UNIVERSITY OF GOTHENBURG

DEPARTMENT OF BIOLOGICAL AND ENVIRONMENTAL SCIENCES

SOIL GROSS NITROGEN MINERALISATION AND FOREST GROWTH IN FOUR HEMIBOREAL FOREST STANDS IN SOUTHWEST SWEDEN

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Front page photo; Linnéa Eriksson, Site S3, "Kaserna", Skogaryd Research Catchment, March 2022.

ABSTRACT

Boreal and temperate forests together make up the largest terrestrial net C sink in the world. They take up carbon dioxide (CO_2) from the atmosphere and store it in plant biomass and soil as they grow, making them crucial in mitigating global climate change. In addition, as climate is warming, the boreal C sink is expected to increase, provided that tree growth is not restricted by e.g. nutrient, especially nitrogen (N), supply. N is an important macronutrient for plants, and is made bioavailable through microbial N mineralisation during decomposition of soil organic matter (SOM). How well microorganisms are able to decompose SOM depends on its elemental ratio between C and N (C:N ratio), making this ratio a measure for site fertility. As a result, both gross N mineralisation and tree growth have been linked to the C:N ratio of soil and litter. Despite this, research on the link between gross N mineralisation rates and forest tree growth is lacking, while methods for estimating gross N mineralisation are more inefficient regarding time and resources compared to measuring soil C:N. Thus, this study investigates the possibility of using soil C:N as a proxy for gross N mineralisation, as well as gross N mineralisation as a driver of tree biomass production, using the ^{15}N pool dilution technique combined with a circular plot forest inventory method commonly used in forest management practices. Field work was conducted in four Norway Spruce dominated forest stands in southwest Sweden, representing a soil fertility gradient, with mean soil C:N ratios ranging between 17-30. Across three of the four forest stands, there were clear relationships between the three parameters. Low soil C:N corresponded with high gross N mineralisation rates, which in turn correlated positively to the estimated biomass production rates. However, these correlations were only significant after accounting for soil C concentrations when calculating the rates of mineralisation. This suggests that the observed positive relationship between gross N mineralisation and tree growth could be applied both ways; that increased concentrations of bioavailable N promotes forest biomass production, while increased tree growth enables higher rates of SOM decomposition and N mineralisation following the addition of labile C to the soil though root exudation (‘priming’). Contrasting this, at the fourth site, the relationship between soil C:N and N mineralisation was close to parallel to that of the other three sites, but elevated, while the connection between gross N mineralisation and biomass growth was inverted; increasing rates of mineralisation were followed by a decrease in biomass production, with a possible explanation for this lying outside the scope of this study. Therefore, based solely on the results found in this study, soil C:N ratio cannot be used as a proxy for gross N mineralisation, nor is it possible to declare gross N mineralisation as the main driver of biomass production. Instead, to close the knowledge gap of how forest ecosystem C sequestration and the forest soil N cycle is connected, this study highlights that more research is required.

Keywords: soil C:N; forest production; ^{15}N isotope dilution; gross N mineralisation; ammonium; soil carbon; soil nitrogen; root exudates; stoichiometric decomposition; boreal forests; priming; microbial C limitation

POPULAR SCIENCE SUMMARY - WHAT MAKES TREES GROW?

Today it is commonly known that forests are important regulators of global climate. They 'breathe in' carbon dioxide (CO_2) from the atmosphere and store it in plant biomass and soil as they grow, making them crucial in mitigating global climate change. In Sweden, these systems are a key, while much-disputed, part of the environmental objectives and climate goals in the country, as well as the EU. But what controls how much trees grow? In addition to factors such as weather, climate and water supply, we find part of the answer by taking a look at the secret lives of soil microorganisms.

The role of the soil nutrient supply

Much like you and I, apart from suitable weather and climate conditions, sufficient water supply and as little stress and disturbances as possible, plants and soil microorganisms need nutrients to grow. How effectively trees create biomass and store C largely depends on the supply of nitrogen (N) in the soil. However, soil N is often bound in large organic molecules that are not available for the trees to take up. To access this N through the root system, it first has to be broken down into smaller, more easily accessible inorganic forms, like ammonium (NH_4^+). This is done during decomposition of soil organic matter (SOM) by soil microorganisms, or microbes, through a process called N mineralisation. How well microorganisms are able to break down SOM, and thereby release inorganic N for plant use, in turn depends on its ratio between C and N (soil C:N). A low soil C:N ratio implies there is enough N to exceed microbial demand, allowing microbes to release inorganic N to the soil, increasing the N supply accessible by plants. Is this process, N mineralisation, the main driver of plant growth? In context of the importance of trees in regulating climate change, I wanted to know more.

The research

One morning in July, 2022, I got into my car to go to Skogaryd Research Catchment, located between Uddevalla and Vänersborg in southwest Sweden. I went there to perform a series of experiments on how the gross rate of N mineralisation differed in the soil between four managed Norway Spruce dominated forests of varying N availability. Earlier the same year, I had been to the same forests to inventory how much tree biomass that had grown there since they were planted. I wanted to examine the connection between the process making N available for plants to take up (N mineralisation) and the growth rate of the trees, as well as look into the possibility of using soil C:N as a proxy for gross N mineralisation rates. Contrary to my initial belief that the N mineralisation rate would be the main driver of tree growth, it turns out that the relationship between the two processes instead might be mutualistic.

A two-way relationship regulated by the soil carbon concentration

Across three of the four forests, it was clear that low soil C:N was connected to high gross N mineralisation rates. These rates were in turn positively correlated to the estimated rates of tree growth at the sites. This all indicated that a higher nutrient supply means higher soil fertility and better conditions for tree growth. But, this was true only after accounting for the different soil C concentrations at the sites in the analysis. The significant influence of soil C on the results points to the possibility of the relationship between tree growth and N mineralisation not only working one way, but both. First, when more N is made available to trees, they can take up and store more C that makes them grow. Second, as they grow, they continuously release part of the C they take up to the soil through their roots. This C is easily accessible to microbes in the soil, providing them with energy needed for decomposition of SOM. Thus, with this extra energy source, microorganisms can break down SOM more effectively, resulting in faster rates of N mineralisation – a phenomenon called '*priming*'.

Contrasting results demands for further investigations

At the fourth site, the relationship between soil C:N and N mineralisation was similar to what I found at the first three sites, but the rates of mineralisation were a lot higher. In addition, I found the connection between gross N mineralisation and biomass growth to be inverted, meaning faster N mineralisation rates were related to a decline in tree growth. So, what does this mean? It certainly does not agree with the idea of a higher nutrient supply leading to faster tree growth. Instead there must be some other factor limiting tree growth at the site, that is beyond the scope of this investigation. Thus, the inconsistent results that emerged from my investigations reject the use of soil C:N ratio as a proxy for N mineralisation, as well as N mineralisation as the main driver of tree growth. Instead, to close the knowledge gap of how forest C storage and the forest soil N cycle is connected, this study highlights the need for further research on this subject.

1. Introduction

Forests cover approximately one third of the terrestrial surface of earth (World Bank Group, n.d.). Currently, these ecosystems act as carbon (C) sinks (e.g. IPCC, 2021; Alberti et al., 2015; Tagesson et al., 2020; Blaško et al., 2022), capturing C from the atmosphere through the process of photosynthesis and storing it in biomass and soil (Pan et al., 2011; Sponseller et al., 2016; Cronan, 2018; Blaško et al., 2022). The boreal forest biome makes up 10-15% of the terrestrial land surface, and store approximately 30% of the global forest C stock (Peichl et al., 2022). This is today, combined with temperate forests, the largest net terrestrial C sink, according to a study by Pan et al. (2011).

Through their ability to sequester C, forests play a major role in the global C cycle, as well as for mitigating global warming, as they are an important part of future *Carbon dioxide remova*l (CDR; IPCC, 2021). Beyond storing C in the living ecosystem, the biomass produced in forests can be used to produce bioenergy, as well as a replacement for materials that demand more energy and lead to larger emissions of CO_2 during production (Swedish Forest Agency, 2021). In Sweden, about two thirds of the surface area consist of forests, making these systems' capacity to sequester C an important, and much-disputed, question connected to mitigating climate change, as well as the environmental objectives and climate goals in Sweden and the European Union (Swedish Environmental Protection Agency, n.d.a, n.d.b). Over the last century, the Swedish forest C sink has increased (IBFRA, 2021), as part of the global terrestrial C sink, which has grown with emissions from human activities since the mid 1900's. The global increase since the 1980s has been attributed to CO_2 fertilisation by the IPCC (2021), meaning an enhanced CO_2 uptake by vegetation as a result of increased levels of atmospheric CO_2. This phenomenon, together with a prolonged growing season resulting from increased air temperatures, could increase the boreal C sink both in plants and soils even further over the coming centuries (IPCC, 2021).

Recent studies show that trees effectively capture and store C throughout their lives as their gross volume increment continues to be stable even at old ages, contrary to previous beliefs (Stokland, 2021; Stephenson et al., 2014). How well trees grow depends on many factors, such as weather and climate, water and nutrient supply, competition between individuals and species, and how sunlight reaches the canopy and foliage, etc. Naturally, these factors, among others such as pests, extreme events and wildfires, can also restrict the growth of the trees, either combined or separately, to different degrees. In higher latitudes, nitrogen (N) has been shown to be one of the most limiting factors to tree growth. It is an important macronutrient for plants as it is an essential building block for chlorophyll, enzymes, proteins, as well as RNA and DNA (Cronan, 2018). Thus, the availability of N, especially in inorganic forms, in soil is highly related to the amount of C that can be taken up and stored in these ecosystems (e.g. Du et al., 2020; Sigurdsson et al., 2013; Sponseller et al., 2016).

There are many examples of how nutrient availability highly constraints tree productivity. In fact, in a study by Vicca et al. (2012) nutrient availability was shown to have a significant impact on the biomass production efficiency (BPE, the ratio of biomass production [within one year] to Gross primary production [GPP]), compared to e.g. climate zone, stand age and forest type which did not have a significant effect. Sigurdsson et al. (2013) reported no effect of elevated CO_2, or air warming on the growth of mature Norwegian Spruce in a Swedish experiment if the trees were not also fertilised. In another study, Strömgren and Linder (2002) showed that a 5^{o}C warming of the soil had a strong positive effect on tree growth, likely due to the increased decomposition and mineralisation rates that follows higher temperatures, which led to increased availability of inorganic N (Strömgren & Linder, 2002; Booth et al., 2005; IPCC, 2021). Terrer et al. (2016) revealed N availability to be the most important

predictor for enhanced plant growth under increased atmospheric CO_2. The actual CO_2 increase only came in third, after plant associated mycorrhizal type, ensheathing the fine root tips of plants and providing these with nutrients. In addition, many climate models now include the terrestrial N cycle in their predictions and show that this results in a lower CO_2 fertilisation effect on C storage of 25-30%, compared to models that do not include the N cycle. Even though results generally fit well with single observations, the data in these models are derived from too few observations due to limitations in e.g. geographical distribution of experiments and scaling problems (IPCC, 2021). Thus, there is a need for more research on the interaction between soil nutrient availability and forest C sequestration capacity, to increase our understanding of how this interaction might change with future environmental changes, as well as changes in climate.

The main source of bioavailable N is through recycling of N in dead organic matter (Cronan, 2018). Even though the nutrients required are present in the soil, the majority are in recalcitrant forms of polymers that are too large to be directly available for uptake by biota (Schimel & Bennett, 2004), e.g. in form of lignin, chitin and protein (Wild et al., 2019). In order to utilise these nutrients, microbes produce and release extracellular enzymes that depolymerize and break down soil organic matter (SOM) into smaller, labile forms (Olser & Sommerkorn, 2007; Schimel & Bennett, 2004). How well microorganisms are able to decompose organic matter is dependent on its elemental ratio between C and N (C:N ratio), which is the C concentration divided by the N concentration (mass or molar; Peichl et al., 2022). Like plants and other organisms, microbes demand C and N in specific ratios to facilitate their metabolism and growth (Olser & Sommerkorn, 2007; Bengtson et al., 2012), and research has found that, while tree growth is often limited by N, microbes are often limited by C (Bengtson et al., 2012; Philips, et al., 2011). Thus, microbes need labile forms of C, such as C in dissolved organic matter (DOM), to use as an energy source for the production and release of these exo-enzymes required for SOM decomposition (Olser & Sommerkorn, 2007; Schimel & Bennett, 2004; Dijkstra et al., 2013). One important labile C source is C released through root exudation, which relieves the C limitation that restricts SOM decomposition, ultimately aiding in breaking apart the unavailable polymers and making them available for biological uptake (Olser & Sommerkorn, 2007; Sponseller et al., 2016).

There is continual competition for nutrients in the soil between microorganisms and vegetation. During decomposition of SOM, N in organic matter (Norg) is mineralised and transformed into bioavailable, inorganic, ammonium (NH_4^+). The NH_4^+ can then be assimilated into microbial or plant biomass (Cronan, 2018). Hence, the rate at which mineralisation of Norg can occur is also connected to the C:N of soil and litter (Olser & Sommerkorn, 2007). Low C:N ratios mean there is more N available per unit C, which in turn often means that there is a surplus of N above the microbial demand. Low soil C:N therefore indicates a more fertile soil (Peichl et al., 2022), as the competition for N in the soil is lower. When there is a surplus of Norg compared to the microbial need for N, mineralised NH_4^+ is released to the soil, making it available for plant uptake (Olser & Sommerkorn, 2007).

The negative relationship between soil gross N mineralisation rates and C:N ratio of litter and soil has been observed in many studies (Accoe et al., 2004; Booth et al., 2005; Mooshammer et al., 2012). Further, research has found the elemental ratio threshold for when there is a surplus or deficit of N compared to microbial demand to be at a resource C:N mass ratio of 20-30. Above this threshold, the majority of the soil N supply would be immobilised into microbial biomass, and below it, net N mineralisation would occur, releasing NH_4^+ into the environment (Mooshammer et al., 2014; Cronan, 2018). Estimating gross N transformation rates is more complicated, costly and inefficient regarding time and resources, compared to measuring soil C:N (Davidson et al., 1992; Cronan, 2018). Thus, using soil C:N as a proxy for gross soil N mineralisation rates would provide an easy and cost effective way to estimate this process rate.

Following the higher soil fertility connected to lower soil C:N ratios, the soil C:N ratio is also linked to the productivity of a forest (Kranabetter et al., 2020; Peichl et al., 2022). Alberti et al. (2015) reveal in their study a direct relationship between forest GPP and the carbon-to-nitrogen (C:N) ratio of the forest soil. The study concluded that a low C:N ratio is connected to higher forest production. Additionally, Peichl et al. (2022) discovered net primary production (NPP) of trees to be the most important driver of annual net ecosystem production (NEP) in managed boreal forests in Sweden, with soil C:N having a significant influence on both variables. Yet, studies investigating the relationship between gross N mineralisation and forest biomass production are lacking.

Taken together it is evident that, to understand the cycling of either C or N, it is required to take the behaviour and impact of the other into consideration (Bengtson et al., 2012; Reich et al. 2006; Högberg et al. 2010). Studying these relationships is of great value for increasing our knowledge of how the carbon and nitrogen cycles interact with each other, both currently and in a changing climate, to be able to make accurate estimations of current and future possible C storage in terrestrial ecosystems.

