Master Thesis

Investigation of nitrogen leaching in managed and unmanaged forests

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Abstract

Danish forests have been experiencing moderate to high nitrogen deposition, resulting in N leaching problems that are very harmful to the environment. In this thesis, soil experiments and gas exchange measurements were conducted for forests with different managements, which were managed forest (Broby Vesterskov) and unmanaged forest (Suserup Skov). The resulting nitrate leaching and N_2O emission data were analysed. N_2O flux was calculated by R code and then these data were analysed by ANOVA, T- test and other statistical methods. The results showed that soil properties were slightly different between the two forests, with both nitrate leaching and N_2O emissions lower in managed forests than in unmanaged forests, possibly because biomass was regularly removed in the managed forest, thereby reducing N input. There was only a slight positive correlation between nitrate leaching and N_2O emission. In addition, it was found that some other factors affecting N_2O emission, such as higher soil temperature, moderate soil water content, canopy gaps effect and edge effect, would increase N_2O emission. These findings would be helpful for municipality to deal with the problem of controlling forest N leaching and find a better management model.

Acknowledgements

This thesis (45 ECTS) was conducted at University of Copenhagen, the Department of Geosciences and Natural Resource Management, as a requirement for completing the Master's project in Environmental Science. The aim of the thesis was to compare nitrogen leaching under different forest managements and explore the factors that influence it.

I would like to thank my supervisor Per Gundersen for his patience, guidance and help in my thesis working process, and my co-supervisor Frederik Nygaard Philipsen, who helped me a lot with R and data calculation. They gave me fully help and support in field work and academics work. Additionally, I would like to extend my appreciation to the biogeochemistry lab for assistance and chemical analysis. Finally, I would like to thank my family and friends for their encouraging, supporting and love during my study.

Content

1. Introduction

1.1 Background

Nitrogen is a significant limiting nutrient in the net primary productivity of terrestrial ecosystems. Since the mid-20th century, human activities such as industrialization, the use of fossil fuels and fertilizers, have been increasing, therefore atmospheric N deposition has shown a rapid increase, even exhibiting a global trend (J. W. Van Groenigen et al., 2015).

The dramatic increase in atmospheric nitrogen deposition will threaten the health and safety of both terrestrial and aquatic ecosystems. N deposited onto the ground accumulates in the soil or into groundwater, then N leaching occurs when the availability of inorganic nitrogen exceeds the demands of plants and microorganisms. After that it will cause many environmental issues, like soil acidification, nutrient loss, water pollution, eutrophication, and increased emissions of greenhouse gases like N_2O (Klaus Butterbach-Bahl et al., 2013).

Forests are a major component of terrestrial ecosystems. From a European perspective, Danish forests receive moderate to high nitrogen deposition, with nearly 70% of the N deposited in forest areas being retained (Per Gundersen et al., 2009). Additionally, forest soils are a source of N_2O emissions (Klaus Butterbach-Bahl et al., 2013). The high N availability of acidic topsoil promotes the formation and emission of N_2O. Therefore, with the increase of N deposition, the emission of N_2O in forest soil increases, which exacerbates the greenhouse effect.

Most Danish forests are under human management, few of them are not, but the area is increasing (P. Gundersen, personal conversation). Unmanaged natural forests always exhibit a more natural vegetation structure and species composition. Managed forests typically undergo human interventions such as regular logging or fertilization, which may alter the chemical properties of the soil. Thus, it is worth exploring the problems that different forest management models may face under increasing N deposition.

1.2 Research objectives

We compiled monthly fluxes of N_2O and nitrate content in forest soils from two forest with different managements in Zealand, Denmark. The effects of managements, soil temperature, soil moisture, canopy gaps and other factors on soil nitrate content and fluxes of the N_2O were investigated.

This study is based on the continued research of (Per Gundersen et al., 2009) and the master's thesis work (Wang Songqing, 2023) Based on previous studies and literature review, we expected soil pH, N availability, soil temperature and moisture to be the most important internal drivers. Based on this framework, the following hypotheses were made in this study:

(i) unmanaged forests are likely to have higher N availability and N leaching compared to managed forests;

(ii) unmanaged forests produce higher N_2O emissions compared to managed forests, especially during the summer months when high soil temperature and moisture;

(iii) high N availability leads to both higher nitrate leaching and to more N_2O emissions.

The thesis is divided into six chapters. Chapters 1 and 2 are an introduction to the thesis and the theory of the topic. In chapter 3 the experimental method is presented, chapters 4 and 5 show the results of the thesis and analyse them, and the last chapters 6 is the conclusion of the whole thesis.

2. Theory

Nitrogen, which makes up about 79% of the atmosphere in the form of dinitrogen gas (N_2), is an essential element for sustaining life and ensuring the normal growth and functioning of organisms. There are two large nitrogen pools on Earth, namely atmospheric molecular nitrogen (N_2) and biologically reactive nitrogen (NO_3-, NH_4+, and organic nitrogen). The interactions between these two N pools are primarily controlled by two key biological processes (Figure 2.1): N fixation and denitrification (Ken Takai, 2019). Reactive nitrogen forms such as ammonium (NH_4+) or nitrate (NO_3-) are essential for optimal plant growth. The conversion of the relatively inert N_2 into reactive nitrogen can be achieved in two natural ways: either by lightning or by biological nitrogen fixation (Gundersen, Schmidt, & Rasmussen, 2006).

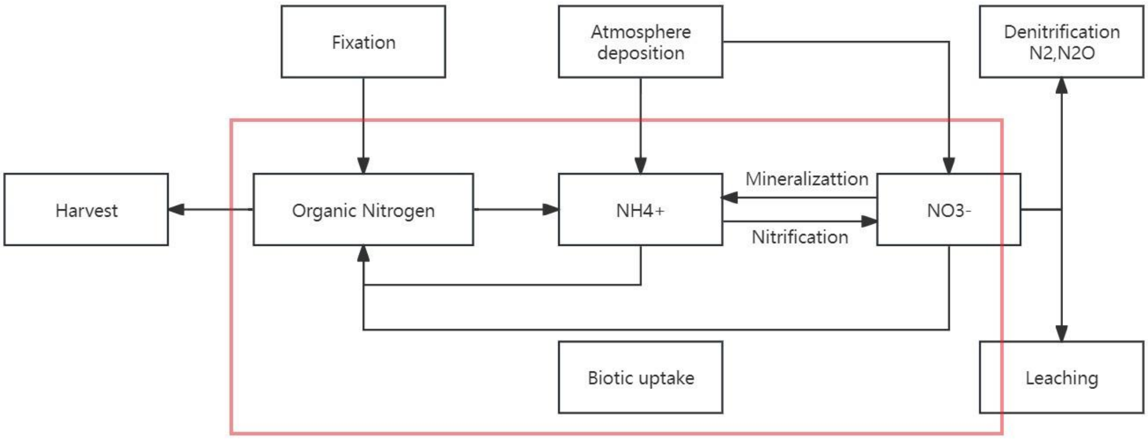

Figure 2.1 Forest N cycle, inside the red box is the internal cycling, outside the box is the external exchange (Redrawn from Gundersen & Rasmussen et al., 1990).

2.1 N cycling

The nitrogen cycling process refers to the transportation, transformation, and turnover of nitrogen between the Earth's atmosphere, biosphere, soil, and hydrosphere. Research into the N cycle started from the mid-19th century, when it was discovered that N was a limiting factor in crop growth. The N cycle on land considered to be a process of N form transformation. More than a century of research has shown that N has an important impact on the productivity of ecosystems around the world and is highly responsive to changes in temperature, precipitation, atmospheric CO_2 levels, and other disturbance regimes (J. W. Van Groenigen et al., 2015).

Nitrogen sources in forest ecosystems are diverse, including natural and anthropogenic sources, such as atmospheric deposition, biological nitrogen fixation, agricultural fertilization, animal manure, and industrial emissions. N deposition in the atmosphere mainly exists in two forms: dry deposition and wet deposition (Lindsay R. Boring et al., 1988). Dry deposition is when nitrogen compounds, including nitrogen, ammonia, nitrates, and volatile organic N compounds, settle directly from the atmosphere to land or water in the form of particles or gases. Wet deposition occurs when N compounds combine with water vapor in the atmosphere to form water droplets or ice crystals, which then precipitate onto land or water bodies. The canopy of the forest traps gases and particles more effectively than the bare ground, so the amount of N deposition in the forest is significantly greater (Gundersen et al. 2006). The broad range of N-in flux from 7 to 38 kg N /ha/yr observed in various Danish studies over the last 20 years was confirmed by the intensive studies in eight stands 2002–2005 (Gundersen et al., 2009).

