycket större än utflödet. Detta beror på att människan har uppfunnit konstgödsel, som i praktiken innebär att kväve tas från luften och stoppas ner i marken. Tillverkningen av konstgödsel är en mycket energikrävande process, eftersom det betyder att man måste bryta N_2-molekylen på konstgjord väg. Inflödet till kvävekretsloppet trappas dessutom upp av att vi använder mer fossila bränslen, framför allt i förbränningsmotorer, och att man idag storskaligt odlar kvävefixerande grödor, särskilt sojabönor, på många håll i världen. Allt detta har lett till att människan idag står för hälften av all kvävefixering på jorden, vilket har ändrat förutsättningarna att leva här på jorden drastiskt. Vi har blivit dubbelt så många människor på jorden sedan 1950-talet, vilket är på grund av att vi nu kan odla mat utan att behöva tänka på de naturliga näringsbegränsningarna i marken. Tyvärr har det ökande användandet av kväve också lett till många miljö och hälsoproblem, t.ex. övergödning av sjöar och hav, luftföroreningar, föroreningar i dricksvatten och utsläpp av växthusgaser, som lustgas (N_2O). Växthusgaser är gaser som håller kvar värmestrålningen från jorden i atmosfären, och gör att jorden är varmare än den

annars skulle varit. Lustgas är en mycket kraftig växthusgas, 265 gånger starkare än koldioxid, och den bidrar även till nedbrytningen av ozonlagret i stratosfären. Utsläppen av lustgas sker naturligt från mark, av mikroorganismer som ingår i kvävekretsloppet, och utsläppen har ökat de senaste hundra åren. Mycket lustgas släpps ut från jordbruksmark, eftersom de kvävegödslas. Men lustgasutsläppen kan även vara stora från skogsmark och det är inte helt klart vad som orsakar och styr dessa utsläpp.

I ogödslade ekosystem är tillgången på kväve för växter framför allt styrd av hur mycket som kommer in genom kvävefixering och deposition (där luftburet reaktivt kväve fastnar på ytor, t.ex. trädens bladverk) samt hur snabbt kvävet omsätts, då särskilt via s.k. mineralisering och immobilisering. Kvävemineralisering (se Figur 24) sker vid nedbrytning av organiskt material (t.ex. växtdelar) då kväve som är bundet i större organiska molekyler (t.ex. proteiner) blir fritt och därmed tillgängligt för växter och mikroorganismer i en enklare form. Immobilisering den motsatta processen, när dessa mindre föreningar görs otillgängliga igen, genom att växter eller mikroorganismer tar upp dem. Om mineraliseringen är större än immobiliseringen kommer det finnas ett överskott av kväve, medan kväve är begränsande om immobiliseringen är större än mineraliseringen. En annan viktig process är nitrifikation, som omvandlar ammonium till nitrat. Nitrifikationen styr ofta hur mycket kväve som släpps ut från ett ekosystem, eftersom kväve i nitratform ofta försvinner ut från ekosystemet. Antingen sker detta genom läckage till grundvattnet, eller genom omvandling till lustgas eller N_2. När nitrat omvandlas till N_2 sluts kvävekretsloppet igen. Mycket med kvävekretsloppet är fortfarande okänt, trots att man länge har vetat om ungefär vilka processer som ingår. Eftersom mycket tyder på att klimatet på jorden håller på att förändras med människans koldioxidutsläpp till i atmosfären, är det också viktigt att förstå vad som händer med kvävecykeln.



Figur 24. Schematisk bild avhur nedbrytning går till. Ett protein är en stor molekyl som består av långa kedjor av aminosyror som är ihopkopplade med varandra. Proteinet bryts ner till mindre kedjebitar (peptider) i en process som kallas depolymerisering. Peptiderna bryts sedan ner ytterligare till fria aminosyror. Aminosyror är organiska föreningar (de innehåller grundämnet kol, C), men i nästa steg sker mineralisering till ammonium och kvävet övergår då till oorganisk (mineral) form. Ammonium är den form av kväve som många växter och mikroorganismer föredrar, men växter kan också ta upp fria aminosyror direkt.