1.2 Aim & Objectives

This study aims to expand our knowledge of the C and N cycles in terrestrial forests. The goal is to understand the connection between nutrient availability and transformation in forest soil, and forest growth. Through ^{15}N pool dilution experiments, combined with forest inventory methods, the influence of the soil C:N ratio on soil gross N mineralisation, as well as the impact of this transformation process on forest biomass production is investigated. The research is conducted in four Norway Spruce dominated forest stands in southwest Sweden, representing a soil fertility gradient, with the objectives to examine if:

1. Soil C:N ratio can be used as a proxy for soil gross N mineralization
2. A higher soil gross N mineralization rate leads to increased forest biomass production

and, thus, test the hypothesis that the process controlling soil inorganic N availability (gross N mineralisation) is in direct relation to soil fertility and forest production.

2. Background

2.1 Soil processes regulating soil N transformation

N exists in the soil in different organic and inorganic forms, making up a number of distinguishable soil N pools. There are many different processes regulating the transformation between and turnover of these N pools (figure 1). Mineralisation of Norg into NH_4^+ occurs during decaying of organic matter, when microbes decompose detritus. Through this process, organic compounds are subject to depolymerisation, meaning large, unavailable polymers are broken down into monomers, e.g. amino acids. These are sequentially, through enzymatic hydrolysis, converted to ammonia (NH_3), which is then transformed into NH_4^+ (Cronan, 2018; Schimel & Bennett, 2004). The free NH_4^+ ions can then either be taken up by and assimilated into plant material, immobilised by and assimilated into microbial biomass, transported through the soil through leaching or adsorbed onto cation exchange sites or other forms of mineral bonds. Another possible pathway for the NH_4^+, as well as Norg, is to be oxidised into NO_3^-, through the process of nitrification. NO_3^- is also available for plant uptake, but is, at the same time, a lot more mobile compared to the conservative NH_4^+, which makes it more susceptible to leaching if not assimilated into plant material. Consumption of NO_3^- also occurs through denitrification, which releases gaseous forms of N into the atmosphere, or dissimilatory nitrate reduction to ammonium (DNRA), which, in turn, also is a production process of NH_4^+ (Cronan., 2018).

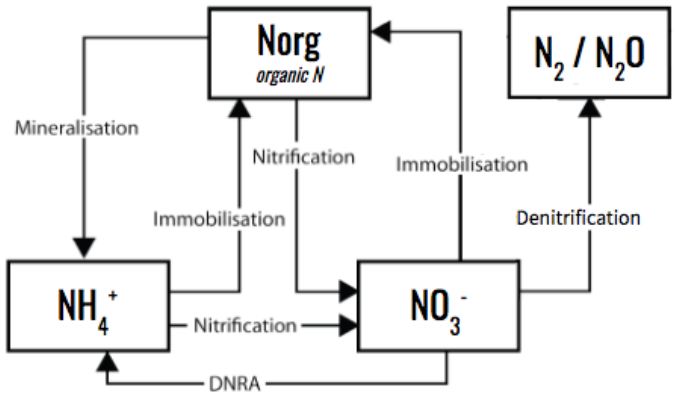

Figure 1. Simplified schematic map of transformation processes in the soil N cycle (Carlsson & Eriksson, 2017).

Huygens et al. (2013) define gross N transformation rates as "*the unidirectional flow of N from one soil N pool to another*" (p. 573). Studying gross rates provides a more complete view on the N cycle than net rates, which a majority of previous research done on transformation rates of N in soil are based on (Davidson et al., 1992). Net transformation rates are the production of a specific form of N minus the consumption of it, or the sum of inflow and outflow of N into and out of a specific N pool (Cronan, 2018). However, the net transformation rates do not necessarily reflect the gross transformation rates, as they can be counteracted by the consumption processes at the specific site. Therefore, looking at net rates will restrain the knowledge of the soil N cycle, as no real estimation of the specific production process can be made (Davidson et al., 1992; Bauters et al., 2019). Davidson et al. (1992) show a clear example of this in a study comparing net and gross mineralization rates in a young conifer plantation and a mature conifer forest. The results show a poor correlation between the rates, as well as net mineralization rates below 14% of the gross rates. Concurrently, there were higher net mineralisation rates in the young plantation compared to the mature forest, where gross mineralisation rates in turn were 2-3 times higher than in the young forest. Results like these emphasise the need to study gross transformation rates rather than net rates, to avoid underestimating the actual rates of mineralization.

2.2 Plant-microbe interactions affecting N supply to trees

The rhizosphere, which is the narrow zone of soil directly surrounding the root system of a plant (Cronan, 2018) supports a large part of the microbial activity in the soil. Here, there is continuous exchange and competition for resources between plants and microbes. Further, the fine root system of most plants is highly associated with different species of mycorrhizal fungi. In northern forests, this association is dominated by ectomycorrhizal (ECM) fungi. The fungi ensheath the majority of the fine root tips, where they receive carbohydrates from the tree. In exchange, they provide their plant host with water and nutrients, as well as produce and release the extracellular enzymes needed for decomposition of SOM (Terrer et al., 2016). Studies show that up to 80% of plant N in boreal forests can be derived from symbiotic fungi (van der Heijden et al. 2008). Though, how much N they provide is depending on the nutrient status of the soil. Studies show that high C exudation rates, which occurs when the soil N supply is limited, increase the fungal N sink, so that a larger fraction of the N the fungi take up is immobilised in its biomass, instead of transferred to the tree. In situations where the plant N supply is higher, root exudation rates decrease, and the ECM fungi transfer a larger fraction of N to the tree. This balance is proposedly sustained through the constant resource competition between the organism (mycorrhizal fungi or plant) and its surroundings, keeping enough nutrient (C or N) to sustain their own growth, but releasing enough to stay an attractive partner in the symbiosis (Högberg et al., 2017).

Rhizodeposits from plant roots also impact the behaviour of other microorganisms, not only mycorrhizal fungi. Despite many studies showing there is a link between root exudates, rhizosphere priming and N bioavailability, the underlying reasons and mechanisms for this are not unequivocal (e.g. Chen et al., 2013; Meier et al., 2017; Bengtson et al., 2012, Wild et al., 2019; Keiluweit et al., 2015; Moreau et al., 2019). One hypothesis commonly discussed in the literature is that of ‘microbial N mining’, where the labile C input resulting from root exudation acts as an important energy source for synthesising extracellular enzymes needed for SOM decomposition (Chen et al., 2013) and thereby mineralising N (Dijkstra et al., 2013). In N deficient ecosystems, such as many boreal forests (e.g. Schimel & Bennett, 2004; Olser & Sommerkorn, 2007; Högberg et al., 2017), plants tend to allocate more C to belowground biomass (Bengtson et al., 2012) to increase their uptake of nutrients limiting their growth (Phillips et al., 2009; Cronan, 2018). This would lead to an increase in root exudation and labile C release (van Groenigen et al., 2015; Bengtson et al., 2012), with the possibility of subsequent rhizosphere priming of microbial SOM decomposition rates (Chen et al., 2013), which in turn lead to increased rates of gross N mineralisation (Bengtson et al., 2012; Drake et al., 2011; Meier et al., 2017; Dijkstra et al., 2013). At the same time, if the N limitation is severe, the additional C transferred to mycorrhizae could strengthen the fungal N sink and, thus, lead to a greater N immobilisation by the fungi, instead of an increased N flow to the plant. This could ultimately lead to a decrease in bioavailable N, rather than an increase (Högberg et al., 2017).

Another theory for what is driving the priming of SOM is that of ‘stoichiometric decomposition’. This theory implies that microbial activity, and thereby SOM decomposition rates, is highest when C and N availability fits the microorganisms’ specific stoichiometric nutrient requirements. Contrary to the ‘microbial N mining’ hypothesis, which would be valid in cases of low soil N supply, the ‘stoichiometric decomposition’ theory would be applicable *also* where N availability is high. In other words, the first would be valid in cases where tree growth is limited by N, the second when microbial growth is limited by C (Chen et al., 2013).

2.3 Defining ^{15}N-labelling and -dilution

The atomic mass of an element is determined by the sum of the number of neutrons and protons in its nucleus. The number of neutrons can vary between atoms of the same element, making up different elemental isotopes. For example, a nitrogen atom with seven protons and seven neutrons in its nucleus has an atomic mass of ≈14, whereas a nitrogen atom with seven protons and eight neutrons in its nucleus has an atomic mass of ≈15. These two isotopes, ^{14}N and ^{15}N, are stable, meaning they will not spontaneously disintegrate over time. Of the two, ^{14}N is lighter and naturally predominant in the soil, as it makes up 99.6% of all naturally occurring N (Cronan, 2018).

By adding ^{15}N as a marker in a specific molecular form to a N source- or product pool in the soil, the pool is *labelled* (Stark, 2000). Monitoring the fate of the ^{15}N enrichment makes it possible to quantify different gross rates of the N transformation processes occurring in the soil (Cronan, 2018). One method of stable isotope labelling commonly used is *^{15}N isotope dilution* (figure 2). As described by Kirkham & Bartholomew in 1954, the technique involves a labelled soil N pool (e.g. NH_4^+) acting as the product pool for the gross N transformation process intended to be quantified (e.g. mineralization). Over time, the natural inflow of newly produced ^{14}N into the product pool through this process will dilute the ^{15}N enrichment, decreasing its relative size compared to ^{14}N. Additionally, the pool size will be reduced due to consumption processes. By monitoring the changes in ^{15}N content at different time intervals, while assuming there is no discrimination between consumption of the tracer substrate and the native N pool in the soil, and that the transformation rates are constant during the time of incubation, the gross transformation rates can be estimated (Cronan, 2018; Booth et al., 2005; Huygens et al., 2013; Braun et al., 2018). However, important to note is that the dilution technique only measures the total gross production and consumption processes affecting the labelled soil N pool, and not the specific transformation processes separately. Thus, the ^{15}N enrichment has to be small enough for the assumption that, once consumed, the heavy isotopes will not be remineralised or transformed back into the labelled pool in other ways (e.g. through the process of DNRA) (Kirkham & Bartholomew, 1954; Huygens et al., 2013; Braun et al., 2018).

N isotope labelling experiments can be done in several ways, both in a laboratory environment and *in situ*. Laboratory incubations can either be done using intact soil cores collected from the field, or soils that have been subject to e.g. mixing, sieving, drying and rewetting, removal of roots, and cold storage. Even intact, field collected soil cores are often stored in a cold environment before ^{15}N labelling and incubation (Rütting et al., 2011). All these treatments affect the soil N transformation rates by altering associated factors in the soil, e.g. soil organic matter chemistry (Meier & Bowman, 2008), microbial communities (Zak et al., 2003), root biomass and rhizosphere response to the surrounding environment (Jackson et al., 2008; Frank & Groffman, 2009), and the mobility and size of soil N pools (Schimel & Bennett, 2004). Among the reported effects are increased production and consumption of NH_4^+ in mixed soils (Booth et al., 2006), promoted and inhibited turnover rates of NO_3^- and NH_4^+, respectively, as a result of cold storage and laboratory experiments of intact soil cores (Arnold et al., 2008), as well as disturbances of the link between soils, roots and associated microbial communities (Frank & Groffman, 2009), compared to undisturbed conditions *in situ*. The latter is also a problem when working in the field, utilising a method of *introduced soil cores*. With this technique, cylinders are inserted into the ground a given time before labelling (Davidson et al., 1991). This way some of the natural response you get from in situ incubation is kept intact at the labelling point, but the response from surrounding areas is lost (Rütting et al., 2011).

For results that mirror natural conditions as close as possible, as well as minimise disturbance of the soil, a method called "*virtual soil core*" injection is preferred. The method is based on a technique used

for examining amino acids in the soil and was developed for estimating gross N transformations rates with ^{15}N stable isotope experiments by Rütting et al. (2011). Through this method, the ^{15}N label is injected directly into the soil without seclusion. After labelling, the ^{15}N enriched area is marked to allow identification for later sampling before any litter is put back to avoid disturbance of natural soil processes and environmental responses. This way the exchange between the labelled soil, plant roots, microorganisms and other environmental factors are kept intact during the incubation period (Rütting et al., 2015; Rütting et al., 2011).

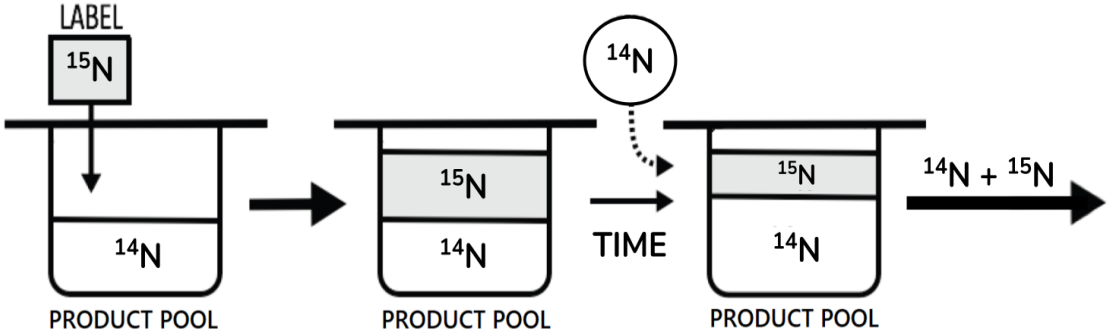

Figure 2. ^{15}N labelling (left) and dilution (middle and right). After the ^{15}N is added to the soil it will decrease in proportion to the ^{14}N naturally transformed into the specific N pool over time. Modified from Carlsson & Eriksson, 2017.

3. Materials & Methods

3.1 Study area: Skogaryd Research Catchment

The fieldwork that forms the basis of this study was conducted in four forest stands across a soil fertility gradient in Skogaryd Research Catchment (SRC) between March - July 2022 (table 1; figure 3). The catchment is located in southwest Sweden, between the cities Uddevalla and Vänersborg, approximately 100 km north of Gothenburg (58°22'N, 12°09'E, 79 m.a.s.l.).

The climate is hemiboreal with a mean annual temperature of 6.2°C, a mean annual precipitation of 709 mm, and ecosystems that can be found here are mires, forests, lakes and streams (University of Gothenburg, 2021; Swedish Infrastructure for Ecosystem Science [SITES], n.d.; Yang et al., 2020). The hemiboreal climate zone is located between the temperate and boreal zones, covering parts of Fennoscandia, the Baltic states, Poland, Belarus and Russia. These forests are characterised by their mixture of coniferous tree species (e.g. *Picea abies* and *Pinus sylvestris*) and deciduous tree species (e.g. *Betula* spp.), and are commonly formed by cultural and natural disturbances (European Environment Agency, 2007).

The research station in the catchment opened and has been run by the University of Gothenburg since 2013 as a part of SITES. Also involved in the research at SRC are LTER (long term ecological research) - Sweden, and ICOS (Integrated Carbon Observation System) Sweden (University of Gothenburg, 2022).

3.1.1 Selection of study sites

The four forest stands making up the study sites were selected in early March 2022, based on previous knowledge about the soil properties in the area (T. Rütting, personal communication, March 2, 2022). To ensure the sites represent a soil fertility gradient, 1-3 soil samples were taken within each forest stand, for initial C:N analysis. For further information about this analysis, continue to chapter *3.5 Soil C and N content*.