Nitrogen output from forests is a critical process in ecosystems that is influenced by a variety of factors, including plant uptake, soil erosion, microbial decomposition, natural disasters, and human activities. For example, N from plants residue is re-released into the soil by decomposition, thus promoting the N cycle within the plant. At the same time, soil erosion and runoff can cause N in the soil to be lost to surrounding water bodies, such as rivers and lakes. In addition, human activities also have an impact on forest N output, such as excessive fertilization and deforestation. These factors work together to shape the flow and circulation of N in forest ecosystems.

2.2 N dynamics

Although the atmosphere is rich in nitrogen, terrestrial ecosystems cannot use it directly (J. W. Van Groenigen et al., 2015). To get enough N, ecosystems must rely on multiple biotransformation processes that convert nitrogen into a bioavailable form. The dynamic nature of N has implications for species in terrestrial ecosystems, which have evolved multiple ways to access, efficiently use, and retain these small amounts of N (J. W. Van Groenigen et al., 2015). Therefore, the input and supply of N are critical for regulating the structure and function of terrestrial ecosystems.

In forest soil ecosystems, N dynamic involves several processes including decomposition, mineralization, nitrification, fixation, denitrification, and plant uptake. N input in the forest ecosystem is mainly accomplished through N fixation process. This process involves microbes breaking down organic matter and minerals in the soil, which releases reactive nitrogen (Lindsay R. Boring et al., 1988).

Nitrates produced by nitrification are highly soluble in water and can, if it not taken up by plants, it would be lost from ecosystems through leaching, or gas loss. On the contrary, denitrification occurs in moist and anaerobic soil conditions. In this process, heterotrophic denitrifiers reduce nitrate (NO_3-) to nitrous oxide (N_2O) and eventually to nitrogen (N_2), which finally release back into the atmosphere (J. W. Van Groenigen et al., 2015). This process effectively recycles N back into the atmosphere.

There is a dynamic equilibrium relationship between nitrogen input and output. The increase of N input will lead to the increase of nitrate level in the forest, which will lead to the increase of nitrate leaching. Nitrate leaching may be considered high at >1 mg N/L in soil water or >2–3 kg N/ha/yr (Gundersen et al., 2006). Nitrate leaching elevation is almost non-existent when throughfall N deposition input is below 8–10 kg N/ha/yr, and it consistently occurs at levels exceeding 25 kg N/ha/yr. When N loss approaches or exceeds N input, the system is considered saturated (Klaus Butterbach-Bahl et al., 2013). The substantial variation in nitrate leaching in response to N input is partly attributed to differences in soil C/N ratio. Higher nitrate leaching is observed when the C/N ratio in the organic layer (C/N-org) is below 25, indicating that C/N-org can serve as an indicator for assessing the risk of nitrate leaching in forest ecosystems under increased N deposition (Gundersen et al., 2009), the lower the C/N ratio, the higher the risk of nitrate leaching in the forest system.

2.3 N_2O

N_2O, a colorless and odorless gas at room temperature, exhibits mild anesthetic characteristic, commonly employed as an anesthetic agent. Additionally, N_2O serves in industrial applications such as gas pressure intensifiers, and in the food industry as a spray for cream mixtures. However, excessive or improper use of N_2O maybe harmful to the environment. Firstly, N_2O is a strong greenhouse gas, with a greenhouse effect about 300 times that of CO_2. It absorbs infrared radiation from the Earth's surface and blocks its radiation into outer space, causing global temperatures to rise and exacerbating global warming and climate change (Klaus Butterbach-Bahl et al., 2013). Secondly, N_2O is a major ozone-depleting substance, and its emission will negatively affect the recovery rate of the ozone layer.

N_2O is a transformation product of nitrogenous compounds in soil sediment and water (Joachim Audet et al., 2020). Soil serves as a major source of N_2O in the atmosphere, with natural soil emissions (6-7 TgN_2O-N/yr) accounting for 56-70% of all global N_2O sources. Forest ecosystems cover approximately 1/3 of the Earth's land surface, and emissions of trace nitrogen gases from forest soils have been identified as significant sources of N_2O and NO in the atmosphere. N_2O emissions from the forest soil are estimated at about 3.6 Tg N/yr, representing 33% of the total global soil N_2O emissions (Changsheng Li et al., 2000).

The emission of N_2O is a process driven by a combination of biological nitrification and denitrification. Each reaction is influenced by a variety of factors, including soil environmental factors. For example, soil temperature, soil moisture, pH, Eh (redox potential) and substrate concentration all have an effect on the reaction (Changsheng Li et al., 2000). The change of the soil temperature will affect the rate of nitrification and denitrification. Generally, higher temperature is conducive to nitrification (between 5-20℃) and denitrification (Changsheng Li et al., 2000). When the soil moisture content reaches the moderate level (60%), the emission of N_2O is the highest (P. Gundersen et al., 2012). Soil pH and Eh values will affect the ecological environment and enzyme activity of soil microorganisms, thus affecting the nitrification and denitrification processes. These factors interact with each other to affect the emission process and rate of N_2O.

2.4 Forest managements on N

Soil management systems can alter soil mineralization and nitrification rates. The impact of different practices in forest management on N_2O emissions is complex and influenced by multiple factors. In forestry management, the application of fertilizer can promote the growth of plants. However, excessive use of nitrogen fertilizer may lead to the enrichment of N in the soil, which increases nitrification and denitrification processes, increases nitrate leaching, and leads to increased N_2O emissions (Eduardo Garcia Cardoso et al., 2011). Different types of forest vegetation have different efficiency of N absorption and release. For example, coniferous forests generally absorb N more efficiently than broadleaf forests, in contrast reducing N accumulation in the soil (Norbertas Noreika et al., 2012). The management of forest vegetation residues (such as leaves and branches) may affect N cycling processes in the soil. For example, removing residues regularly can reduce N accumulation in the soil, thereby lowering N_2O emissions. Implementing soil conservation measures such as forest cover and reducing soil erosion helps maintain N cycling balance, consequently reducing N_2O emissions. Danish forests are under moderate to high nitrogen deposition, unmanaged old forests could lead to high N-availability as there is no harvest removal of N and minor net tree growth to take up nutrients (P. Gundersen, personal conversation). Whereas managed forests are held in phases of high growth by regular thinning, they take more nutrients like N, which is then again removed with biomass.

3. Methodology

3.1 Sites description

The aim of this study was to measure and analyse nitrate content and N_2O emissions from forest soils, thus to assess the effects of management models. Investigate from three aspects: evaluate soil N_2O emissions, measure deep soil nitrate concentration and measure top soil total-N concentration before and after mineralization.

This study selected two forests (Suserup Skov and Broby Vesterskov forest) located in Zealand, Denmark for research, see the location in the Figure 3.1. The two forests represent different forest types, vegetation types and management models. Suserup Skov forest is an unmanaged forest while Broby Vesterskov is managed (Figure 3.2).

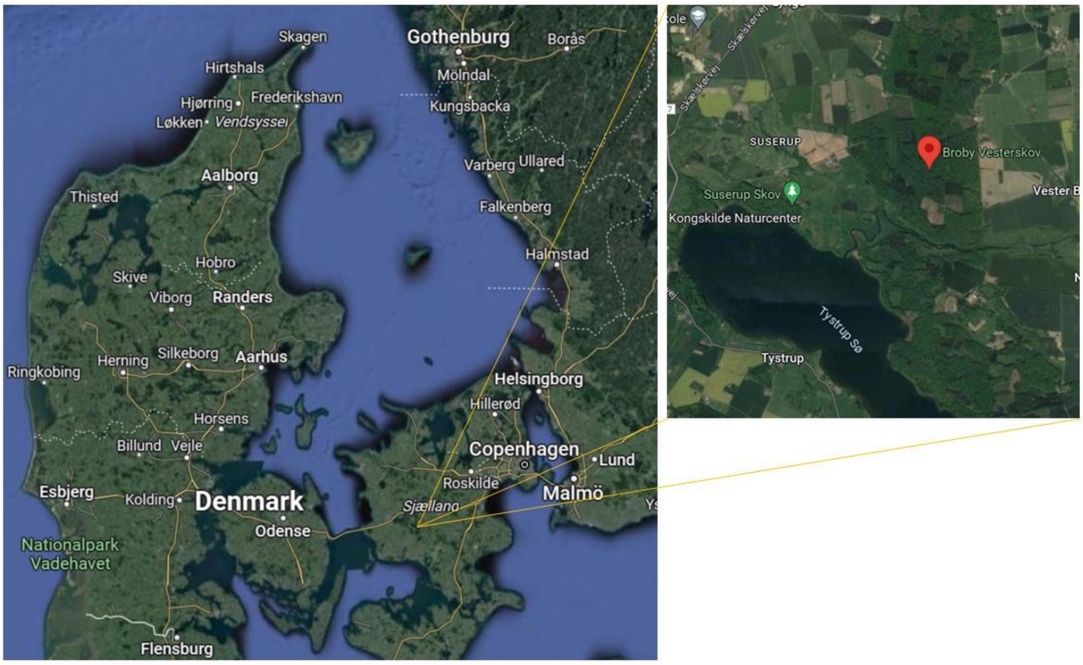

Figure 3.1 Forest locations in Denmark. The scale of the left image is 1:50 km, and the scale of the right image is 1:1 km (Google Map).