En del tror att reaktivt kväve kanske kommer att bli mer otillgängligt i t.ex. skogsekosystem när det blir mer koldioxid i luften, eftersom träd då ökar sitt koldioxidupptag och kan växa mer effektivt. Om detta sker kommer mer kväve bindas in i träden och fortsätta vara otillgängligt för mikroorganismer och andra växter.

Metoder

Vi har undersökt omvandlingarna i kvävekretsloppet i s.k. naturliga ekosystem (skogar och hedmark) för att ta reda på vilka processer som är viktiga i dessa system och vad i miljön som påverkar dem. Vi har tagit jordprover från olika slags ekosystem och sedan gjort laboratorieexperiment där vi tittat på kväveomvandlingarna. För att undersöka detta har vi använt stabila isotoper. Isotoper är varianter av grundämnen som har samma egenskaper men olika atomvikt. Kväve har två stabila (icke radioaktiva) isotoper, ^{14}N och ^{15}N. 99.6% av allt kväve man hittar i naturen är ^{14}N, vilket gör att ^{15}N kan användas som en ”spårare” i experiment, eftersom den är så ovanlig. Det funkar ungefär som en slags infärgning. Eftersom isotoperna väger olika mycket kan man senare skilja på dem i analyser. Och när man tittar på den här ”infärgningen” över tid kan man räkna ut hur snabbt de olika processerna i kvävekretsloppet går. Det kan man både göra för hand, med hjälp av en s.k. analytisk modell, och med hjälp av dator i en s.k. numerisk modell. I en analytisk modell kan man bara räkna ut en hastighet åt gången medan numeriska modeller kan räkna ut flera hastigheter samtidigt. Vi har använt en numerisk modell eftersom det är det som efterliknar verkligheten bäst.

Resultat

Vi undersökte klimatförändringarnas effekt på kvävekretsloppet i ett fältexperiment där man simulerat hur man tror att klimatförändringarna kommer vara i Danmark år 2075. I experimentet blev ytor behandlade med högre koldioxidhalt, värme och torka (genom utestängning av regnvatten). Vi såg att omsättningen av kväve var annorlunda på de ytor som fått en ensam klimatbehandling (t.ex. ökad koldioxidhalt) jämfört med de ytor som fått en kombination av två eller flera behandlingar (t.ex. högre koldioxidhalt i kombination med tork- och värmebehandling). Det visar att det är viktigt att göra experiment med flera klimatbehandlingar i kombination när man undersöker klimatförändringar, eftersom det finns många mekanismer som samspelar och motverkar varandra (**artikel I**). Vi såg också att nitrifikationen i en norrländsk skog begränsades av kraftig kvävegödsling (**artikel II**). Vad detta beror på kan vi inte svara på i denna studie, men det skulle kunna ha att göra med att nitrifikationen i detta vanligtvis näringsfattiga ekosystem sköts av svampar, och att dessa blir negativt påverkade av hög kvävehalt. I ett trädslagsexperiement, där man planterat skog med olika trädslag såg vi att kväveomsättningen skiljer sig mellan granskog och bokskog (**artikel III**), framför allt i omsättningen av nitrat, som är mycket högre i bokskogen. Vi såg också att vilken typ av organiskt material som mineraliserades

skilde sig mellan olika jordar (**artikel IV**), vilket förmodligen beror på mängden nedbrutet organiskt material (humus).