Table 1. Study site coordinates

Site	Coordinates
Stand 1 (S1)	58°21'47.0"N 12°08'56.0"E
Stand 2 (S2)	58°22'16.8"N 12°09'00.2"E
Stand 3 (S3)	58°21'17.4"N 12°09'25.0"E
Stand 4 (S4)	58°22'24.2"N 12°08'41.6"E

Three plots with a 15m radius were defined within each forest stand (A, B and C; figure 4), except for S1, where two plots were already marked out and used for forest inventory previously the same year. The locations for the plots were chosen randomly, except for efforts made to avoid any existing or previously used forest roads. The plots were later used for estimating forest biomass production, measuring the soil C and N content, as well as the C:N ratio, and for conducting stable isotope dilution experiments to estimate the gross N mineralisation rate. For estimations of biomass production at S1,

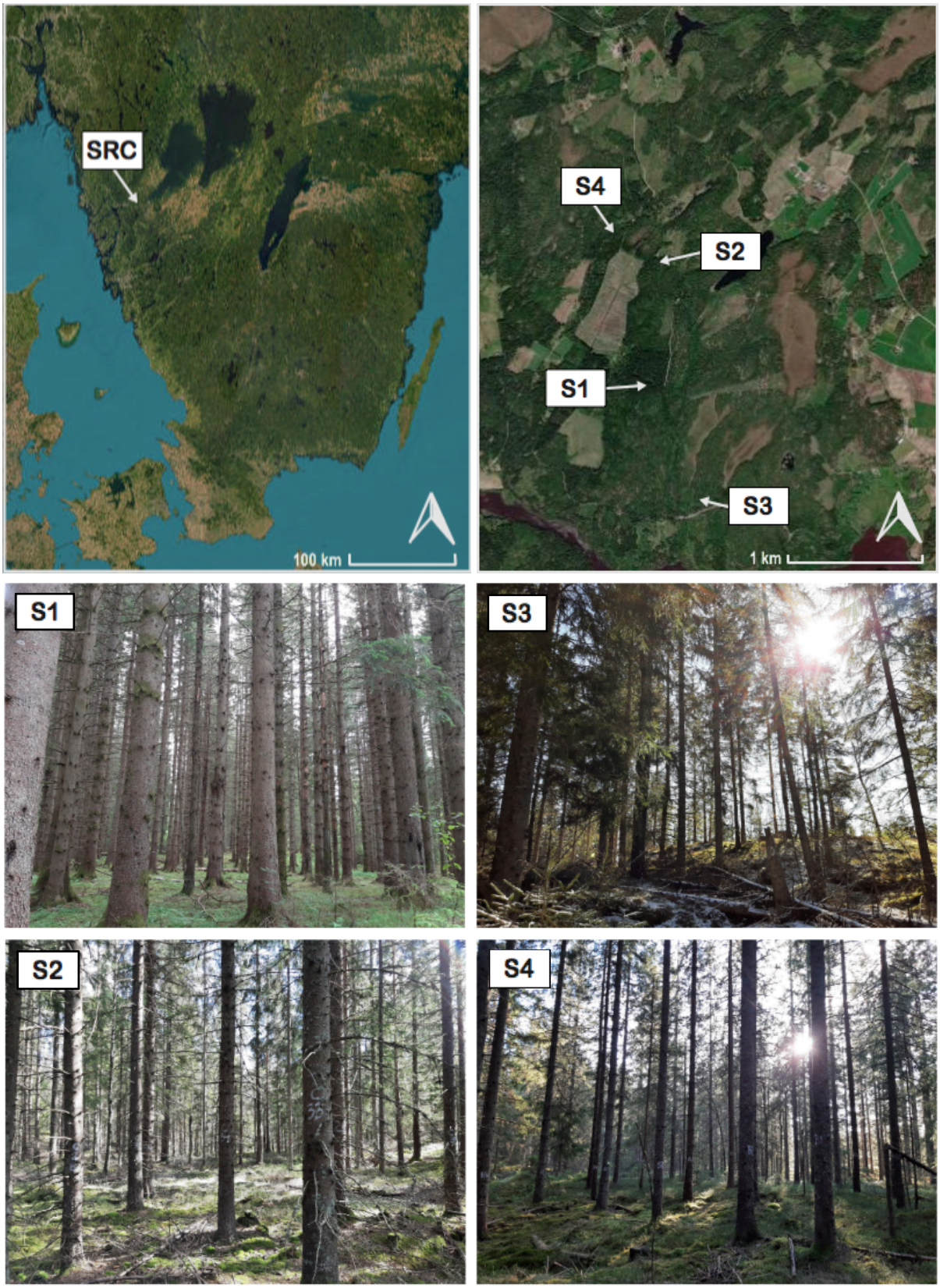

Figure 3. Maps showing the location of the study area (top left; Skogaryd Research Catchment [SRC]) and study sites (top right), and pictures of the four forest stands S1 (middle left), S2 (bottom left), S3 (middle right) and S4 (bottom right). Map source: modified from SITES - SITES Station Map (https://meta.fieldsites.se/station/?station=/resources/stations/Skogaryd&icon=). Photo source: Linnéa Eriksson, March 2022.

only data from the two pre-existing plots (S1A and S1C) were used, based on the spatial homogeneity of the tree growth at the site. As a result, plot S1B was excluded from all statistical analyses including biomass production. For soil C, N, C:N and soil gross N mineralisation, a third plot was defined between the two pre-existing plots (S1B).

S1 consists of a forest planted on former agricultural land on mineral soil. At the remaining sites the soil is more or less organic, with sporadic characteristics of podzols occuring at S2 and S4. The terrain at S1 and S4 is flat. At S2 and S3 the sampling plots are located uphill in a mixed terrain. In the end of 1700s and beginning of 1800s, parts of the forest in the area were used for coaling, and remnants of coal pits can be found in the soil at S4 (Hill, 1999). The overstory vegetation at all sites is dominated by Norway Spruce (*Picea abies*), with varying occurrences of Scots Pine (*Pinus sylvestris*) and Birch species (*Betula* spp.) (Table A1).

3.2 Estimation of forest biomass production

In this study, forest biomass is defined as the standing dry weight (d.w.) of living trees with a mean diameter at breast height (DBH = 130 cm above the ground) of ≥10 cm. An estimation of the above ground biomass was done using a basic circular-plot inventory method developed for forest management, slightly modified from Ranneby et al. (1987). Within each of the 11 plots, the DBH of all trees was measured by taking two measurements at a 90^{o} angle using a calliper. The mean of the two measurements was used as the input variable in the single tree biomass functions presented in Marklund (1988) and Petersson & Ståhl (2006). Thereafter, the d.w. of all measured trees within each forest stand were added and divided by the surface area of the inventory plots to get a value of d.w. biomass (ton) per area (t ha^{-1}) within each stand.

To estimate the forest biomass production, first the ages of the forest stands were estimated. Information provided in the forestry plan covering the area (L-G. Svensson, Fryxell - Langenskiöldska Foundation [Stiftelsen], personal communication referenced by T. Rütting, May 4, 2022), information based on soil fertility estimations made during the forest inventory (in Swedish: *Översiktlig skogsinventering [ÖSI]*) in 1991 by The Swedish Forest Agency (S. Sjöberg, personal communication, August 3, 2022), and aerial photographs provided by The Land Survey from 1965 (https://geolex.lantmateriet.se/), 1960 and 1975 (https://minkarta.lantmateriet.se/) were all reviewed and compared. The resulting best estimate of the age of each stand was then used to divide the value of d.w. biomass (t ha^{-1}) within each plot, to get an approximate growth rate index (t ha^{-1} year^{-1}). The method was based on a study by Stokland (2021), reporting the annual volume increment of Norway Spruce trees to be rather constant during the lifetime of the tree, after reaching an age of approximately 30 years. The same calculation was done for all ages within an uncertainty range of ±5 years around the best estimate of the stand ages, to create an uncertainty interval that was later used when analysing the data. The calculations were done only including the Norway Spruce trees measured at the study site, due to [1.] they are the dominating tree species at the sites, [2.] they were planted and would thus represent the true age of the stands, and [3.] that one of the two tree species studied in the article by Stokland (2021) was Norway Spruce.

3.3 Stable isotope experiments

3.3.1 ^{15}N labelling

Gross N mineralization rates were estimated through stable isotope dilution experiments in situ, conducted in late July, 2022. An aqueous solution of 98 atom% ^{15}N labelled ammonium sulphate ($(^{15}NH_4)_2SO_4$) was prepared as the *label* (Kirkham & Bartholomew, 1954; Huygens et al., 2013). The

label was added to the soil to correspond to approximately 20% of the native soil NH_4^+-pool, which was measured in June, 2022. The specific concentration was decided to avoid disturbances in the transformation rates, e.g. substrate induced increases in microbial NH_4^+ immobilisation, while still enabling reliable measurements of changes in ^{15}N enrichments over the incubation period (Davidson et al., 1991).

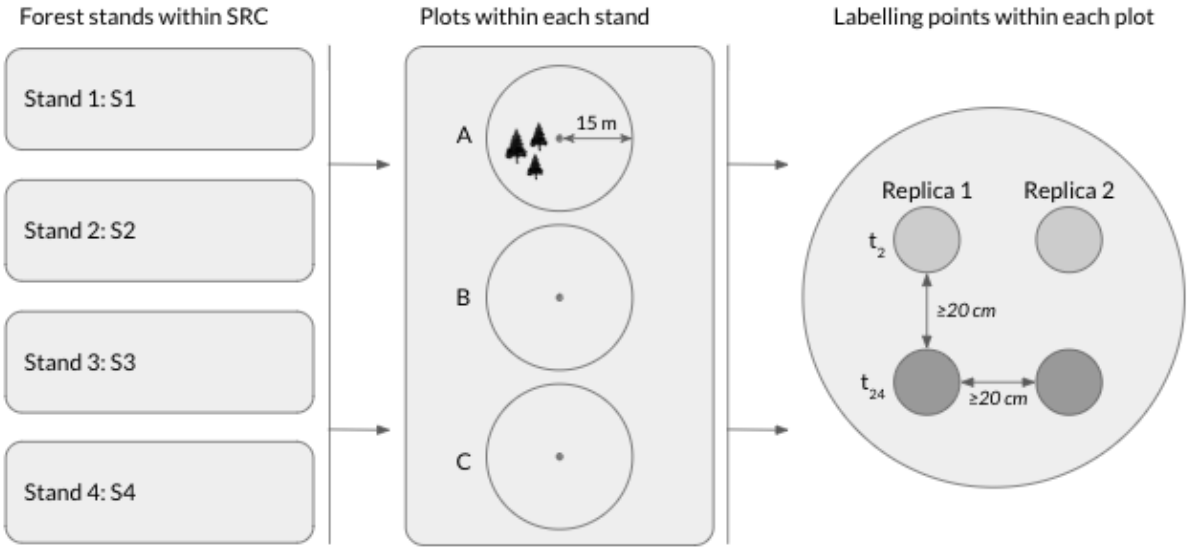

Figure 4. Schematic map of the study sites, divided into forest stands (S1, S2, S3 and S4), plots (A, B and C, e.g. S2B) and labelling points (1 and 2, as well as t2 and t24), in Skogaryd Research Catchment.

In this study, the "*Virtual Soil Core*" ^{15}N labelling technique was used (Rütting et al., 2015; Rütting et al., 2011). Within each plot, two replicas of two time steps each (t_2 and t_{24}) were labelled with $(^{15}NH_4)_2SO_4$, making up 12 labelling points in total within each forest stand (figure 4). The labelling points were selected randomly, except for efforts made to avoid large roots and stones that would prevent effective and complete sampling of the soil. The minimum distance between each point was 20 cm, and any litter on top of the soil was removed. Thereafter, a 100*100 mm PVC plastic plate with 19 inlets was placed on the soil (figure 5A). The plate was fixed with four plastic sticks, one in each corner. Through each of the holes, the soil was injected uniformly with 1 ml of $(^{15}NH_4)_2SO_4$ per injection using a BRAND™ Dispensette™ S analog-adjustable bottletop dispenser with a 90 mm long 22 G (approx. 0.7 mm in diameter) pencil point whitacre (side port) needle attached to the discharge tube. The side port needle aids an even distribution of the ^{15}N label in the soil (Davidson et al., 1991) and prevents the needle being clogged by soil particles. After the injections were made, the plastic plate was removed. Then the t_2 labelling points were sampled directly (see section *3.3.2 Sampling and extraction*), while the litter was placed back on top of the soil of the t_{24} samples for minimal disturbance of the natural processes. The four plastic sticks were left in the soil to mark the spot that would later be sampled.

To minimise the risk of sampling untreated soil, as well as avoiding lateral diffusion of the label solution, the 19 holes in the injection plate together cover an area larger than the diameter of the sampling tubes later used for collecting the soil samples. However, due to immediate sampling, the t_2 labelling points were only injected through the innermost seven holes. Any labelling solution injected through the outermost 12 holes was judged to have little to no impact on the result, as the dispersion of

the solution would have been minimal in that short time. The t_{24} labelling points were injected through all 19 holes, making up a total of 7 ml and 19 ml of $(^{15}NH_4)_2SO_4$ added per labelling point, respectively.

3.3.2 Sampling and extraction

After labelling, soil cores of the labelled soil were taken and extracted at two time points, two and 24 hours. The first sample (t_2) was taken directly after labelling to confirm the initial NH_4^+ content and ^{15}N enrichment, and the second (t_{24}) to determine the change in these concentrations over the incubation time.

At the time for sampling, any litter was again removed from the labelled soil before a 100*100 mm PVC plastic plate with a centred 60 mm in diameter hole was placed on top of it (figure 5B), to make sure the sampled soil core would only contain isotopically enriched soil. Through the hole, a 60 mm in diameter sampling tube was pushed down 80-90 mm into the soil, creating a soil core sample of the labelled soil (figure 5C). All soil cores were put into plastic bags and brought to a field laboratory for extraction.

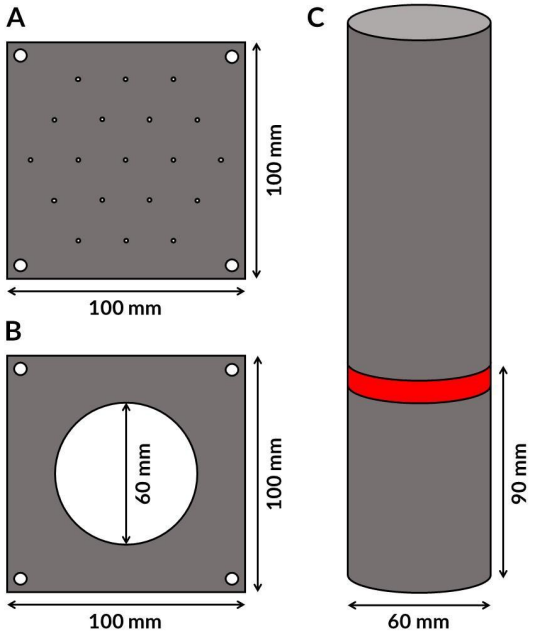

Figure 5. Schematic illustration of equipment used for the "virtual soil core" labelling method, showing the A) injection plate, B) sampling plate, and C) sampling tube.