Figure 3.2 Left side is Suserup Skov, right side is Broby Vesterskov forest (Personal photo, 09.2023).

3.1.1 Suserup Skov

Suserup Skov is an unmanaged near-natural temperate deciduous forest in Denmark with a long history and little human impact, covering an area of 19.2 hectares. The forest located in the centre of Zealand at 55º22´N, 11º34´E (Jens Emborg et al., 2000). It is bordered by the southwest lake Tystrup, while the eastern and northern parts are connected to agricultural fields which have been abandoned for 20 years (Lise Dalsgaard, 2007).

It was initially managed as a minimal intervention forest park and later designated as a non-intervention forest in 1961.The forest has a long history and is characterized by a mix of tree species, with beech being the dominant species, occupying 53% of the basal area (Shaaban Ghalandarayeshi et al., 2017). The earliest recorded forests in Suserup Skov were dominated by Tilia (lime) trees. Today, however, the primary trees in woodland are Fagus sylvatica (beech) and Fraxinus excelsior (ash), with some Quercus robur (oak) and Ulmus glabra (elm) (Gina E. Hannon et al., 2000).

The climate is cool-temperate with a mean annual temperature of 8.8 °C and a mean annual precipitation of 674 mm, although precipitation remains roughly the same throughout the year, it usually increases in late summer and autumn (Shaaban Ghalandarayeshi et al., 2017). Most of the soil in this forest is loamy soil, developed from fertile glacial deposit with approximately 20% clay (Jens Emborg et al., 2000). The pH value of the soil is 3.7 and C/N ratio value is 30.3 in the organic layer and 15.1 in the mineral layer. The clay content in the first 50-100 cm is around 12% and the thickness of the organic layer is around 2 cm (Gundersen, et al., 2009).

The entire forest has been divided into three parts for better study, with the eastern part less disturbed by humans than the western part which is used for grazing (Jacob Heilmann-Clausen et al., 2007). Therefore, our investigation was carried out in the eastern part A.

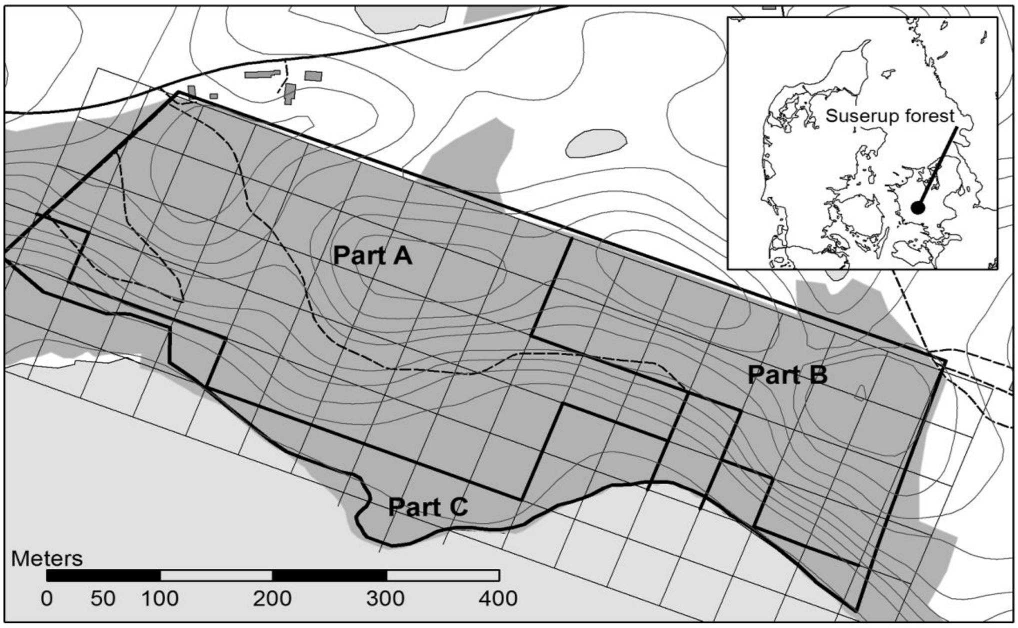

Figure 3.3 Suserup Forest. The original 50×50 m sample grid is shown on the map (Nord-Larsen et al., 2019).

Four central plots in Suserup Skov were used for soil sampling and soil N_2O gas exchange. Each small plot was set around the center of the measurement points in four directions to form a 10m circle, a total of 16 survey plots. In order to study the effect of a dry-to-wet gradient on gas emissions, 7 plots were set up on the hillside to understand the relationship between N_2O emissions and soil moisture. The following Figure 3.4 shows Suserup Skov's sampling plots.

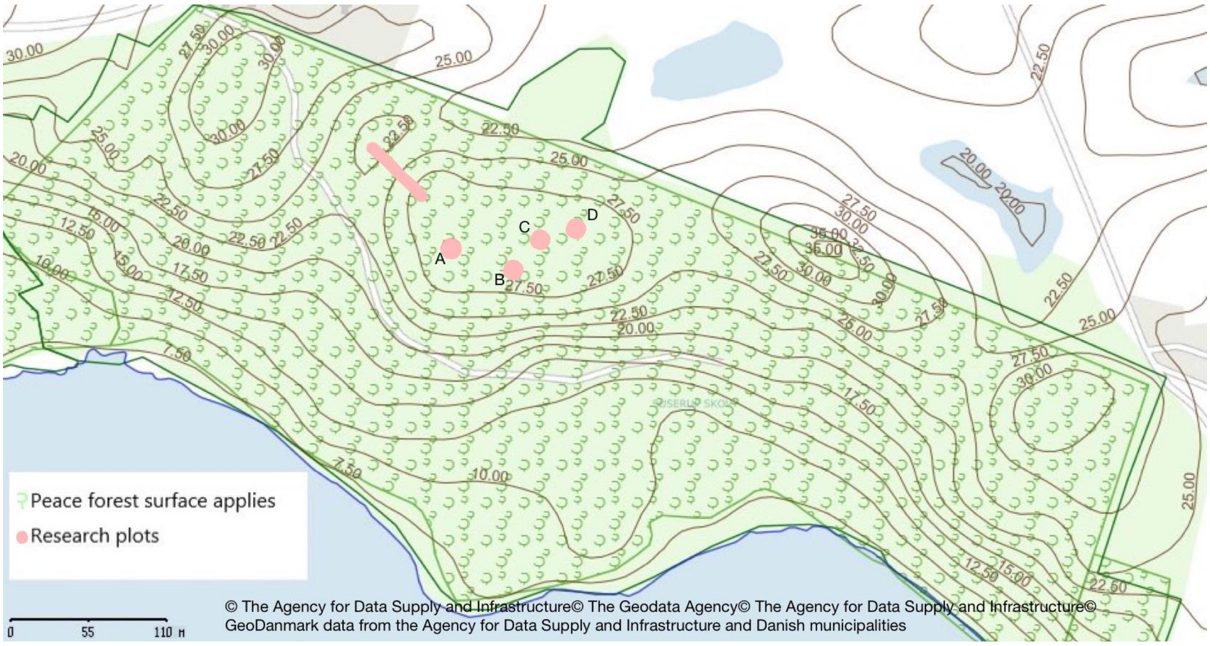

Figure 3.4 Slope test and long-term monitoring plots in the Suserup Skov (SDFI kortviewer).

3.1.2 Broby Vesterskov

Broby Vesterskov forest covers an area of about 4.5 km^2, near farmland in the north and the Suså river in the south, and located in the southeast part of the Sorø Sønderskov forest region at 55º38´N, 11º59´E, about 5 km from Sorø. It is also close to the northeast of Suserup Skov with a distance of approximately 1.7 km.

The geological features of the area are related to the last ice age, when large areas were ice-free, while other areas were covered by a kilometer-high ice sheet. The ice-free areas were covered by large amounts of meltwater, leaving behind raw materials in various forms, from fine sand and clay to large and small gravel and stones. There are a wide variety of trees in the area: a mix of fir, cypress, oak, beech and so on, all of them must be well protected and managed by the municipality's employees or owners (Sorø Kommune, n.d.).

We placed the sample plots also on the higher ground in the northern part of the area dominated by beech (Figure 3.5). The plots from two forests were all on the edge of forest. In this way, the forest environments and soil properties of the two forests are comparable.