Avhandlingen tar också upp några svårigheter med att genomföra dessa experiment, särskilt i jordar där det finns väldigt lite kväve. Ett av de största problemen är att man måste förändra systemet för att kunna undersöka det. Eftersom man tillsätter kväveisotoper till jorden kan det göra att mikroorgansimerna blir mer aktiva, vilket gör att hastigheterna blir överskattade, vilket vi såg i **artikel II**. Ett annat problem gäller det s.k. återfinnandet (”recovery”) av den tillsatta kväveisotopen. För att kunna mäta isotopsammansättningen måste man kunna få ut all tillsatt kväveisotop från jorden igen en kort stund efter man har tillsatt den. Det gör man genom extraktion, där jorden blandas med en saltlösning. Det mineraliska kvävet (ammonium och nitrat) i jorden hamnar då i lösningen, och sedan filteras jorden bort. Men vi har sett att en stor del av kvävet inte går att extrahera ut igen, utan stannar kvar i jorden, trots att vi tillsatt saltlösningen direkt efter tillsättningen av kväveisotopen. Det kan bero på att mikroorganismer snabbt tagit upp kvävet, eller att det fastnat (adsorberats) på lerpartiklar eller organiskt material i jorden. I **artikel II** såg vi att ju mer kväve som fanns i jorden från början, desto mer av det tillsatta isotopkvävet gick att extrahera ut igen. I **artikel III** testar vi olika sätt att korrigera för om inte all kväveisotop kan extraheras ut eftersom det kommer att påverka resultaten. I praktiken kan båda dessa problem innebära att man i vissa jordar inte kan utföra denna typ av experiment med dagens metodik eftersom kvävehalterna är så låga och man förändrar systemet för mycket genom att undersöka det.

Acknowledgements

TACK / THANK YOU / TAK / BEDANKT / KIITOS:

Min handledare **Tobias Rütting**, för att du trodde på mig och gav mig möjlighet att göra det här projektet. Tack för ditt tålamod och all praktisk hjälp och stöttning genom åren. Min bihandledare **Leif Klemedtsson**, för din entusiasm och ditt kontaktnät, vilket gör att du ibland får saker att hända inom minuter, det är inspirerande! Min examinator **Håkan Pleijel**, för att du har funnits i bakgrunden hela tiden, inte direkt inblandad i mitt projekt, men det har alltid känts som att jag kan vända mig till dig om det skulle vara nåt. Min mentor **Sofia Thorsson**, som kom in sent i mitt arbete men i precis rätt tid. Trots (eller kanske tack vare?) att det inte är ditt forskningsområde har jag fått nya insikter och perspektiv på min forskning av dig, och jag har sett fram emot våra luncher!

Per Ambus, thanks for giving me the opportunity to come to Risø and for your support. I really learned a lot. **Anja Nielsen**, **Nina Thomsen**, **Mette Flodgaard** and **Bente Andersen** are greatly acknowledged for helping me out in the lab (and for being great people) and **Sabine Reinsch** and **Petra Lachouani** for interesting discussions and company. Great thanks also **to Klaus Steenberg Larsen** who have answered tons of questions about Brandbjerg and who provided me with photos from the site. **Lars Vesterdal**, **Per Gundersen** and **Christian Riis Rasmussen** are greatly acknowledged for valuable input and practical help with work in Vallø. **Maija Marushchak**, **Jenie Gil**, **Christina Biasi**, **Richard Lambert**, **Maarit Liimatainen** from the University of Eastern Finland, Kuopio, thank you for your collaboration, it was great to find other people struggling with the same type of methodological problems.

Pascal Boeckx and **Samuel Bodé**, thank you for good input and ideas. **Stijn Vandevoorde**, for analyzing a lot of amino acid samples. And a very special thanks to **Katja van Nieuland** – for preparing and analyzing the majority of my samples (I know how much work it is!) and for putting up with many detailed questions about the methods. Thank you also for being an amazing person, always helpful, funny and enthusiastic, and for cheering me up after I crashed a 5 liter volumetric flask with calcium chloride in your laboratory. Again: I'M SORRY! (I still think about this incident every time I prepare chemical solutions).