At the laboratory, each soil core was cleared from stones and roots for five minutes per sample. The remaining soil was weighed and put into glass containers. For extraction of soil N, 100 ml 1M potassium chloride (KCl) solution was added to the soil samples of 50 g each, before being shaken for one hour on a shaker table at 125 RPM (revolutions per minute). The mixtures were then filtered, and the extracts were put into plastic containers (Rütting et al., 2015; Rütting et al., 2011). All extracts were brought back for further analysis at the department of Earth Sciences at University of Gothenburg.

3.3.3 Sample preparation and analysis

To analyse the N content and its isotopic composition, the samples were prepared for isotope ratio mass spectrometry (IRMS) analysis with the micro diffusion technique, described by Brooks et al. (1989). Of each soil extract, 10 ml was pipetted into 60 ml plastic cups. A Whatman glass microfiber filter (GF/A diameter 47 mm, 100 circles CAT 1820-047) was cut into small pieces with a scalpel, which were thread onto separate pieces of Bårebo 0.4 mm thick stainless-steel wire cut to fit the inside of the plastic cups. The wires were, two at a time, placed hanging across the top of a separate 60 ml plastic cup used for preparation of the filters. Two drops of 10 µl of 5M sulfuric acid (H_2SO_4) were pipetted onto each filter paper to make the filters acidic. After that, 0.2 mg magnesium oxide (MgO) was added to each 10 ml extract, making the solution basic and transforming the NH_4^+ in the sample into NH_3. Rapidly, to avoid losing any NH_3 into the room, a steel wire with an acidic filter was placed in the cup and the lid was screwed on tightly (figure 6). To mix the MgO with the solution, the cup was held against the desk while being twisted back and forth carefully to not get any of the solution onto the filter paper. The NH_3 gas rises into the cup, reaching the acidic filter paper. H_2SO_4 lowers the pH, transforming the NH_3 back into NH_4^+, while capturing it on the filter. To avoid contamination from the

air, the prepared filters stayed in the open for a maximum of 10 minutes before they were confined in the plastic cup together with the extracts.

After three days of incubation, the plastic cups were opened, and the filter papers were carefully removed and placed on empty silver capsules to dry for two days. When dry, each filter paper was enclosed in a separate silver capsule, which then were put into separate tin capsules (Brooks et al., 1989), before being combusted in a coupled elemental analyser (Sercon Europa EA-GSL) and IRMS (Sercon 20-22). Thereafter, the isotopic composition of the N_2 gas resulting from the combustion was analysed by the instrument (Sercon, n.d.), and the resulting data were later processed and analysed in Microsoft Excel (version 2209).

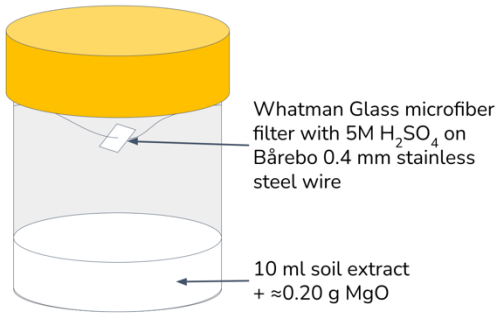

Figure 6. Sample preparation with the micro diffusion technique.

For every 10 sample extracts, two reference samples were prepared. First, 83.696 mg of $(NH_4)_2SO_4$ (5,5 atom% ^{15}N) was mixed into 100 ml 1M KCl. The solution was then diluted by mixing 10 ml into 90 ml 1M KCl. Lastly, each reference sample was made from 2 ml of the diluted solution and 8 ml 1M KCl, making up an approximate concentration of 0.017 mg $(NH_4)_2SO_4$ (5,5 atom% ^{15}N) ml^{-1} sample. All reference samples then followed the same procedure for sample preparation and IRMS analysis as the extracts (Brooks et al., 1989).

3.4 Gravimetric Water Content

The gravimetric water content (GWC) is defined as the mass water per mass dry soil. It is needed to calculate the dry mass of the soil cores, which in turn is required to estimate the gross N mineralisation rate. To measure the GWC, an additional ≈20 g of each soil core was brought back to the University of Gothenburg. The samples were dried at 40°C for 21 days before being reweighed to calculate the loss of water mass. Traditionally, GWC is calculated from samples dried at 105°C for approximately one to three days (e.g. Davidson et al., 1991). The reason the soil was dried at 40°C for a longer time period was to protect the organic matter in the samples, as these were later used in the analyses of soil C and N content, and C:N ratio.

3.5 Soil C and N content

Soil samples to analyse the soil C and N content, as well as the C:N ratio, was taken in two turns, first in early March to support the selection of the study sites, and second in late July, in conjunction with the ^{15}N stable isotope experiment, for a more detailed analysis. The initial analysis consisted of 8 samples taken at different depths at the four different sites: S1 (one sample), S2 (two samples), S3 (two samples), and S4 (three samples). The second analysis consisted of four samples from all of the three plots at each of the four sites, apart from plots S2C (one sample), S3B (three samples), S3C (three samples) and S4B (three samples), making up 42 samples in total. The missing samples were due to the mass of the soil core being too low to take a separate GWC sample, which also made soil C and N content impossible to analyse. To eliminate the risk of overestimating the soil N content due to the ^{15}N enriched injections, t_2 and t_{24} samples were analysed separately at two different occasions to later be compared. No difference in N content between the two sets of samples was detected (t-Test, two sample assuming unequal variances, $P = 0.93$).

After weighing the dry GWC samples, the soil was finely ground using a ball mill (RETSCH MM 400 Mixer Mill). Then, ≈10 mg of each soil sample, as well as several reference samples of 15 mg each, were weighed and put into separate tin capsules. All samples were combusted in the same combined elemental analyser and IRMS as described above. In the instrument, the N_2 and CO_2 resulting from the combustion are separated and measured, resulting in data of the N and C content in % of the sample soil dry mass (Nilsson et al., 2015). All C:N ratios in this study are expressed as mass ratios.

3.6 Bulk density

To calculate the extractability of the added $^{15}NH_4^+$ tracer (^{15}N recovery rate), as well as the gross N mineralisation rate per area (see section *3.7.2 Extractable NH_4^+-N & gross N mineralisation rates)*, the bulk density of the soil was measured at all four sites in early September 2022. Two samples were taken from the top 5 cm of the soil within each of the three plots at each site, using a cylinder with a defined volume of ≈206 cm^3. The wet soil was dried at 105°C for three days. Then the samples were weighed and the dry weight (g) per cm^3 was calculated (Rütting et al., 2015).

3.7 Data analysis

3.7.1 Forest biomass production

To further account for uncertainties regarding the ages of the forest stands, the soil C:N ratio was correlated against the calculated Norway Spruce growth rates for all estimated ages within the uncertainty interval (estimated age ± 5 years) using MATLAB (R2020a & R2022b; figure 7). For each of the 11 suggested ages, the data were grouped on site level, according to the assumption that each specific site was of the same age, resulting in three groups per site. Within each group, the data were paired on plot level, as the biomass growth rate and soil C:N data were plot specific. This was done for all sites and plots except site S3, which instead was divided into 22 groups. The first 11 age groups represented plots S3A and S3B. The remaining 11 represented only S3C, which had been estimated to be younger than the other two plots at this site. Based on the assumption that the relationship between tree growth and soil C:N previously seen in the literature (Kranabetter et al., 2020; Peichl et al., 2022; Alberti et al., 2015) is also valid at the sites of interest in this study, all 161.051 combinations of groups were tested to find the combination with the strongest relationship to soil C:N, and, through this, the most likely age of each of the sites. Plot S4A was excluded from all statistical analyses including soil C, N and C:N ratio in the study, on account of the occurrence of charcoal in the soil, as a result of the coal pits used in the 1700s and 1800s (Hill, 1999). Due to these remnants, the soil C, soil N and soil C:N analyses of this specific plot are not representing the soil organic matter, and are therefore not comparable with the remaining plots. Further analyses including forest biomass growth in this study were conducted for two species categories, first accounting for all trees within each plot (All Species), and second only including Norway Spruce.

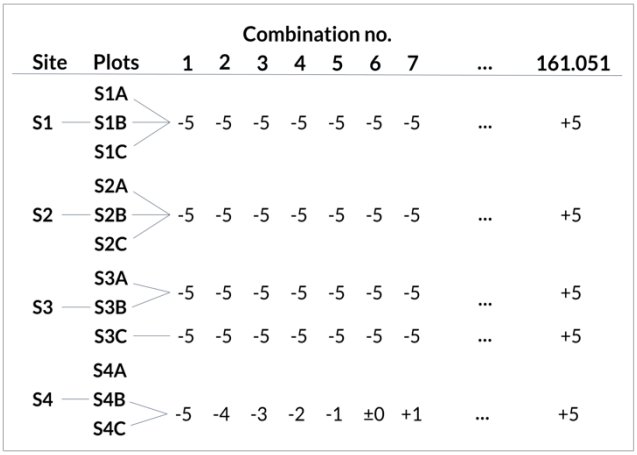

Figure 7. Schematic visualisation of the soil C:N-biomass growth rate regression model used to determine the final forest stand ages used further on in the study.

3.7.2 Extractable NH_4^+-N & gross N mineralisation rates

The NH_4^+-N content (µg) and ^{15}N fraction of each sample resulting from the IRMS analysis were drift corrected and calibrated against the known concentration of NH_4^+-N in the reference samples. The liquid content of each sample was calculated by adding the water content retrieved from the GWC measurements to the volume KCl added for N extraction. Thereafter, the µg NH_4^+-N g^{-1} dry mass was calculated for each sample by first dividing the NH_4^+-N mass from the IRMS analysis by the volume of the analysed KCl extract (10 ml), then multiplying it by the liquid content, and then dividing it by the soil dry mass of the specific sample. The µg NH_4^+-^{15}N g^{-1} dry mass was then calculated by multiplying the µg NH_4^+-N g^{-1} dry mass with the ^{15}N fraction. Additionally, the mean concentration of extractable NH_4^+-N of the two t_{24} samples was calculated to determine the NH_4^+-N pool of each plot.

The differences in ^{15}N concentration between the time steps were used to calculate the inflow of N into the NH_4^+ pool, hence, the gross N mineralisation rate. To calculate the gross N mineralisation rates, the following equations from Kirkham & Bartholomew (1954) were used:

$$m = \frac{M_0 - M}{t} \frac{log\ H_0 M / H M_0}{log\ M_0 / M} \qquad [a]$$

where m = gross mineralisation rate (µg N g^{-1} d.w. d^{-1}), M_0 = µg of total NH_4^+-N ($^{14}N^{+15}N$) per g dry soil at t_0, M = µg of total NH_4^+-N ($^{14}N^{+15}N$) per g dry soil at t_{24}, H_0 = µg NH_4^+-^{15}N per g dry soil at t_0, H = µg NH_4^+-^{15}N per g dry soil at t_{24}, t = incubation time (days between t_0 and t_{24}), and log = natural logarithm (base e).

On some occasions, this equation returned negative values for gross N mineralisation. As gross rates represent the production, i.e. a one-way process, negative gross rates are not possible (e.g. Booth et al., 2005). One possible reason for this could be the large spatial heterogeneity that has to be considered when working in a natural field environment (e.g. Schimel & Bennett, 2004), evident by large differences in NH_4^+-N content within the same plots, between the time steps, as well as examples when the NH_4^+-N content was higher at t_{24}. In these instances, the gross mineralisation rate was assumed to be equal to the gross consumption rate, based on results in previous studies (Christenson et al., 2009; Bengtson et al., 2012; Booth et al., 2005). The mean total NH_4^+-N ($^{14}N^{+15}N$) content was calculated from the concentrations at t_2 and t_{24} and equation [a] was substituted by the following equation, slightly modified from Kirkham & Bartholomew (1954):

$$m = c = (M_0 / t) log H_0 / H \qquad [b]$$

where c = gross consumption rate (µg N g^{-1} d.w. d^{-1}). This modification was done for one sample replica taken at S2B, both replicas at S2C, one at S3A, one at S3B, and one replica at S3C.

To further account for spatial variations in soil conditions as well as increase the comparability between the different parameters of interest in this study, gross mineralisation rates were also calculated accounting for soil C concentration (µg NH_4^+-N g^{-1} soil C), as well as per area (g NH_4^+-N ha^{-1}). This was done by recalculating the NH_4^+-N ($^{14}N^{+15}N$, and ^{15}N) concentration using the soil C measurements and the soil bulk density data, before inserting the new concentrations in the same equations as above. The resulting gross rates calculated for both replicas from plot S2C still came back negative. This was

assumed to be due to measurement errors or a result of large spatial variability that could not be accounted for in the analyses, and these samples were excluded from further analyses including gross mineralisation in the study.

The extractability of the added $^{15}NH_4^+$ label was calculated using the following equation:

$$^{15}Nrecovery = \frac{^{15}Nexcess}{label_N 0.99/CV*BD} \quad [c]$$

where ^{15}Nexcess = mass of extracted NH_4^+-^{15}N (µg ^{15}N g^{-1} dry soil) from one sample, minus natural abundance (0.3663%, e.g. Davidson et al., 1991; Cronan, 2018), $label_N$ = mass of added N per soil core (µg N), 0.99 = label ^{15}N % enrichment, CV = soil core volume (cm^3), and BD = bulk density (g cm^{3-1}).

3.7.3 Statistics

All rates, concentrations and C:N ratios were calculated per labelling point, then used to compute a mean value for each plot. This value was then used in the statistical analyses of the study. All mineralisation rates are expressed in mass per day, and biomass growth rates are expressed as mass per year. The statistical difference between the variables and rates between the four sites was determined using a single factor ANOVA. The relationships between the variables and rates were visually and statistically analysed through correlation and regression analyses, using the Data Analysis Toolpak in Microsoft Excel (version 2209) and MATLAB (R2020a & R2022b). After initial analyses of the whole data set, visual analyses led to the data being divided into two groups; *Group S1-3*, including all data from sites S1, S2 and S3; and S4, which was analysed separately. The confidence level used for determination of statistical significance was set to 95% for the ANOVA, and 90% for correlations between variables, as working with in situ measurements involves a large spatial heterogeneity in the investigated and surrounding environment that will inevitably impact the results. Correlations significant at higher CI (95%, 99%, as well as 99.9%) are specified in the results section. In some cases the data was also log transformed prior to the statistical analysis in order to avoid overlooking any relationships disguised by this spatial variability. To account for the possibility of bias and analyse for multicollinearity between the three parameters in focus (soil C:N, gross N mineralisation and forest biomass growth), gross N mineralisation and forest biomass growth was analysed in conjunction with soil C:N as a control variable using multiple linear regression. The analysis was followed by an analysis of the variance inflation factor (VIF) for the independent variables, with a threshold of 5 for determining multicollinearity.

4. Results

4.1 Differences between sites in concentrations and rates

There were significant differences between all sites for all variables and rates, except gross N mineralisation (Nmin) g^{-1} dry soil, extractable NH_4^+-N g^{-1} dry soil d^{-1} and excess ^{15}N recovery (%; table 2). Thus, these were excluded from further analyses comparing the sites. The site mean gross N mineralisation rate ranged between 100 - 1260 g N ha^{-1} and 6.5 - 53.8 µg N g^{-1} soil C, while the extractable NH_4^+-N ranged between 726 - 2144 g N ha^{-1} and 27.9 - 90.7 µg N g^{-1} soil C (table 3). The forest biomass growth rate ranged between 3.4 - 7.53 t ha^{-1} for All Species, and 2.89 - 7.53 t ha^{-1} for Norway Spruce only, and the soil C:N ratio measured to a mean value per site between 17 - 30. Within Group S1-3, the highest mean Nmin rate (g N ha^{-1} d^{-1} and µg N g^{-1} soil C), lowest soil C:N and fastest forest biomass growth rate (All Species) was found at S1, followed in order by S3 and S2. The growth rate for Norway Spruce was slightly higher at S2 than S3 (3.15 and 2.89 t ha^{-1} yr^{-1}, respectively). S3 was shown to have the highest C and N concentrations in the soil (% of dry weight), almost twice of what was found at S2, which in turn had a more than twice as high concentration of soil C as what was found at S1, but only a ca 30% higher N concentration, compared to the same site.