Figure 3.5 Broby Vesterskov forest, the red area is the selection areas (Google Map).

In order to be able to compare soil and gas exchange monitoring in long-term with Suserup Skov, 4 plots with similar geographic characteristics in the northern part of Broby Vesterskov were selected. Similarly, each plot was set up with four survey points around the centre in four different directions, but one extra point in a canopy gap at point C, resulting in a total of 17 survey plots. Figure 3.6 shows the sampling plots in Broby Vesterskov.

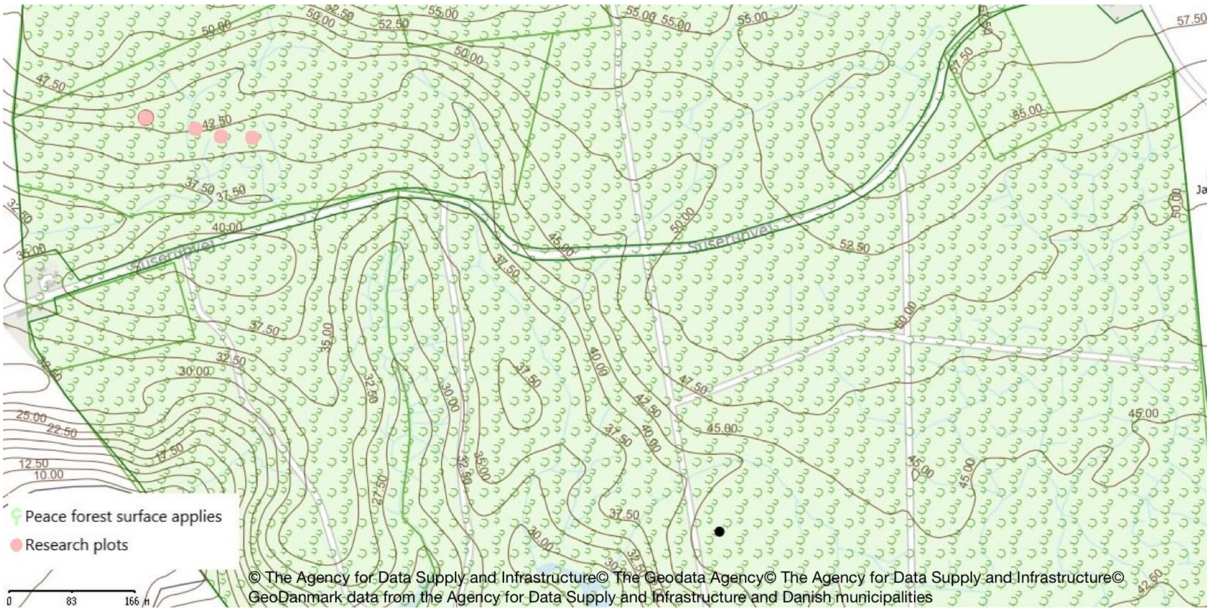

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Figure 3.6 The long-term monitoring plots in the Broby Vesterskov (SDFI kortviewer).

3.2 Experiment design

3.2.1 Gas exchange measurements

In the gas monitoring, N_2O emissions were quantified along four cardinal directions (east, south, west, and north), starting from the central point. Gas collection was facilitated using the 8200-01 Smart Chamber, a portable, self-powered 20cm measuring chamber designed for evaluating the spatial variability of soil gas fluxes. The Smart Chamber, when connected to the LI-COR-7820 Trace Gas Analyser through a conduit, allows the seamless flow of gas from the chamber to the analyser. Subsequently, the Smart Chamber calculates real-time gas flux, providing immediate data on a mobile device, recording gas flux for a 3-minute duration per measurement. And it is equipped with Stevens HydraProbe soil moisture and temperature probe, temperature thermistor, and integrated GPS measurements (LI-COR.com, n.d). These auxiliary data are captured concurrently with gas flux data. Ultimately, the N_2O fluxes are calculated through an R-package (Karelle Rheault et al., 2024).

The experimental setup involved placing a cylinder, enclosed by plastic and compacted with sandbags, at the designated measurement plot. The depths of the cylinder's surface from the soil were measured, and the chamber, connected to the analyser, was securely put on the cylinder. The Smart Chamber's built-in probe was employed to automatically detect soil temperature and moisture when it was inserted into the soil. Since November 2023, a consistent cylinder arrangement has been installed at each plot to ensure measurement stability.

Figure 3.7 Cylinders of different periods, covered with litters on the left and plants on the right.

The measurement shall be made in strict accordance with the guidelines specified in the operational manual or instructions for use. This included precise startup and termination procedures for the Smart Chamber. To minimize possible deviations in gas emissions from soil compression, movement within the measurement area should be minimized both before and during measurement. Furthermore, measurements were deliberately avoided in bad weather, such as heavy rain. This was taken to ensure the preservation and integrity of the measurement results.

3.2.2 Soil experiments

The overall process of the soil experiment is shown below Figure 3.8. Soil sampling was conducted in December 2023. A total of 33 plots at four sites (A, B, C, D) in each of the two forests for both topsoil and deep soil samples were sampled, all with two replicates. Around each gas sampling point, an 4.5 cm thick soil auger was hammered to a depth of 10 cm, and this portion of the soil was selected to be used as a top soil sample, subsequently a 3 cm thick soil auger was hammered to a depth of 90 cm, and soil at a depth of 75-90 cm was selected to be deposited as a deep soil sample; The two replicate samples were bulked and meant to ensured coverage of an area of 1/2 m^2 around the gas measurement sites. Each soil sample was bagged, labeled, and stored in a foam box before being brought into the laboratory to be prepared for the next step.

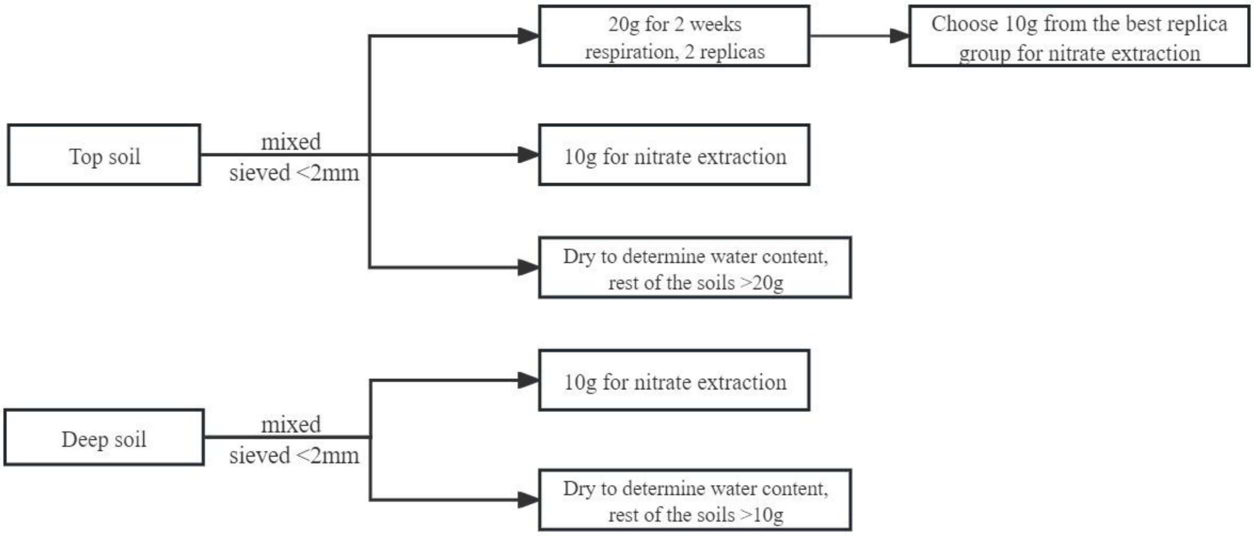

Figure 3.8 The process of soil experiment.

The soil samples were first mixed and sieved through a 2 mm mesh sieve. For top soil, 20 g soil were placed in cups ready for the respiration experiment, with two replicates; 10 g soil were stored in 50 ml plastic tubes and placed in a freezer to await nitrate/ammonium extraction; the remaining soil, which should be at least 20 g of soil, were weighed and placed in paper bags and then dried in an oven at 55 ℃ for 2-3 days, and then weighed again to compare the difference in mass of the soil samples to determine the soil water content. For deep soil, 10 g soil should be stored in 50 ml plastic tubes and put into a freezer to wait for the nitrate extraction test; the remaining no less than 10 g of soil should be used for drying, and the difference between the before and after mass should be compared to determine the soil water content as well.

a. Soil Respiration Experiment

The principle of the soil respiration rate test device is that the hydroxide solution in the small cups above the experimental jar absorbs CO_2 to form carbonate ions and causes a decrease in the conductivity of the solution, and this change in conductivity can be used to calculate the rate of CO_2 production from soil respiration. These jars could be placed in a water bath at a constant temperature for long term, extensive monitoring.