Från SLU: **Charlotta Erefur**, **Hjalmar Laudon** och särskilt **Ulla Nylander** för fältprovtagningen i Flakaliden. Tack också till **Sune Linder**, som alltid är redo att svara på minsta fråga om gödslingsförsöket, samt **Joachim Strengbom** för dina foton.

All my colleagues who have brightened my working environment over the years, in no particular order: **Anna Hedenrud**, **Thomas Berg Hasper**, **Angelica Lorentzon, Maria Grundström**, **Brigitte Nyirambangutse**, **Lisa Adolfsson**, **Johanna Bergkvist**, **Shubhangi Lamba**, **Philipp Schleusner**, **Ruud Scharn**, **Ardo Robijn**, **Hongxing He**, **Frida Lindstein**, **Lorenzo Minola**, **Aifang Chen**, **Janka Konarska**, **Juliane Kellner, Yumei Yu**, **Tinghai Ou, Shiho Onamura**, **Mauricio Fuentes**, **Mohammad Aurangojeb**, **Axel Sjöqvist**, **Robert Björk**, **Johan Uddling Fredin**, **Sophie Rychlik**, **Michelle Nygren**, **Marcus Giese, Bengt Liljebladh**, **Mats Olvmo**

Louise Andresen, tack för gott samarbete i labbet och i fält. **Per Weslien** och **David Allbrand**, jag är väldigt tacksam för alla små och stora saker ni hjälpt mig med genom åren. **Vivian Aldén** och **Aila Schachinger** för all hjälp i labbet, både med analys och problemlösning. Tack också **Josefina Carlberg,** jag har lärt mig mycket av dig om analysinstrument (och att man inte behöver vara så rädd för dem!). **Åsa Kasimir**, för att du introducerade kvävecykeln för mig när jag var student på miljövetarprogrammet. Det öppnade verkligen en ny värld för mig, trots att jag inte visste det då!

Stort tack till er som hjälpt mig i slutet av skrivprocessen: **Ezra Haaf**, **Gustaf Peterson**, **Lina Rasmusson**, **Magdalena Wallman** och **Mats Björkman** som alla läst min avhandling och gett konstruktiva kommentarer och råd (och extra tack till Mats för att du är en klippa överlag!). **Nils Wallenberg** som slängde ihop en GIS-karta på tre minuter, det hade tagit mig en vecka att göra samma sak. **Sofia Rybrand**, som tecknat den fina omslagsbilden (och som blev entusiastisk av att teckna jord, det gjorde mig glad!).

Therese Brolin, tack för att du bara funnits där och satt lite perspektiv på saker. Jag saknar dig i Göteborg! **Emelie Lindquist** och **Tage Vowles**, mina vänner som jag hittade genom doktorerandet, utan er skulle de här åren varit så mycket tråkigare. Och Tage, tack för all språkgranskning du har gjort åt mig.

Mamma och **pappa**, som alltid ställt upp för mig och varit stolta. Tack vare er envishet med fjällvandringar och tältsemestrar blev jag till slut genuint intresserad av naturen, även om det tog lite tid. **Inger** och **Stig Brolin**, för att ni finns där och alltid ställer upp för mig och vår familj. Särskilt tack till Inger för alla fredagar du varit med barnen för att jag skulle kunna skriva. **Josefina Fogelin**, käraste vän, långt borta men alltid i mitt hjärta (och i min telefon 😘). Ingen muntrar upp mig som du efter en dålig dag på jobbet.

Och **Andreas**, min klippa, bästa vän och partner. Utan ditt stöd, uppmuntran och det faktum att du alltid gör ditt yttersta för oss och vår familj hade jag inte fått ihop det! Och **Ivar** och **Sally**, älskade skruttisar, ni tvingar mig att vara i nuet och inte grubbla så mycket över jobbet. Tack vare er vet jag vad som *verkligen* är viktigt. ♥

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