Table 2. Statistical differences between sites, resulting from a Single factor ANOVA. Tree DBH and Biomass d.w. tree-1 are divided in 5 sites (S1, S2, S3AB, S3C, and S4) due to stand age differences within S3. Soil C, N and C:N did not include data from plot S4A.

Variable	P =	F value
Nmin (µg N g^{-1} dry soil d^{-1})	0.093	2.49
Nmin (g N ha^{-1} d^{-1})	0.013	4.77
Nmin (µg N g^{-1} soil C d^{-1})	0.009	5.24
Soil C (%)	0.003	5.65
Soil N (%)	0.007	4.84
*NH_4^+-N (µg g^{-1} dry soil)**	0.113	2.29
*NH_4^+-N (g ha^{-1})**	0.021	4.18
*NH_4^+-N (µg g^{-1}soil C)**	0.019	4.27
Soil C:N	< 0.001	34.47
Tree DBH	< 0.001	14.83
Biomass d.w. $tree^{-1}$	< 0.001	63.16
Excess ^{15}N recovery (%)	0.272	1.42

*mean of extractable pools in t_{24} samples

The highest mean Nmin rate of all four sites was found at S4 (table 3). Of all plots at S4, S4B had the lowest soil C:N ratio, as well as the highest Nmin rate. The samples from the plot had a soil C concentration of more than five times what was found at the other two plots, and soil N was more than six times higher. S4A had the highest soil C:N of the site, as well as the lowest Nmin rate, and S4C was positioned between the other two plots for both variables. As for biomass growth, compared to Group S1-3 the pattern at S4 is reversed, with the highest growth rate found at S4A and the lowest at S4B.

Table 3. Site mean of all measured variables and modelled rates, as well as plot specific values for all plots within S4 (SD).

	Site				Plot within S4		
	S1	**S2**	**S3**	**S4**	**S4A**	**S4B**	**S4C**
Soil C (%)	5.64 (0.69)	12.92 (9.93)	22.47 (13)	19.4 (20.46)	7.27 (0.64)	43.02 (0.54)	7.9 (2.37)
Soil N (%)	0.34 (0.04)	0.47 (0.4)	0.93 (0.52)	0.76 (0.91)	0.19 (0.03)	1.81 (0.11)	0.28 (0.09)
Soil C:N	17 (0)	27 (1)	24 (2)	30 (8)	39 (3)	24 (2)	28 (2)
GWC (%)	31.4 (5.8)	48.9 (0.3)	101.4 (62.6)	171.2 (225.6)	18.6 (3.3)	430.3 (30.3)	64.7 (23.2)
Bulk Density (g cm^{-3})	0.75 (0.05)	0.55 (0.14)	0.34 (0.26)	0.49 (0.37)	0.85 (0.15)	0.12 (0.01)	0.52 (0.25)
*NH_4^+-N (g ha^{-1})**	1345 (926)	1019 (640)	726 (606)	2144 (537)	1600 (164)	2674 (945)	2158 (546)
*NH_4^+-N (µg g^{-1} soil C)**	52.4 (29.9)	42.7 (15.9)	27.9 (12)	90.7 (36.3)	49 (3.6)	108 (39)	115.1 (62.8)
Nmin (g NH_4^+-N ha^{-1} d^{-1})	698 (343)	100 (51)	524 (305)	1260 (564)	830 (1)	1898 (242)	1051 (346)
Nmin (µg NH_4^+-N g^{-1} soil C d^{-1})	31.1 (12.3)	6.5 (0.4)	18.4 (2.5)	53.8 (24.9)	26.9 (1.8)	76.2 (10.5)	58.2 (37.8)
Biomass growth (t ha^{-1} yr^{-1}); All Species	7.53 (0.97)	3.4 (1.02)	3.64 (0.42)	3.6 (0.64)	4.2 (-)	2.92 (-)	3.67 (-)
Biomass growth (t ha^{-1} yr^{-1}); Norway Spruce	7.53 (0.97)	3.15 (1.06)	2.89 (0.55)	3.39 (0.49)	3.9 (-)	2.92 (-)	3.34 (-)
*Forest stand ages ** (yrs)*	55	55	47 ($S3A^+B$) 35 (S3C)	71			

*mean concentration of t_{24} samples

**resulting from regression analysis described in section 3.7 Data analysis

4.2 Soil C:N and forest biomass production

The relationship between soil C:N and Norway Spruce biomass growth was shown to be significant for all forest stand ages within the uncertainty interval (±5 years), as well as for both species categories ($P < 0.05$). This supports a robust relationship between biomass growth and soil C:N. The strongest relationship (*Norway Spruce*: $P = 0.001$, $R = -0.84$; *All Species:* $P < 0.001$, $R = -0.86$; figure 8) determined the final stand ages used to calculate the forest biomass growth rates used further in the study (table 3). Plot S3C was estimated to be 35 years old, which increases the uncertainty in the estimated biomass growth rate at the specific plot (see method description in section *3.2 Estimation of forest biomass production*). This was taken into consideration when performing additional analyses of the results.

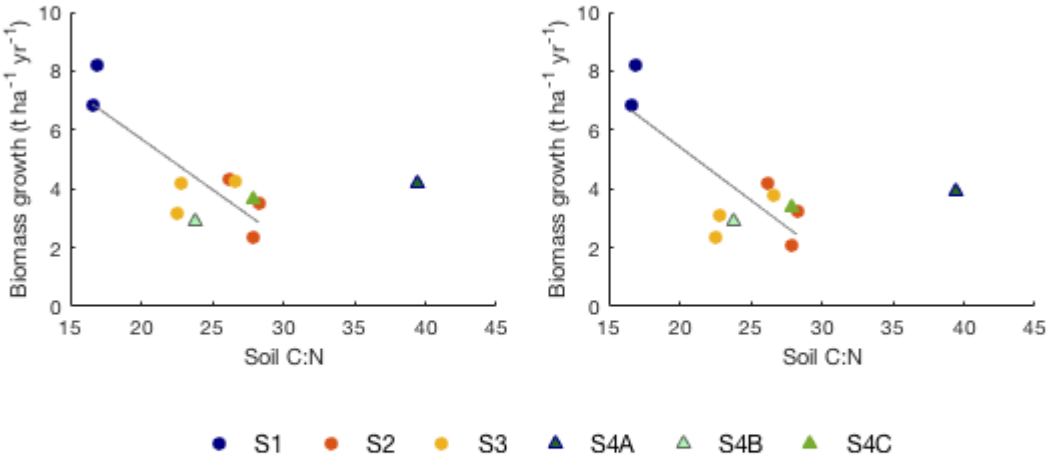

Figure 8. The relationship between soil C:N and forest biomass growth (t dry wood ha^{-1} yr^{-1}) for the species categories All Species (left) and Norway Spruce (right). Circles represent Group S1-3 and triangles S4. The solid line represents linear regression for Group S1-3, S4B and S4C.

4.3 Soil gross N mineralisation and forest biomass production

There was a significant positive relationship between the soil C based gross N mineralisation rate (µg NH_4^+-N g^{-1} soil C) and forest biomass production for both tree species categories within Group S1-3 (figure 9; table 4. For a full list of linear regression analysis, see table A2.). Looking at the areal based gross N mineralisation rate (g NH_4^+-N ha^{-1}) there was no significant relationship with either of the two tree species groups. However, the data deviating from the linear regression between Nmin ha^{-1} and biomass growth is from plot S3C, where the biomass growth rate was likely underestimated, due to the young age of the forest stand (table 3). Rerunning the analysis excluding this plot resulted in a significant relationship also between Nmin ha^{-1} and biomass growth, while the relationship with C based Nmin remained slightly stronger of the two (table 4).

Within S4, there was a near flat, but significant, negative relationship between Nmin (µg NH_4^+-N g^{-1} C) and the growth rate of Norway Spruce at S4 (figure 9; table 4) No other significant relationships between the variables were found at the site (data not shown). The analyses show no significant relationship between the soil gross N mineralisation rate and forest biomass production when including all four forest stands ($P > 0.13$). In addition, no correlations were found between the extractable NH_4^+-N pool

and biomass growth within Group S1-3, but the area based NH4+-N pool at S4 was significantly correlated to forest biomass production in both species categories (table 4).

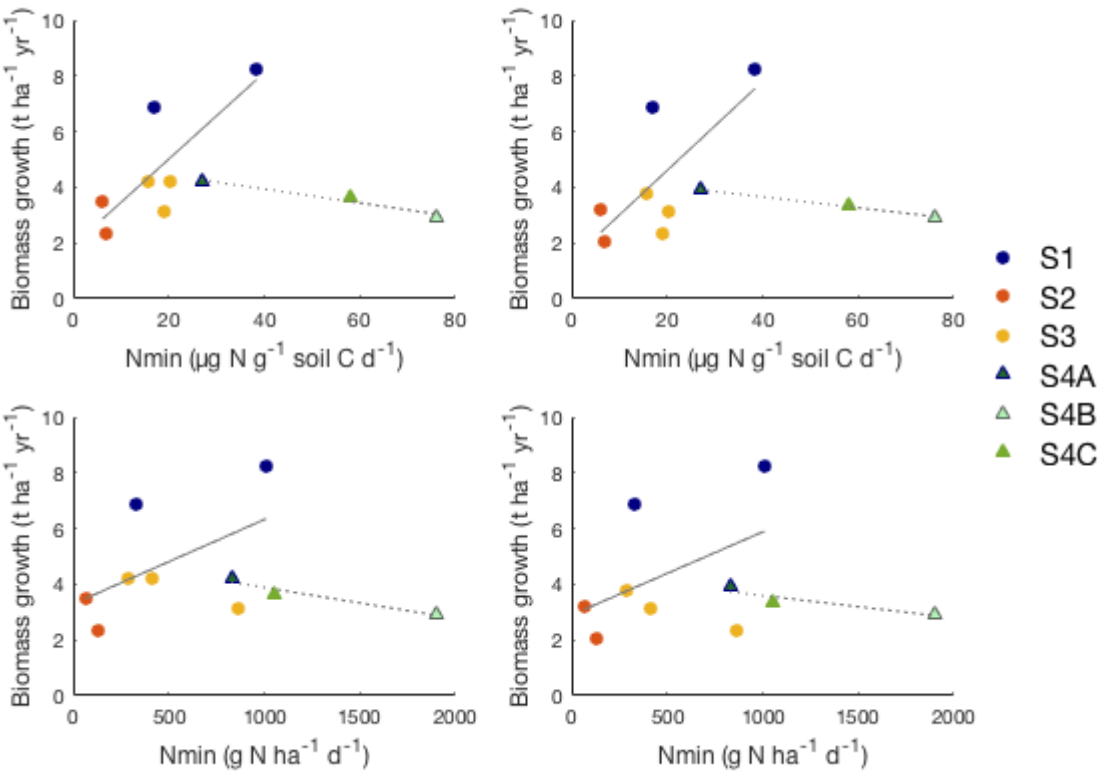

Figure 9. The relationship between soil gross N mineralisation and forest biomass growth. Nmin is expressed as µg NH_4^+-N g^{-1} soil C d^{-1} (top) and g NH_4^+-N ha^{-1} d^{-1} (bottom), and biomass growth is expressed as t d.w. ha^{-1} yr^{-1} for the species categories All Species (left) and Norway Spruce (right). Circles represent Group S1-3 (solid line represents linear regression) and triangles S4 (dotted line represents linear regression).

4.4 Soil gross N mineralisation and soil C, N and C:N

Two sample plots from site S3 and one from S4 were shown to have organic soil, with an organic matter content in the samples of > 30% (C content > 29%; S3B, S3C, & S4B). Thus, these plots were excluded from the regression analyses before looking into the relationship between Nmin and soil C and N. No regression analysis of S4 was carried out in this case, due to there only being two sample points available.

For mineral soil, there was no significant relationship between soil C and log transformed Nmin. However, the relationship between soil N and log transformed Nmin was strong (table 4; figure 10). A significant negative correlation was found between log transformed Nmin (µg N g^{-1} soil C) and soil C:N for Group S1-3, meaning a lower soil C:N ratio corresponds to a higher Nmin rate, and vice versa. No regression analysis was done for S4 following the unrealistic soil C:N ratio at plot S4A, but visual analysis suggests there is a similar, close to parallel but elevated, relationship at this site.

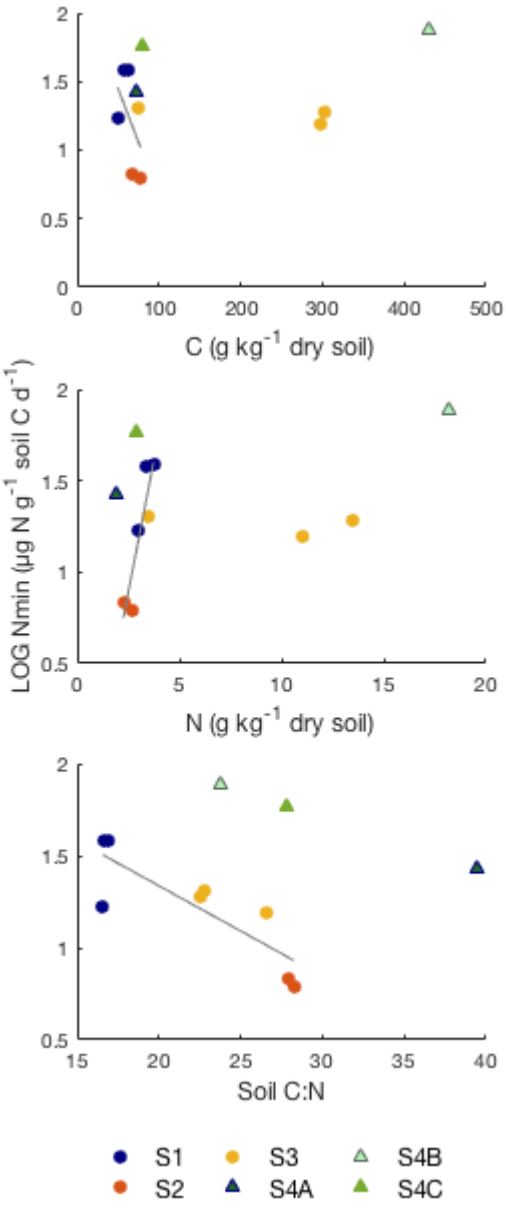

Figure 10. The relationship between log transformed gross soil N mineralisation (µg NH_4^+-N g^{-1} soil C) and Soil C and N (g kg^{-1} dry soil), and soil C:N. Circles represent Group S1-3 (solid line indicates linear regression) and triangles represent site S4.