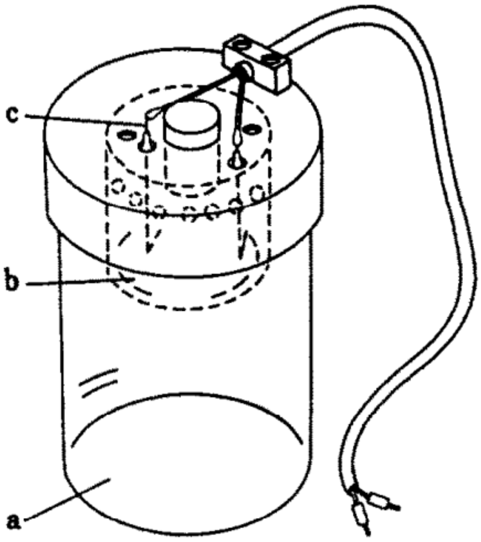

Figure 3.9 “a” is an experimental jar for placing the soil, “b” is a small cup above for placing the solution as a conductivity cell, and “c” are two platinum electrodes fixed to the lid of the cup by melting the surrounding plastic (Anders Nordgren, 1988).

Each jar contained 20 g topsoil with labels, for a total of 66 experiment groups, which were randomly placed in the water bath, and 2 additional blank control groups. 10 ml of 0.3 M KOH solution was aspirated with an automatic pipette and injected into the small cup above, then the lid was tightly closed and the experiment was started. The temperature of the instrument water bath was kept at 15 °C. The CO_2 measurement curves were checked regularly the first days to be sure all cells were functioning. After three weeks, the measurements were turned off and the CO_2 accumulation data retrieved. Since there were two replicates of each soil sample, we compared the resultant curves of the two replicates and selected the better one for the nitrate extraction experiment (took 10 g soil).

Figure 3.10 The soil respiration rate test device in the lab (personal photo).

b. Nitrate extraction experiment

Each sample was stored in a 50 mL plastic tube, 20 mL of 0.1 M KCL solution was added to the soil sample and then shaken on orbital shaker in a 160 times/minute mode for one hour to mix it well. The samples were then centrifuged at 2000 rpm for 10 minutes and filtered through a Cellotron filter to obtain a clarified solution in a glass tube. Finally, the solution was analysed by flow injection analysis (FIA) to obtain the NH_3- and NH_4+ concentration in the solute. The net mineralization rate of soil can be calculated by measuring nitrate content before and after respiration experiment. The equation is:

$$Net\ Nmin = \left[(NH_4^+ - N + NO_3^- - N)_f - (NH_4^+ - N + NO_3^- - N)_i\right]/T_d$$

where the subscripts “ i ” and “ f ” denote the concentration measured before (initial) and after (final) incubation respectively, and “ T_d ” denotes the incubation time in days.

c. soil property determination

Soil pH was measured using sieved soil after 2-hours extraction in a 0.01 M $CaCl_2$ solution at a ratio of 5:1 (extractant to mineral soil) using a Radiometer combination-electrode GK2401 (Radiometer, Copenhagen, Denmark). A portion of the dried topsoil was crushed (not less than 10 grams) in a Retsch Planetary Ball Mill PM400 and then placed in a brown glass bottle for C/N determination. Soil for nitrogen and carbon analysis was analysed for total carbon and nitrogen via dry combustion (Dumas method) in a FLASH 2000 EA NC Analyzer (Thermo Fisher Scientific, Waltham, MA, USA). The soil phosphorus content was determined after 0.1 M H_2S0_4 extraction as ortho-P (PO_4- P) concentration by the salicylate method on an auto-analyser (AA500, SEAL Analytical, Germany).

3.3 Calculations and statistics

N_2O fluxes were calculated using the R package. With the R code, two different models, HM (Gaussian model) and LM (Linear model) were used and the best model was selected to obtain the most accurate value of N_2O emission fluxes.

Use the new R package “go Flux”, which allows users to easily import raw data from a variety of instruments, including LI-COR and Smart Chamber. It supports both linear (LM) and non-linear (HM) flux calculation methods for accurately calculating fluxes of various greenhouse gases such as N_2O.

Although the linear regression approach (LM) is commonly used to estimate greenhouse gas fluxes, the method tends to underestimate pre-deployment fluxes. In fact, the effect of the chamber on the gas flux needs to be considered, and when the gas concentration in the enclosed chamber increases or decreases, nonlinearity is expected due to changes in the gas gradient between the soil and air in the chamber (Karelle Rheault et al., 2024). Among the many alternatives to LM that have been developed, the HM method is a good choice. The LM method is suitable for simple flux estimation scenarios, while the HM method is more suitable for complex cases.

In most cases, a model with a higher R^2 fit is used to determine the N_2O flux. However, when dealing with N_2O flux data, it is very important to consider changes in background values, requiring the background N_2O level in the environment to be subtracted from the actual observed N_2O concentration. The HM model is easily affected by the background at relatively low fluxes, while the LM model shows higher stability at low fluxes (Wang Songqing, 2023).

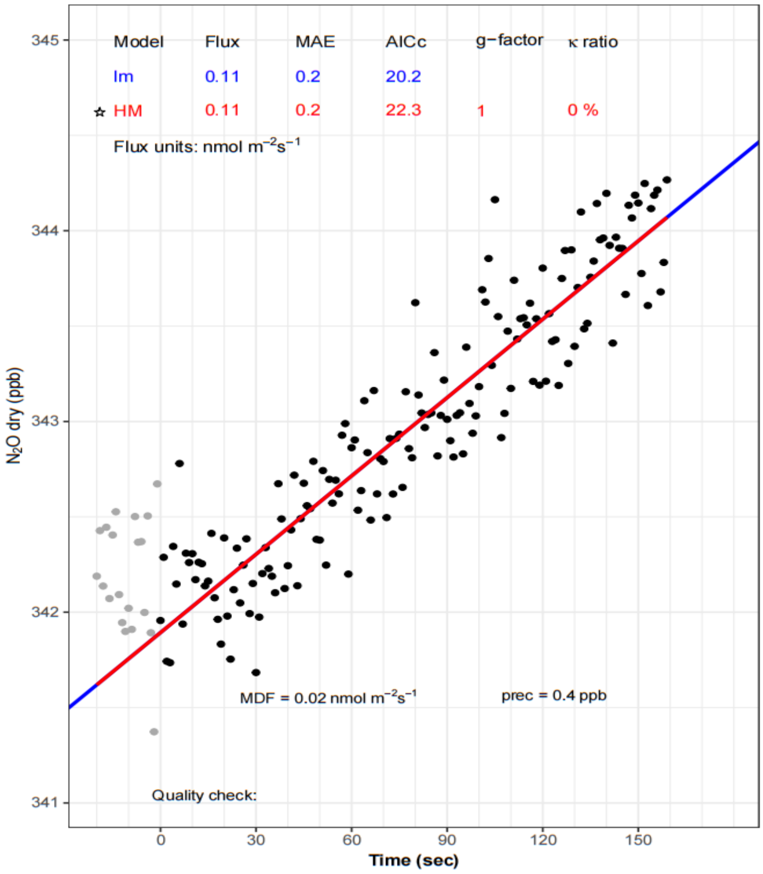

Figure 3.11 An example of N_2O flux in plot A1, Suserup, December 2023.

All data were shown as “average ± standard error” and then be statistic analysed. ANOVA and T-test were used to determine whether there were any significant differences between or within two forests, with nitrate concentration, N_2O emissions and other factors as the variables. A significance level of $P \leq 0.05$ was set, meaning that results were reported as significant difference if they had a $P \leq 0.05$ or marginally significant when $0.05 < P \leq 0.1$.

4. Results

According to the experiment design, each forest was set with four sampling points A, B, C and D, and each point was set with four plots around the centre point. SA means Suserup plot A, BA means Broby plot A, and so on. They were all under canopy shelter in the same environment basically, except for Broby's plot C5, which was in the canopy opening, set up to compare with C1-4 plots to illustrate the effects of canopy cover and gap. In order to ensure the fairness of the experimental results, C5 data was not included in the average calculation and comparison of all the following results.