4.4 ^{15}N recovery

Less than 2% of the added NH_4^+-^{15}N label could be recovered in 38% of the samples, and less than 3% in 79% of the samples. As previously mentioned, there was no significant difference in excess ^{15}N recovery between the sites. The multiple regression analysis used to analyse for multicollinearity between gross N mineralisation and soil C:N revealed the inclusion of soil C:N improved the strength of the relationship between mineralisation and biomass growth (adjusted R^2 = 0.69). Subsequently, the VIF value for the independent variables imply multicollinearity is not a major issue affecting the results of the regression (VIF = 2.5).

Table 4. Pearson correlation coefficients of the relationships between the different rates of gross N mineralisation and soil C and soil N (g kg^{-1} dry soil), soil C:N, estimated biomass growth rates for all species and Norway Spruce only (t ha^{-1} yr^{-1}), as well as the relationship between mean extractable NH_4^+-N at T_{24} and biomass growth in both species categories. Grey area shows rerun correlations excluding S3C. Asterisks indicate significance (see below).

	Group S1-3				S4		
	N	**Nmin (g N ha^{-1})**	**Nmin (µg N g^{-1} soil C)**	**Excl. data**	**N**	**Nmin (g N ha^{-1})**	**Nmin (µg N g^{-1} soil C)**
Soil C ^{LOG10}	6	-0.52	-0.48	S2C S3B S3C	-	-	-
Soil N ^{LOG10}	6	**0.86****	**0.92*****	S2C S3B S3C	-	-	-
Soil C:N ^{LOG10}	8	**-0.76****	**-0.85*****	S2C	-	-	-
Biomass growth; All Species	7	0.51	**0.79****	S1B S2C	3	-0.97	-0.97
Biomass growth; Norway Spruce	7	0.45	**0.73***	S1B S2C	3	-0.92	**-1****
Biomass growth; All Species	6	**0.83****	**0.85****	S1B S2C ***S3C***		-	-
Biomass growth; Norway Spruce	6	**0.79***	**0.80***	S1B S2C ***S3C***		-	-
	N	**Extractable NH_4^+-N (g N ha^{-1})**	**Extractable NH_4^+-N (µg N g^{-1} soil C)**	**Excl. data**	**N**	**Extractable NH_4^+-N (g N ha^{-1})**	**Extractable NH_4^+-N (µg N g^{-1} soil C)**
Biomass growth; All Species	7	0.46	0.58	S1B S2C	3	**0.99***	0.75
Biomass growth; Norway Spruce	7	0.51	0.64	S1B S2C	3	**0.99****	0.86

* P < 0.1

** Significant at the < 0.05 probability level

*** Significant at the < 0.01 probability level

**** Significant at the < 0.001 probability level

^{LOG10} vs. log transformed Nmin

S1B - No biomass data

S2C - No Nmin data

S3B & S3C – organic soil

S3C - Uncertainty in biomass growth rate estimation due to young age of the forest stand

5. Discussion

In this time of climate and environmental change, it is important to know more about what processes are linked to C sequestration and storage capacity of terrestrial ecosystems, and what parameters affect these relationships. Coincidentally, there is great value in finding and developing easy and available methods for exploration of these controls. Thus, the objectives of this study were to investigate the relationship between the soil C:N ratio, the rate of soil gross N mineralisation and forest biomass growth across a soil fertility gradient.

5.1 Differences between sites in concentrations and rates

The gross N mineralisation rates at the SRC sites fall into the range of 100 – 1000 g ha^{-1} d^{-1}, that has been observed in other boreal forest ecosystems (Högberg et al., 2017; table 3). It is clear that S1 is by far the most productive of the three sites in Group S1-3, as it has the lowest soil C:N, highest Nmin rates and highest forest biomass growth rate for both species categories. These results correspond well to the history of the site, as the high soil fertility creates suitable conditions for agricultural purposes. Looking only at the sites within Group S1-3, it seems clear that the relationship between soil C and N (i.e. the soil C:N ratio) is a more important factor for soil fertility and N transformation than the actual nutrient concentrations (figure 10). Following S1, S3 and S2 are rather consistent in the pattern in soil C:N and Nmin, as well as biomass growth rates. Two deviations from this pattern are the larger extractable NH_4^+-N pool and the higher Norway Spruce growth rate at S2, compared to S3. An explanation for the comparably large NH_4^+-N pool at the site would demand further sampling and analyses that have not, unfortunately, been included in this study. The slower Norway Spruce biomass production at S3 could be a result of two factors; a higher fraction of Spruce trees at S2 than S3, rather than a specific decline in spruce growth rates at the site (table A1), or that the forest at S3C is younger than the other sites (table 3). To account for the smaller fraction of Spruce trees, it could have been useful to include the species density into the equation while calculating the growth rate for the Norway Spruce species category. Regarding the age of the forest at S3C, the potential problem lies in the method used for estimating the forest growth rates. Stokland (2021) reports the annual volume increment of Norway Spruce trees to be rather constant during the lifetime of the tree, though starting from an age of approximately 30 years. Before 30 years old, the yearly volume increment increases with the age of the tree. In the regression model used for estimating the ages of the forest stands, plot S3C was estimated to be 35 years old. The smaller, and rising, annual increment between 0-30 years naturally affects the calculated growth rate for this specific plot, leading to a probable underestimation of the biomass productivity, relative to the other plots. This is discussed further in sections *5.3 Soil gross N mineralisation and forest biomass production* and *5.6 Robustness of analyses and possible method limitations*, below.

Within S4, although not fitting onto the same line of best fit as Group S1-3, all parameters followed the same patterns, except for biomass growth. This was shown to have an inverse relationship to Nmin and soil C:N. The Nmin rates at the site were fastest of all estimated in this study. In fact, in two of the three plots they were estimated to exceed the previously mentioned range of 100 – 1000 g ha^{-1} d^{-1}. The fast Nmin rates were also followed by the largest extractable NH_4^+-N pools of all sites. The divergent observations from this particular site are discussed further in section *5.4 S4 as a consistent deviation.*

5.2 Soil gross N mineralisation and soil C:N

Soil C:N ratio was shown to be significantly negatively correlated to the log transformed C based Nmin rate within Group S1-3, revealing that the rate of Nmin is faster in more fertile soils, and vice versa

(figure 10, table 4). This corresponds to what has been found in previous research (e.g. Booth et al., 2005; Christenson et al., 2009; Mooshammer et al., 2012). In their review of 100 studies conducted in different types of ecosystems, Booth et al. (2005) found a significant relationship between soil C:N ratio and gross N mineralisation only when soil C:N was analysed in conjunction with soil C, which coincides with the findings of the present study. There was no significant correlation between soil C:N and Nmin g^{-1} dry soil (original data and log transformed, $P > 0.4$; table 4), but after accounting for differences in soil C in the analysis, the relationship became significant. Furthermore, Nmin g^{-1} dry soil was not significantly different between the sites investigated in this study, despite the differences between the sites in other respects (e.g. soil C:N, C and N concentrations, etc.). This points to show that Nmin based on soil dry mass is a poor measurement of the transformation process when used in comparing sites with differing soil properties.

Contrasting the results found in Booth et al. (2005), the soil C:N ratio within Group S1-3 from SRC is also significantly correlated to log transformed areal based Nmin at the sites (table 4). Though, the correlation is not as strong as for Nmin based on soil C. As there is a significant difference in soil C concentration between the sites, this could be connected to the site specific bulk density. Soil bulk density is connected to the organic matter content of the soil. Where SOM concentrations, and thereby C concentrations, are high, bulk density is normally low (e.g. Hossain et al., 2015) which has also been observed in this study (table 3). Soil organic C is also used as an input variable to estimate the bulk density of a soil with varying pedotransfer functions (Acutis & Donatelli, 2003). As soil C concentration is a determinant for the bulk density of the soil, Nmin ha^{-1} still has some signal of the soil C concentration in the calculated rates. Comparing this relationship with the lack of one between soil C:N and Nmin based on dry soil mass, the results of this study again point to organic matter and soil C to be highly important for the cycling of soil N.

After removing the organic soil samples from the linear regression analysis, the soil N concentration was shown to have a strong, positive relationship with the log transformed Nmin rate, which is consistent with results of previous research (figure 10, table 4). At the same time, soil C content was shown not to be related to Nmin. This differs from results presented in previous studies (e.g. Accoe et al., 2004; Booth et al., 2005). Nevertheless, when compared to the analysis of the same relationship in e.g. Booth et al. (2005), which is based on a much greater sample size, the results of this study falls within the range of their data, but spread around the regression line (true also for soil N and C:N). Thus, it could be that a relationship between soil C and Nmin would also appear here if the sample size were increased.

The link connecting a higher soil N concentration and a lower soil C:N ratio to higher rates of Nmin found in this study supports previous conclusions, that a higher substrate supply means that more N can be mineralised (Booth et al., 2005; Mooshammer et al., 2012; Högberg et al., 2017). When resource C:N is high, microbes are increasingly limited by N, leading to high microbial N immobilisation. Mooshammer et al. (2014) found the elemental ratio threshold for when one or the other of the processes is favoured to be at a resource C:N mass ratio of 20, below which net N mineralisation would be favoured, and above, net N immobilisation. Other studies have suggested this threshold to be at a resource C:N mass ratio of 20-25, and some at 30 (Cronan, 2018). Moreover, where C:N ratios are low, there is less C available per unit N, which could exacerbate the microbial C limitation (Mooshammer et al., 2012; Högberg et al., 2017). For example, Demoling et al. (2007) state that bacteria could be limited by available C in soils with a C:N ratio up to 28, which is slightly higher than the soils with the highest C:N ratio of Group S1-3 in this study (table 3). In this case, the microbial C use efficiency (CUE) would increase (Alberti et al., 2015), as a larger fraction of metabolised C is assimilated into microbial biomass, compared to what is respired as CO_2 (Schimel et al., 2022). N mineralised through

SOM decomposition would be in excess of the microbial demand, compared to the C mineralised in the same process, creating a microbial release of inorganic N to the soil (Olser & Sommerkorn, 2007; Mooshammer et al., 2012). This gives rise to the question if the potential Nmin rate based on Norg supply might not be met if the supply of labile C is limited. Hence, rather than it simply being a question about nutrient supply, it may be more about the stoichiometry of the nutrients, and the composition of SOM, available (Booth et al., 2005; Mooshammer et al., 2012).

Högberg et al. (2017) reported, in their review, results from three boreal sites near Betsele in northern Sweden, with differing soil N availability (soil C:N of 38.1, 22.9 and 14.9, respectively). They observed large changes in Nmin rates and soil N retention following shifts in microbial community and C:N. The observation also included a shift in the microbial community from fungal to bacterial dominance with decreasing soil C:N, which has also been noted in other studies (e.g. Chen et al., 2013; Dijkstra et al., 2013). The fungi-to-bacteria ratio at the N rich site was found to be extremely low, which could also be seen in the low microbial C:N ratio indicating a microbial dominance by bacteria. The low soil C:N measured at the N rich site is only slightly lower than what was found at S1 in SRC (17). The two sites are also comparable in terms of extractable NH_4^+ (1345 g ha^{-1} in SRC, 1962 g ha^{-1} near Betsele), but very different when comparing Nmin rates (698 g N ha^{-1} d^{-1} in SRC, 4300 g N ha^{-1} d^{-1} near Betsele). In fact, all sites at Betsele had daily gross N mineralisation rates exceeding the site specific total extractable NH_4^+ pool, while in SRC, extractable NH_4^+ exceeded the daily mineralisation rates at all sites (table 3). Sites S3 and S2 in SRC had soil C:N ratios slightly higher than the intermediate N site at Betsele, as well as higher extractable NH_4^+, but not even half of the mineralisation rate measured at the site. At least part of these large differences in Nmin rates are probably due to the different methods used for the stable isotope labelling; in this study it was done *in situ* without disturbing the natural soil conditions while the results in Högberg et al. (2017) are derived from first sampling the soil, then removing the roots and mixing the sample, before adding the ^{15}N solution. As it is known that this kind of treatment increases production and consumption of NH_4^+ (Booth et al., 2006), the higher rates are not directly comparable with the findings of the present study, but highlight the importance of *in situ* measurements to avoid overestimating the N turnover in soils. Nevertheless, the observed relationships between the soil C:N ratios and Nmin rates are consistent between the studies; a lower soil C:N ratio corresponds to a higher rate of Nmin.

5.3 Soil gross N mineralisation and forest biomass production

The statistical analyses of this study confirm there is a relationship between Nmin and forest biomass production within Group S1-3, where a higher Nmin is related to increased forest growth. This corresponds well with the relationships between both variables and soil C:N previously shown. As low soil C:N coincides with both increased forest biomass production and faster Nmin, a positive relationship between forest biomass growth and Nmin was expected. Even though research on the connection between Nmin and forest growth is lacking, many studies present strong correlations between inorganic N availability and plant productivity. Vicca et al. (2012) show in their analysis of 49 forests with varying nutrient availability across the boreal, temperate and tropical biome, that forests with more available N use a larger fraction of their GPP for biomass production, compared to forests with low nutrient supply. However, they do not report any significant differences in GPP between the nutrient classes per se. They suggest this difference in productivity instead is due to changes in C allocation to i.a. root exudates and mycorrhizal symbionts, which would increase in N poor, and decrease in N rich systems. Other studies have found significant relationships between soil N and GPP, as well as with forest above ground NPP-to-GPP ratio (Alberti et al., 2015). Not to mention all reports on how fertilising forests with inorganic N increases biomass production (Blasko et al., 2022; Sponseller

et al., 2016), such as the up to fourfold increases in Norway Spruce stem growth following long term fertilisation, reported by Bergh et al. (1999). Interestingly, the results of this study show no correlations between biomass growth and extractable NH_4^+-N. Even though the NO_3^- pool has not been measured here and is thus not included in the analysis, this points to Nmin as a better way of estimating the inorganic N supply to trees than the actual pool sizes.

Like the previously discussed relationship between Nmin and soil C:N, the influence of Nmin on biomass growth rate is also related to the C concentration of the soil. Including all plots within Group S1-3 except S1B (no biomass data) and S2C (negative Nmin data), the relationship between Nmin and forest biomass growth is significant only when looking at Nmin based on soil C, and not per area (figure 9; table 4). However, removing plot S3C from the regression analysis makes the relationship significant also for Nmin ha^{-1}, though C based Nmin still has a stronger relationship, even if only slightly, with biomass growth also after removing this data point. In addition, there is no way to predict what the estimated growth rates at the plot would have been if the forest there would be of the same age as the other two plots at the site. Thus, it is impossible to say for sure how it would have impacted the regression analysis if the forest at the site were older.

Due to C based Nmin consistently having a stronger influence on the forest growth compared to areal based Nmin, with or without the influence of the biomass growth data from S3C, it seems that soil C concentrations are important also in this relationship. As trees consistently exude C from their roots (Heinemeyer et al., 2012), these findings point to the possibility of biomass growth and Nmin mutually influencing each other (figure 11). Instead of a one way relationship between the rates, the observed relationship between Nmin and tree growth could also go the other way around; that the subsequent C exudation from belowground biomass following tree growth, despite the observed decline with lower soil C:N (Vicca et al., 2012; Alberti et al., 2015; Högberg et al., 2017), positively influence the Nmin at the SRC sites (e.g. Bengtson et al., 2012).

The evolutionary gain from releasing valuable carbon compounds to the soil through the roots has been questioned in previous literature (e.g. Dijkstra et al., 2013; Högberg et al., 2017). Studies show exudation from roots to have a strong positive effect on decomposition of SOM, and thereby also on N mineralisation. It provides an additional input of labile C to the soil which is necessary for microbial synthesis of extracellular enzymes (e.g. Bengtson et al., 2012). These enzymes are needed for depolymerisation of SOM, which is commonly viewed as the bottleneck for the northern forest soil N cycle (Sponseller et al., 2016). Meier et al. (2017) found root exudates to have a positive priming effect, foremost enhancing decomposition of fast-cycling SOM pools. The study concluded that the effect of added labile C was present in both soils with low and high N availability. Yet, they concurrently state that the rate of root exudation in a natural environment probably would decrease in soils where N supply is not limited, due to a lower C allocation to belowground biomass. This decline in rhizodeposition from roots is commonly seen also in other studies (e.g. Vicca et al., 2012; Alberti et al., 2015; Högberg et al., 2017). Adding to this, some studies alternatively suggest this decrease in root exudation could be due to a shift in belowground C allocation from fine- to coarse root biomass, and not only due to increased tree C allocation to above ground biomass. Other studies have instead observed increased growth rates in both coarse and fine roots, but declines in the fraction of mycorrhizal root tips (Högberg et al., 2017). Regardless of what causes the decline in exudation rates, this would not mean they stop entirely. Instead, plants continuously release C into the rhizosphere, even though the C allocation to fine roots and mycorrhizae is lower. Studies show an average exudation rate of 17-20% of total net C uptake through photosynthesis (Heinemeyer et al., 2012), or 50% of all plant C allocated to belowground biomass (Nguyen, 2003). With this in mind, it is reasonable to assume this constant, ‘background’, root

exudation, while comprising a smaller fraction of NPP compared to in N limited soils, to increase with increased biomass production.

The soil C:N of the sites investigated in this study, apart from S1, are somewhat centred around, or below, the reported thresholds for the elemental ratio determining microbial assimilation or release of inorganic N (Mooshammer et al., 2014; Cronan, 2018). Solely based on this, they could be classed as having an intermediate N supply, and not suffer from the limited N supply seen in many other boreal forests (e.g. Schimel & Bennett, 2004; Olser & Sommerkorn, 2007; Högberg et al., 2017). Root exudates where N supply is not limiting could provide good conditions for microbial stoichiometric decomposition of SOM. While some studies conclude that priming of SOM declines with increasing soil N (e.g. Bengtson et al., 2012), other studies instead report a shift in the priming phenomena (Chen et al., 2013; Drake et al., 2013). Instead of using the addition of labile C for SOM decomposition to match the microbial need for N, when N availability is sufficient microorganisms might instead use it to further increase their C supply. Additionally, studies show that a coincidental release of N through root exudation has a positive effect on N mineralisation rates, compared to the addition of C alone (Drake et al., 2013; Yan et al., 2023). This has been attributed to exudates better matching microbial demand for both C and N, and thereby enabling both microbial growth and production of exo-enzymes, which are both rich in N. That the addition of N would increase the priming of SOM contradicts the vast research done concluding excess N decreases priming. However, most of these results are based on the addition of inorganic N, which do not reflect natural conditions. The enhanced priming effect from an addition of amino acids seen in a recent meta-analysis by Yan et al. (2023) supports the conclusions that additional N can enhance the priming of SOM also in natural environments, which could be explained by the microbial 'stoichiometric decomposition' theory. Assuming these mechanisms are valid also at the sites in SRC, they could provide a partial explanation for the relationship between biomass growth, Nmin and soil C:N found in this study, for which soil C appears to be an important regulator.

Furthermore, of the three sites covering a fertility gradient near Betsele in northern Sweden, Högberg et al. (2017) found that it was the site with intermediate N supply that showed the highest number of ECM species. The authors suggested this could confirm a higher tree C allocation to ectomycorrhizal associations at this site, compared to the N-poor site. With more N available in the soil, this would lead to a larger transfer of N back to the plant (Högberg et al., 2017). The soil C:N at the intermediate-N site was just below soil C:N found at sites S2 and S3 (C:N of 27 and 24, respectively). The soil C:N at S1 (17) was closer to the high-N site at Betsele, where the authors observed a decline in C allocation to mycorrhizae and a subsequent decline in ECM biomass. The smaller fungal biomass was saturated faster by the larger N supply, and thus supported a higher N transfer to the tree. As ECM fungi also produce and release exo-enzymes needed for depolymerisation of SOM (Terrer et al., 2016; Högberg et al., 2017), an enhanced symbiotic ECM root association in combination with higher soil N could also positively affect the Nmin rates at the site. Assuming that similar conditions regarding root C allocation and ECM fungal associations prevail also at the sites studied at SRC, this may contribute to enhanced rates in tree N supply and growth at the sites, as well as increased Nmin rates related to the observed tree biomass productivity.

In summary, the enhanced microbial C limitation following decreasing soil C:N ratios would increase their need for labile C. Meanwhile, a low soil C:N ratio would mean a higher substrate supply, leading to increased availability to mineral N, and through that also increased tree growth. Even though higher tree biomass in general could produce larger quantities of C exudates, as discussed above, these will still be at a rather constant level in relation to the tree biomass (Heinemeyer et al., 2012; Nguyen, 2003). Nonetheless, the relative importance of the root exudate C for microbial breakdown of SOM in the soils

would increase where C:N is lower. Additionally, the suggested associations between roots and ECM fungi would affect the N supply to trees, and thereby their growth, differently at the sites; the large ECM biomass at S2 and S3 could support a large N transfer to host trees, while the increasing fungal N saturation would be the main driver at S1, despite the smaller fungal biomass. At all sites, the ECM fungi could also contribute positively to Nmin rates, following C exudation from tree roots. Taken together, this could serve as an explanation for how the observed negative relationship between soil C:N and Nmin relates to the proposed subsequent rhizosphere C deposition following tree biomass production, and possible microbial 'stoichiometric decomposition' priming effect (figure 11). With the previous discussion in mind, decreasing soil C:N together with root exudates relative to the higher biomass growth could provide conditions where the exudate C and the higher soil N availability matches microbial nutritional demands. The labile C addition to the soil would aid in widening the soil N cycle bottleneck of SOM depolymerisation, leading to SOM decomposition and soil gross N mineralisation closer to their full potential rates. Obviously, the lack of data on microbial communities, as well as quantity and quality of root exudates, limits the possibility to draw any definite conclusions from this analysis. In order to make a more robust conclusion, more research is needed.

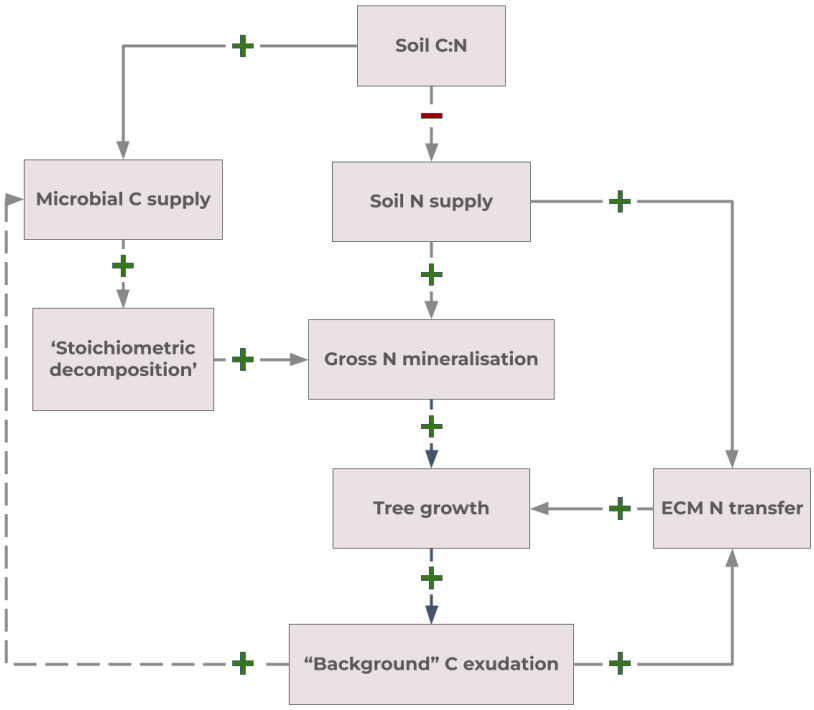

Figure 11. Conceptual representation of the proposed two-way relationship between soil gross N mineralisation and tree growth at intermediate N supply. Plus-signs represent positive (reinforcing) relationships and minus-signs negative (inverse) relationships.

5.4 S4 as a consistent deviation

How the results from S4 repeatedly differ from those found within Group S1-3 was quite surprising, and suggest there are other factors affecting the investigated relationships that are still unknown. The remnants from the coal pits used at S4A in the 18th and 19th century (Hill, 1999) clearly affected the soil C:N within the plot. The high C:N ratio (39) reflects the large amount of charcoal in the soil samples, rather than the concentration of organic C and N (table 3). The exclusion of plot S4A from all statistical analyses including soil C, N or C:N was thus necessary, however unfortunate.

Without taking into account any statistical analysis, looking at the relationship between soil C:N and Nmin for the plots within S4, it follows the same pattern as the same relationship within Group S1-3, where a lower soil C:N coincides with faster Nmin rates. Although, compared to the Nmin rates found for Group S1-3, all rates at S4 are elevated, seemingly making S4 the most fertile of all four sites (figure 10). Soil C and N concentrations at S4B are very high compared to the other plots, as well as the other sites (table 3). It also has the highest soil moisture. Both high concentrations of organic matter and soil moisture have been associated to higher Nmin rates before, as long as the soil is not saturated (Högberg et al., 2017). Coincidentally, this is the plot with the highest Nmin rate of them all. The areal, as well as C based Nmin rates at the plot were four times faster than those at S3, even though the plot and site share the same soil C:N (24; second to lowest of all sites). However, in plot S4C, where soil C and N content is relatively low, GWC is intermediate and soil C:N is high (28; second to highest of all sites, excl. S4A), Nmin is second to highest of all plots and sites. At this plot, the areal and C based Nmin rates are ten times greater than at S2, where the soil C:N was measured to 27. This gives rise to a number of questions this study can only attempt to answer through speculation. Most importantly, these results suggest there are other factors not investigated in this study that influence the Nmin rate, as well as its relationship with soil C:N at the studied sites, that has not been included in this investigation. Thus, based solely on the results found in this study, soil C:N ratio cannot be used as a proxy for Nmin. Instead, more research on what controls the relationship between the two variables is needed.

Regarding the connection between Nmin and biomass growth, the general pattern that appears while looking at the data is opposite to the relationship within Group S1-3, namely that an increase in the Nmin rate corresponds with a decrease in biomass production (table 3, figure 9). The statistical analyses revealed the relationships to be very strong ($R > -0.86$; table 4), but only significant between C based Nmin and the growth rate of Norway Spruce ($P = 0.048$, $R = -1$). However, due to the limited amount of sample points, the lack of statistical significance was expected. Despite this, the visual analysis, together with the strong correlation coefficients, could suggest there is a relationship to be found between the variables, but that more research and a larger sample size is needed to come to a definite conclusion.

Similar patterns, opposite those found for Group S1-3, can be seen when isolating S4 in the analysis of the connection between biomass production and soil C:N, even though S4B and S4C fit well into the overall correlation (figure 8). It appears here that at this specific site, an increase in soil C:N corresponds to an increase in forest biomass production (see also table 3). The unrealistic soil C:N at S4A makes it impossible to draw any conclusions from this relationship only. Nonetheless, the fact that the pattern repeats itself through both Nmin and soil C:N, and how these two variables previously have been shown to predict tree biomass growth, yet again points toward some unidentified variable affecting the productivity at the site. Peichl et al. (2022) found in their study on controls of the C balance in managed boreal forests that soil C:N explained NEP and NPP well on a landscape scale, but that the results differed when dividing the forests into age groups. Soil C:N was shown to remain an important predictor for NPP in forests up to 58, and above 131, years old. All sites within Group S1-3 fit into the lower end of this category (35-55 years). For forests in the age groups between 61-105 years, where S4 is included (71 years), direction, such as north–south and east–west gradients, was more important in regulating NPP. Without the ability to say that the same is true for the sites of interest in this study, this highlights the fact that there might be other factors controlling tree growth at S4 that have not been analysed here.

The high Nmin rates at the site point to soil conditions that meet microbial need for N. That the Nmin rates are so much faster here than at the other sites could be due to there being a large difference in the microbial community between them. The slower rates of tree growth, taken together with the rather large NH_4^+ pool sizes (table 3), could imply that there is low competition for N between microbes and

trees. It could also indicate a large supply of labile C enabling fast rates of mineralisation in excess of microbial and plant demand (Högberg et al., 2017). But, despite this low competition, the tree growth does not increase. Something else seems to be limiting the forest biomass productivity at this specific site; something that makes this limitation more severe with decreasing soil C:N. Perhaps the trees at S4 have weaker associations with ECM fungi, slowing down the N supply to the roots (Högberg et al., 2017). Perhaps there is a lower base saturation at the site, leading to mineralised NH_4^+ getting adsorbed onto cation exchange sites, and through this becoming unavailable to trees (Cronan, 2018). However, the high concentrations of extractable NH_4^+ vote against this. Additionally, studies show abiotic immobilisation to have a marginal role in boreal ecosystems, compared to biotic immobilisation (e.g. Högberg et al., 2017). Of course, a limitation of the tree growth at the site could be associated with other things than the soil nutrient supply or microbial community, such as competition, chemical stress, possible historical disturbances, or temporal shifts in local weather and climate that has not affected the younger sites (Cronan, 2018). Nonetheless, these are all only theories of what could impact the tree growth rates differently at this site, compared to the remaining three. The divergence in Nmin rates between the sites in SRC could simply be a result of the small sample size. However, in the present situation, the results from S4 points to soil Nmin not being a strong enough determinant to control biomass growth rates. To understand what is causing this apparent contradictory behaviour, further measurements and analyses are needed.

5.5 Implications for future CO_2 sequestration capacity and climate

The ecosystems of the world are largely shaped by the specific environmental, biogeochemical and climatic conditions governing at their geographical location. A change in climatic conditions and weather events, as well as the biogeochemical cycling of elements, will inevitably impact how these ecosystems function, including rate of plant growth within them (Cronan, 2018). The capacity for trees to produce biomass from photosynthates is highly dependent on the nutrient status of the soil (Vicca et al., 2012). Hence, the rhizosphere priming of SOM following root C exudation previously discussed in this analysis is important to consider while discussing the boreal forest C storage under elevated atmospheric CO_2. Research shows that plants tend to increase the root C exudation under higher temperatures and enhanced atmospheric CO_2, potentially leading to declines in the soil C stock (e.g. Bengtson et al., 2012; Phillips et al., 2009). In N deficient ecosystems, while having a positive effect on the supply of bioavailable N, the enhanced decomposition of SOM creates a microbial surplus of C resulting in increased microbial respiration of C stored in the soil, and thus, release of CO_2 to the atmosphere. This increased respiration could potentially create a positive feedback loop, further increasing global warming and climate change (Wu et al., 2016). Yet, many studies discuss that the CO_2 fertilisation effect and increased temperatures, together with a greater nutrient availability, might stimulate the plant C sink so that it makes up for the increased C respiration entirely (Bengtson et al., 2012; De Graaff et al. 2006; Dijkstra et al. 2008; Zak et al. 2011), or that the increased C input to the soil by plants would lead to higher turnover rates of the soil nutrient pools, but that the pool sizes will remain more or less the same (Kuzyakova et al., 2019). Ultimately, the effect of increased tree C allocation to root exudates largely comes down to the availability of nutrients essential to the tree for assimilation of C into its biomass. Thus, this is important to consider in context of the C storage capacity of high latitude forests in a changing climate.