4.1 Soil properties

Soils were all collected in December 2023. The C/N in soil was 14.7±0.2 and pH was 4.07±0.08 in unmanaged forest Suserup, while C/N was 15.0±0.3 and pH was 3.84±0.06 in managed forest Broby. The pH value between two forests showed significant difference (P=0.035), while C/N ratio between two forests had no significant difference (P=0.4). PO_4-P in 0.1M H_2SO_4 in Suserup was almost 6.6 times more than in Broby, its showed significant difference between two forests (P<0.0001). Notably, Broby C5 showed an extremely high value compared with other plots in Broby, this could be due to the effect of canopy gaps.

	pH in $CaCl_2$	PO_4-P in 0.1M H_2SO_4 (mg/kg)	C/N
Suserup	4.07 ± 0.08	226 ± 13	14.7 ± 0.2
Broby (except C5)	3.84 ± 0.06	34 ± 4	15.0 ± 0.3
Broby C5	4.08	150	15.5

Table 4.1 Shows the soil properties in Suserup Skov and Broby Vesterskov. All the plots were under canopy shelter, except Broby C5 which was in the canopy opening.

4.2 Nitrate concentration under different forest managements

Nitrate concentrations in soils vary considerably under different forest managements. It tended to be lower in forests under sustainable management models compared to unmanaged forests.

The differences in soil nitrate concentrations in unmanaged forest Suserup and managed forest Broby were compared below.

4.2.1 In deep soil

For the nitrate concentration in deep soil, we compared the data from two different forests and illustrated their differences in the Figure 4.1 below. In Suserup (unmanaged forest), the average nitrate concentration was 19.7 ± 2.6 mg/L, whereas in Broby Vesterskov (managed forest), it was 6.6 ± 1.1 mg/L. Particularly, of the four plots of Suserup, only plot A differs from the other three in having a low content of 4.8 ± 0.8 mg/L. The average level of the four plots in Broby was low, but the highest was 10.8 ± 2.1 mg/L in plot A and the lowest was 2.4 ± 0.1 mg/L in plot D. This disparity may reflect variations in soil chemical composition and biological activities across different forest ecosystems.

To validate the significance of these differences, we conducted ANOVA-test, which revealed that the difference in nitrate concentration in the inner plots of two forests were statistically significant (P=0.003 in Suserup, P=0.03 in Broby), and the difference between two forests was also significant(P<0.0001).

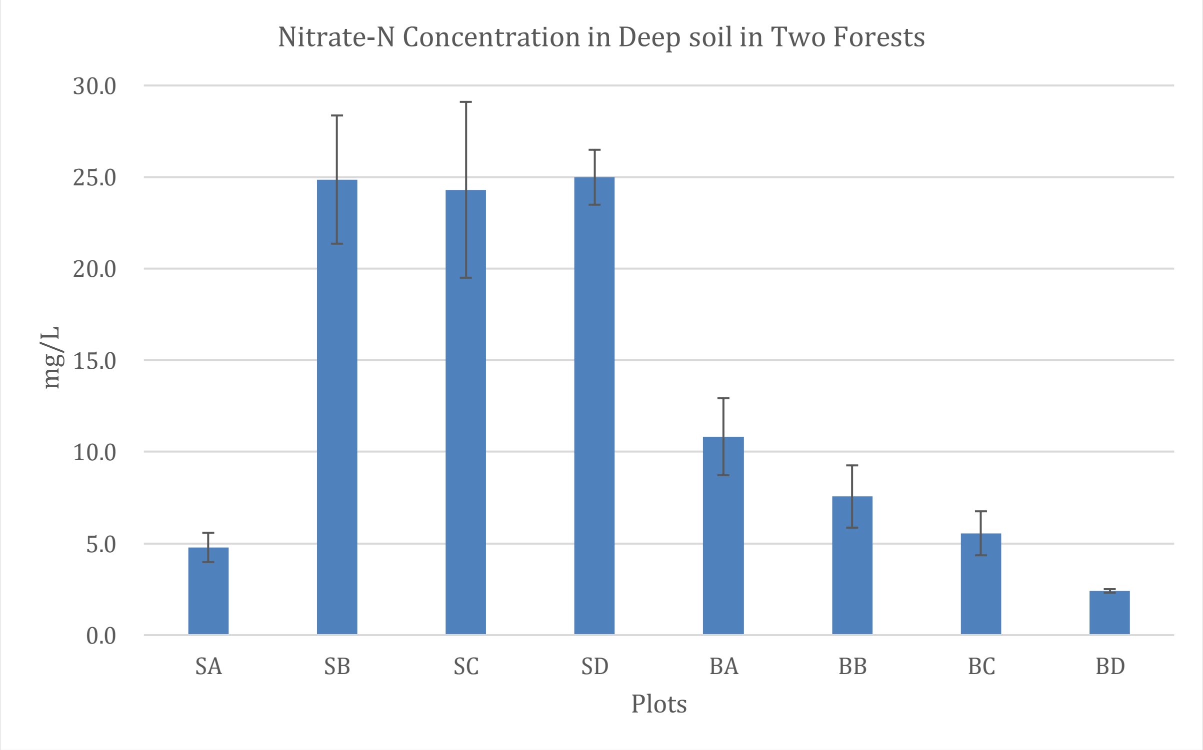

Figure 4.1 Nitrate concentration in deep soil (90cm deep) cross over Suserup and Broby forest in December 2023.

4.2.2 In top soil

The total-N concentration (including nitrate and ammonia nitrogen) of top soil in two different forests was plotted and compared (Figure 4.2). In Suseup, the average total-N concentration before respiration of the four plots was 7.4 ± 0.7 mg/L, compared with 5.9 ± 0.4 mg/L in Broby.

Respiration experiments were then performed to assess the changes in total-N content before and after treatment. The results showed significant increase during incubation in both forests. In Suserup, the average total-N concentration after respiration increased to 23.7 ± 2.6 mg/L. In Broby, it increased to an average of 18.4 ± 1.2 mg/L. Overall, for soil samples taken from the two forests, the total-N concentration after respiration was around 3.1 times higher than that before respiration. The nitrate concentration between two forests showed marginally significant difference before the respiration experiment (P=0.076), also after the respiration experiment (P=0.076).

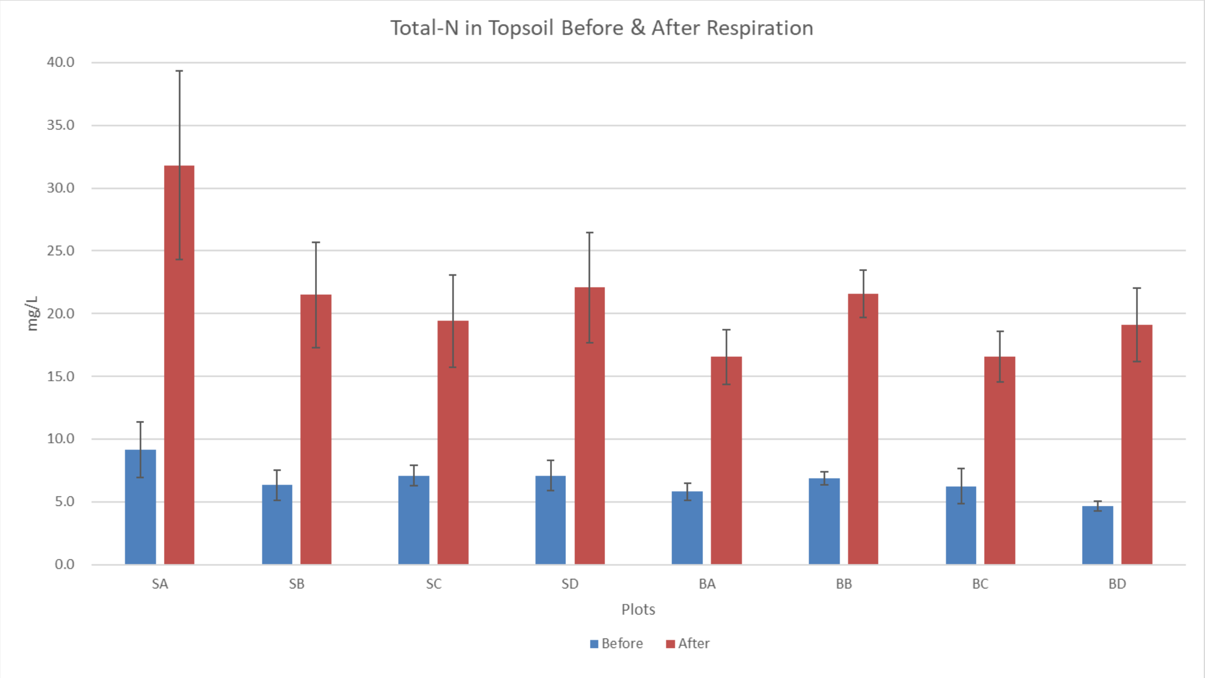

Figure 4.2 Total nitrogen concentration before and after respiration in top soil (10 cm deep) cross over Suserup and Broby forest, in January 2024. Blue bars means before, while red bars means after respiration.