Terrer et al. (2016) revealed that trees limited by N show a positive growth response to elevated atmospheric CO_2 only in ecosystems where plants are associated with ectomycorrhizal fungi, such as boreal and temperate forests. These results were, however, based on systems where the N limitation was not as severe as in many boreal systems, such as the low-N site investigated by Högberg et al.

(2017). Combining the analysis by Högberg et al. (2017) and Terrer et al. (2016), perhaps trees in intermediate N systems, such as the SRC sites investigated in this study, where ECM fungi are abundant, would indeed show a positive growth response and CO_2 sequestration capacity as a result of increased atmospheric CO_2, possibly counteracting global warming. Meanwhile, in many other boreal forests the response could be reversed, followed by an increased progressive nitrogen limitation (PNL). Many studies regarding plant response to elevated atmospheric CO_2 have discussed the concept of PNL, which means that the enhanced biomass production due to elevated atmospheric CO_2 and the associated increased plant N demand would exacerbate the N limitation and thereby decrease the forest C sequestration capacity, both in tree biomass and soils. The discussion about how ECM fungi would assimilate a larger fraction of available soil N instead of transferring it to their tree hosts in very N poor systems could contribute to this idea, but the ECM response to elevated atmospheric CO_2 is not yet fully known (Högberg et al., 2017). Nevertheless, the observed priming effect following elevated CO_2 in N limited ecosystems may, instead of directly leading to PNL, work as a strategy towards mitigating it (Phillips et al., 2009; Dijkstra et al., 2013).

Contrasting the increased soil respiration in low N systems, priming in an N rich environment would not lead to the same increased mobility of soil C, but instead an elevated release of inorganic N, enhancing the tree C sequestration capacity. In these systems microbes are increasingly limited by C, creating a need to assimilate the mineralised C instead of releasing it (Mooshammer et al., 2014). This would increase the fraction of C taken up through photosynthesis allocated to long-lived woody biomass, while decreasing the relative autotrophic respiration that does not contribute to the forest C sink (Vicca et al., 2012). However, Alberti et al. (2015) concluded that in fertile forests with a high N supply, root C exudation could in fact increase the soil C sink, rather than decreasing it. The study found a significant connection between low soil C:N and increased soil C stocks, resulting from a larger proportion of root C exudates being sequestrated in the soil. This was suggested to be a result of the increased microbial CUE. If the same conditions apply for elevated CO_2 induced increases in root C input to soils, it could lead to a greater soil C stock in these ecosystems. Regardless of if the elevated CO_2 uptake ends up increasing the soil C stock, or that of tree biomass, it is evident that the nutrient balance in the soil is a crucial component regulating the forest ecosystem reaction to elevated atmospheric CO_2, and thus global climate.

5.6 Robustness of analyses and possible method limitations

While the true mechanisms behind the relationship between tree biomass growth and Nmin found in the present study are difficult to establish, it is evident that the nutrient balance in the soil is an important factor affecting the relationship. Högberg et al. (2017) suggest that it is the access to N substrates that control plant nutrition, rather than which forms of N the trees can utilise, and that plants can only choose a preferred form of N when the availability is high enough. Considering this, it could be that soil C:N drives the tree growth, and not Nmin per se, and that Nmin is simply a consequence of the balance between C and N availability in the soil. Nevertheless, it can also point to a sufficient and continuous N supply that allows the access to mineral N to control the biomass production. An additional analysis of the connection between tree growth and presence of e.g. amino acids in the soil would provide a clearer picture of this possibility. Still, the multiple regression analysis and the subsequent VIF analysis indicate there is not a major issue with multicollinearity between Nmin and soil C:N, revealing that soil C:N is the most important factor impacting biomass production of the two, and that there is a separate impact on biomass growth from Nmin.

The method used to estimate the biomass production at the four sites in SRC was based on a report by Stokland (2021), in which the annual volume increment of Norway Spruce trees were found to be rather

linear throughout the lifetime of the tree. This linearity, however, does not begin until the trees are above approximately 30 years old. Before that, the annual volume increment is lower, which is natural due to the size of the tree, and gradually increases until it reaches the threshold for linear growth. The forest in one of the plots (S3C) was estimated to be 35 years old, which naturally would make the biomass production there lower, compared to the other plots where the forest was older. This age related growth bias creates an uncertainty in the resulting growth rate, and makes it less comparable against the other plots. Nevertheless, insead of looking at it as a means of estimating real biomass production, it should be seen as a method for creating an index for the production rates. To improve the accuracy of the method, a function to normalise the annual growth against forest stand age should be included. However, this and other ways to estimate forest biomass production were beyond the scope of this study.

The extractability of the added ^{15}N at t_2 was low, which could indicate errors somewhere in the chain of experiment, such as during sampling (e.g. accidental extraction of untreated soil), analysis (e.g. undiscovered problems during micro diffusion or IRMS analysis), or calculations (e.g. lack of comparability between ^{15}N samples, representing the top 9 cm of the soil, and bulk density samples, representing the top 5 cm). However, there is another possible reason for this low extractability. The substrate is added to the product pool and is thus assumed not to impact the production rate and the inflow into that pool. Though, it may stimulate consumption rates, as has been discussed in several studies. A fast, sometimes immediate, consumption of the added ^{15}N label has been commonly observed, and implies there is a considerable short-term flux following labelling. This would not be sustained over longer periods, and has thus not been observed in net N mineralisation experiments, that usually last over longer periods of incubation (Christenson et al., 2009).

Discussions about possible explanations for these rapid initial NH_4^+ consumption rates are plenty. Booth et al. (2005) discuss if adding the ^{15}N to the soil in liquid form could possibly stimulate consumption rates in the context of increased soil moisture, rather than increased substrate supply. Some studies have reported only a limited contribution to the fast NH_4^+ consumption to be a result of abiotic retention or nitrification (Christenson et al., 2009) or cation exchange capacity (Högberg et al., 2017), while others report clay fixation as one of the major processes behind it (Braun et al., 2018). However, most agree that rapid microbial immobilisation of the added substrate substantially contributes to the fast consumption (Christenson et al., 2009; Braun et al., 2018; Högberg et al., 2017). Furthermore, research has connected the ^{15}N recovery to soil nutrient availability, where the initial immobilisation in a soil rich in N was approximately 20%, while it at an N poor site was approximately 80% (Högberg et al., 2017). The extractability of excess ^{15}N in the present study was even lower, following an immediate consumption of > 80% of the added ^{15}N label.

Put into context of the previous findings discussed, the low ^{15}N extractability at SRC would imply all sites have a limited N supply. Nevertheless, the lack of significant differences in excess ^{15}N recovery between the four SRC sites indicate that it is not related to the soil nutrient status in this case. Hence, at these specific sites, the fast initial NH_4^+ consumption rate might not be a result of mainly biotic, but abiotic, immobilisation. While clay fixation can occur within hours of adding the label into the soil, microbial uptake can occur within minutes, or even seconds (Braun et al., 2018, and references therein). Thus, insead of moving the soil samples to a field laboratory and so delaying the time of extraction, as was done in this study, extracting the soil samples immediately after labelling and sampling in the field would be preferred for a more accurate result. Still, without additional measurements, it is difficult to draw any conclusions about what is controlling the rapid label substrate consumption, though it does question the assumption that both the added substrate and native soil N pool of interest are consumed at the same rate, which is a necessity for conducting ^{15}N dilution experiments.

5.7 Conclusions

The capacity of northern forests to sequester C and mitigate global climate change is highly dependent on the interaction between the soil C and N cycles. The significant inverse relationship between soil C:N and gross N mineralisation rates within Group S1-3 in SRC adds support to previous findings showing mineralisation to be faster in more fertile soils. Concurrently, it further emphasises the important role of soil C on this connection, as it regulates the significance of the relationship. Thus, the results of this study agree with previous research, pointing to organic matter and soil C to be highly important for the cycling of soil N.

Furthermore, the results reveal there is a connection between gross N mineralisation and forest biomass production across three of the four sites at SRC; a relationship in which the two rates could be mutually influencing each other. A higher substrate supply followed by faster gross rates of mineralisation increases the N pool available to plants, enabling enhanced tree growth. Meanwhile, the labile C addition from larger 'background' root exudation following tree growth could alleviate the microbial C limitation resulting from low soil C:N and provide good conditions for microbial stoichiometric decomposition of SOM. In other words, decreasing soil C:N together with root exudates relative to the higher biomass growth could provide conditions where the exudate C and the higher soil N availability better matches microbial nutritional demands, widening the bottleneck of SOM depolymerisation on the soil N cycle, and leading to SOM decomposition and soil gross N mineralisation closer to their full potential rates. Additionally, possible strong ECM fungal associations may further contribute to enhanced rates in tree N supply and growth at the investigated sites, as well as increased mineralisation rates related to the observed tree biomass productivity. However, this is based on the assumption that the ECM fungal community resembles that of similar sites investigated in previous studies, as no such measurements have been taken at the sites in SRC.

Within the same three forest stands as mentioned above, there was no correlation between biomass growth and extractable NH_4^+-N, pointing to gross N mineralisation as a better way of estimating the inorganic N supply to trees than the actual pool sizes. Moreover, due to the lack of a significant difference between the sites, the results point to N mineralisation rates based on soil dry mass as a poor measurement of the transformation process when used in comparing sites with differing soil properties, and argues for the inclusion of soil C concentration in the analysis.

Despite the clear associations between soil C:N, soil gross N mineralisation and biomass production within Group S1-3 in SRC, the consistent divergence, and at times opposite relationships, of S4 suggest there are other factors not investigated in this study that influence the N mineralisation rate and tree biomass production, as well as its connection to soil C:N, at the studied sites. Thus, based solely on the results found in this study, soil C:N ratio cannot be used as a proxy for soil gross N mineralisation, nor is it possible to declare gross N mineralisation as the main driver of biomass production. Even though it could be that the divergences in mineralisation and biomass production rates between the sites in SRC are simply a result of the small sample size, in the present situation the results from S4 point to a need for further measurements and analyses on what controls the relationship between the variables. Thus, the results of this study reject the hypothesis that the process controlling soil inorganic N availability (gross N mineralisation) is in direct relation to soil fertility and forest production. Instead, to expand our knowledge on these connections and their implications for the C sequestration and global climate change mitigation capacity of northern forests, this study highlights that more research is required.

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Table A1. Description of tree species composition resulting from circular plot inventory.

		n (% of site n)	*Age* (years)	*DBH* cm* (SD)	*Basal area* ($m^2\ ha^{-1}$)	*Density* (stems ha^{-1})
S1	***Norway Spruce***	65 (100%)	55	37.7 (8.8)	54	460
	Scots Pine	-	-	-	-	-
	Birch	-	-	-	-	-
	Other Deciduous	-	-	-	-	-
S2	***Norway Spruce***	132 (88.6%)	55	21.5 (7.2)	25	622
	Scots Pine	3 (2%)		28.5(0.9)	1	14
	Birch (spp.)	14 (9.4%)		14.9 (4.6)	1	66
	Other Deciduous	-	-	-	-	-
S3A & S3B	***Norway Spruce***	64 (79%)	47	24.3 (6.8)	23	453
	Scots Pine	7 (8.7%)		31.3 (5.8)	4	50
	Birch	9 (11.2%)		18.8 (5.1)	2	64
	Other Deciduous	1 (1.2%)		13.8	0	0
S3C	***Norway Spruce***	27 (75%)	35	19.9 (4.9)	13	382
	Scots Pine	4 (11.1%)		25.8 (3.3)	3	57
	Birch	5 (13.9%)		17.2 (4.2)	2	71
	Other Deciduous	-	-	-	-	-
S4	***Norway Spruce***	107 (93.9%)	71	27.9 (6.5)	33	505
	Scots Pine	2 (1.8%)		41.4 (10.0)	1	9
	Birch	3 (2.6%)		17.9 (4.4)	0	14
	Other Deciduous	2 (1.8%)		29.3 (3.0)	1	9

*Diameter at breast height

Table A2. Linear regression analysis of all variables and rates included in the study.

Group S1-3

Dependent variable *Independent variable*	N	Slope	Intercept	R =	R^2 =	P =	Excluding
Biomass growth (t ha^{-1} yr^{-1}); All Species							
Soil C:N ratio	8	-0.36	13.05	-0.86	**0.74**	0.006	S1B - No biomass data
Nmin (µg NH_4^+-N g^{-1} soil C)	**7**	0.16	1.90	0.79	**0.63**	0.034	S1B & S2C - No biomass data, no Nmin data
Nmin (g N ha^{-1})	**7**	0.003	3.29	0.52	**0.27**	0.236	S1B & S2C - No biomass data, no Nmin data
Nmin (µg NH_4^+-N g^{-1} soil C)	**6**	0.16	2.11	0.85	**0.73**	0.031	S1B, S2C & S3C - No biomass data, no Nmin data & uncertain biomass data
Nmin (g NH_4^+-N ha^{-1})	**6**	0.005	2.86	0.83	**0.69**	0.040	S1B, S2C & S3C - No biomass data, no Nmin data & uncertain biomass data
Biomass growth (t ha^{-1} yr^{-1}); Norway Spruce							
Soil C:N ratio	8	-0.38	13.15	-0.81	**0.66**	0.014	S1B - No biomass data
Nmin (µg NH_4^+-N g^{-1} soil C)	**7**	0.16	1.40	0.73	**0.53**	0.063	S1B & S2C - No biomass data, no Nmin data
Nmin (g NH_4^+-N ha^{-1})	**7**	0.003	2.90	0.46	**0.21**	0.304	S1B & S2C - No biomass data, no Nmin data
Nmin (µg NH_4^+-N g^{-1} soil C)	**6**	0.17	1.66	0.80	**0.65**	0.054	S1B, S2C & S3C - No biomass data, no Nmin data & uncertain biomass data
Nmin (g NH_4^+-N ha^{-1})	**6**	0.006	2.41	0.79	**0.63**	0.060	S1B, S2C & S3C - No biomass data, no Nmin data & uncertain biomass data
LOG Nmin (µg NH_4^+-N g^{-1} soil C)							
Soil C:N ratio	8	-0.05	2.32	-0.85	**0.72**	0.008	S2C - No Nmin data
Soil C (g kg^{-1} dry soil)	**6**	-0.02	2.23	-0.48	**0.23**	0.336	S2C, S3B, S3C - No Nmin data, org mat outliers
Soil N (g kg^{-1} dry soil)	**6**	0.58	-0.55	0.92	**0.84**	0.01	S2C, S3B, S3C - No Nmin data, org mat outliers
Nmin (µg NH_4^+-N g^{-1} dry soil)							
^{15}N recovery at T2	**16**	0.46	-0.33	0.87	**0.75**	< 0.001	S2CT2-A, S2CT2-B (No Nmin)