By subtracting the initial from the final total-N concentration divided by the total time, net N mineralization by the soil in different plots per day during the respiration experiment was obtained (Figure 4.3). The average net N mineralization value of the four plots of Suserup was 0.86 ± 0.10 mg/kg/day, and the average value of Broby was 0.66 ± 0.05 mg/kg/day, among which Plot A of Suserup was the highest, with 1.20 ± 0.28 mg/kg/day. The net N mineralization value between Suserup and Broby showed marginally significant (P=0.1) difference.

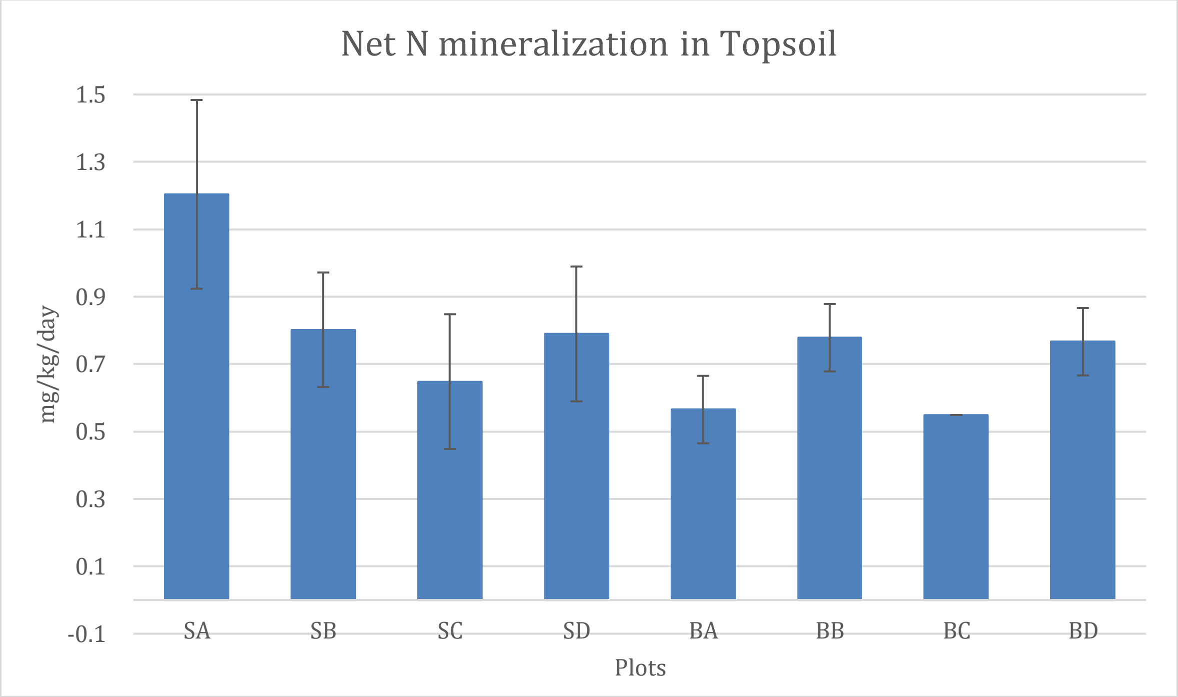

Figure 4.3 Net N mineralization by top soil during respiration time, Total-N (end-initial) / Time (end - initial) = mg/kg/day. Cross over Suserup and Broby forest, at 15 ^{o}C.

The CO_2 captured by the soil respiration rate test device was used to calculate the daily CO_2 production. From Figure 4.4, Suserup had a higher CO_2 production rate at 0.44 ± 0.05 mg/kg/day, while its 0.33 ± 0.04 mg/kg/day. The CO_2 production rate value between Suserup and Broby showed significant difference (P=0.046).

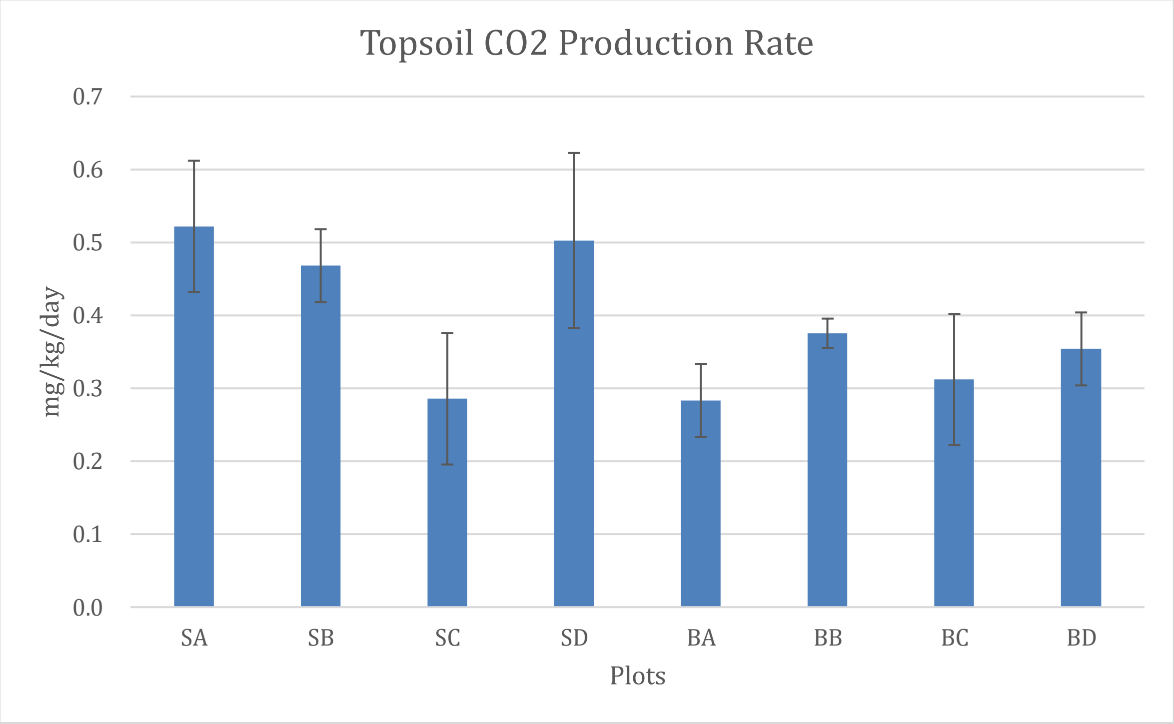

Figure 4.4 Top soil CO_2 production rate at different plots during respiration experiments. Cross over Suserup and Broby forest, at 15 °C.

Then daily CO_2 production could be divided by net N mineralization over the same period. This was done to consider the effect of soil nitrogen content on CO_2 production. The high value could mean that the CO_2 production per unit of soil was high relative to the soil net N mineralization over the same period. This could indicate a relatively high rate of decomposition of organic matter in the soil, or a higher level of microbial activity, resulting in more CO_2 release. From Figure 4.5, it was observed that the values for Suserup, particularly in plot B and D, exceeding 0.7 ± 0.2. The mean value in Suserup was 0.58 ± 0.08 higher than in Broby which was 0.51 ± 0.04. But there was no significant difference between the two forests (P=0.43).

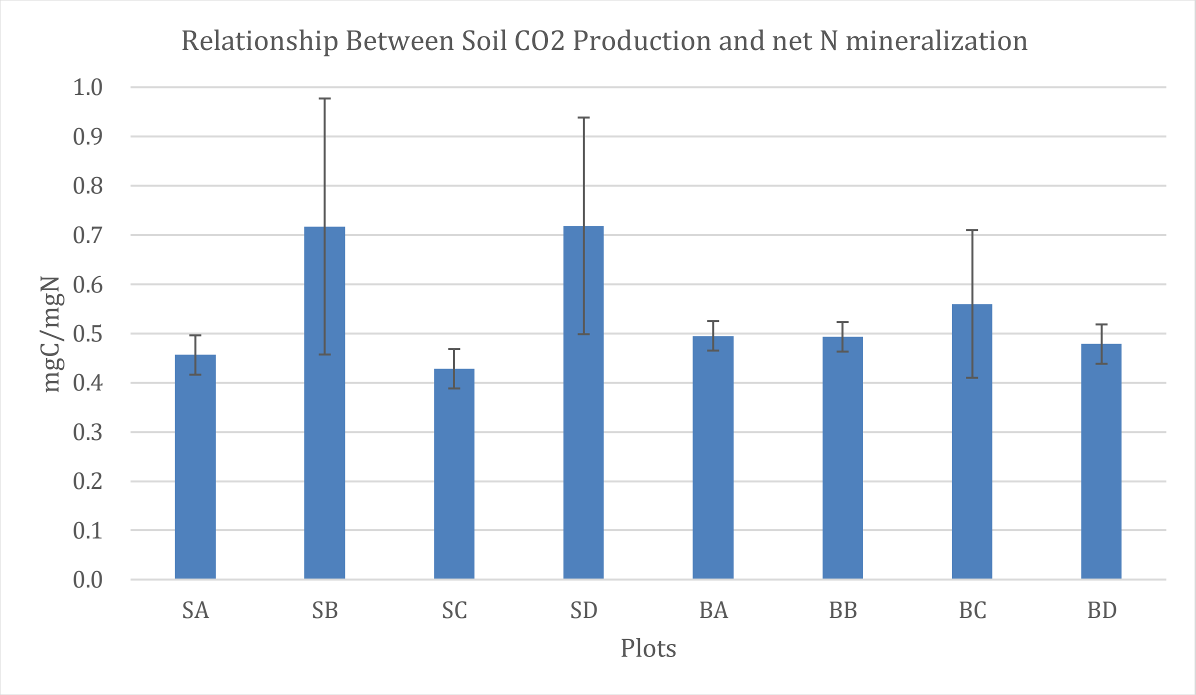

Figure 4.5 Shows the soil CO2 production/the net N mineralization at different plots. Cross over Suserup and Broby forest, at 15 °C.

4.3 N_2O fluxes

The N_2O emission fluxes from forest soils were examined by gas exchange experiments to determine the presence of N_2O release from soils, and find differences in time and location.

4.3.1 Monthly variation

The release of N_2O in soil was a dynamic process, as illustrated in Figure 4.6. In August, the N_2O emission fluxes from soil in both forests peaked, with Suserup exhibiting levels approximately 11 times higher than those in Broby. However, by September, the emissions from Suserup sharply declined to levels similar to those in Broby. Subsequent months saw both forests maintaining relatively low emission levels, except for February 2024, when Suserup's emissions reached to 0.33 ± 0.04 nmol/m^2/s before rapidly dropping to 0.006 ± 0.001 nmol/m^2/s. In March and April 2024, the average N_2O emissions from both forests approached zero. Interestingly, some plots even recorded negative N_2O fluxes (e.g. Broby C2 N_2O flux was -0.00301 nmol/m^2/s in March 2024), indicating N_2O uptake from the atmosphere during colder weather. There was no significant difference in the monthly variation of N_2O emissions between two forests ($P=0.169$).

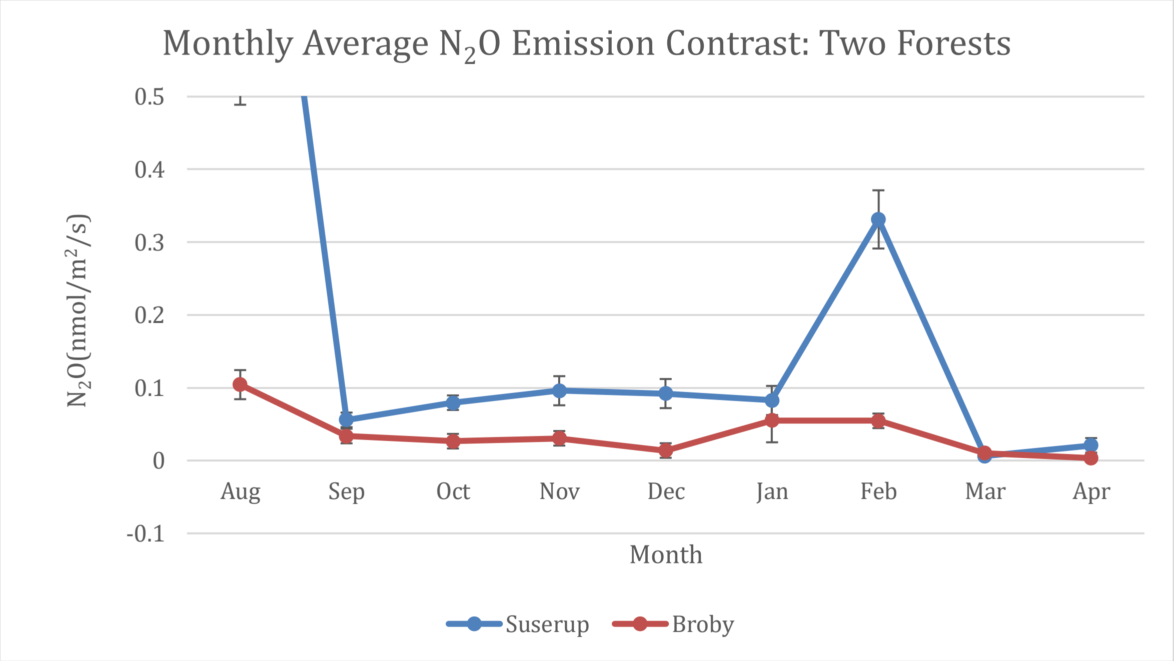

Figure 4.6 The monthly average N_2O emissions from two different forests, compared from August 2023 to April 2024.

4.3.2 Impact of forest managements

Differences in managements between the two forests also influence N_2O emissions. From August 2023 to April 2024, the average N_2O fluxes in Suserup was 0.203 ± 0.079 $nmol/m^2/s$, approximately 5.5 times higher than Broby's average fluxes of 0.037 ± 0.005 $nmol/m^2/s$. The following Figure 4.7 provides a detailed breakdown of the average N_2O emissions from different locations in two forests. It was evident that plots B, C, and D in Suserup exhibited notably high emissions, with plot C peaking at 0.408 ± 0.312 $nmol/m^2/s$. In contrast, emissions from all four sampling points in Broby remained consistently low and stable. There was a marginally significant difference of average N_2O fluxes between two forests (P=0.06).

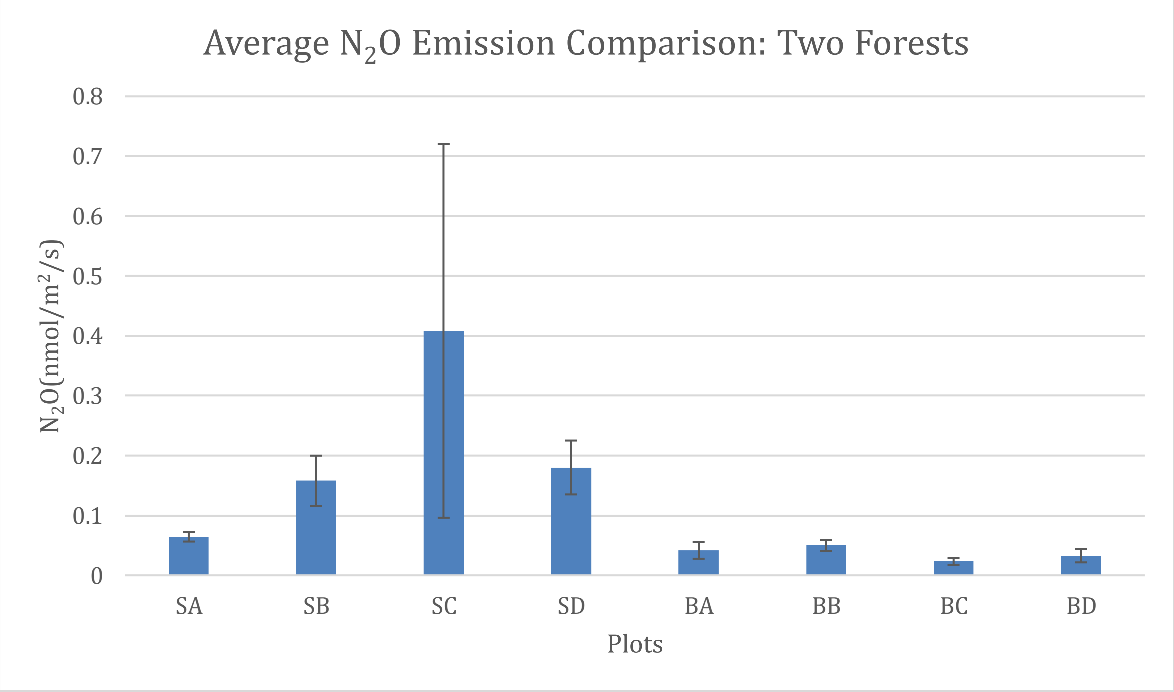

Figure 4.7 The average N_2O fluxes from August 2023 to April 2024 at different sampling plots in the two forests.

4.3.3 Inorganic N - N_2O

In general, a positive correlation is expected between inorganic N and N_2O emission (Huai Yang et al., 2017). But this relationship did not appear in our study clearly. For the top soil total-N in Suserup, there seems to be a weak relationship from Figure 4.8 (R^2=0.15), but there was no significant correlation from topsoil in Suserup (F=0.16). There were no correlations between the variables for deep soil in Suserup and Broby (Figure 4.9).

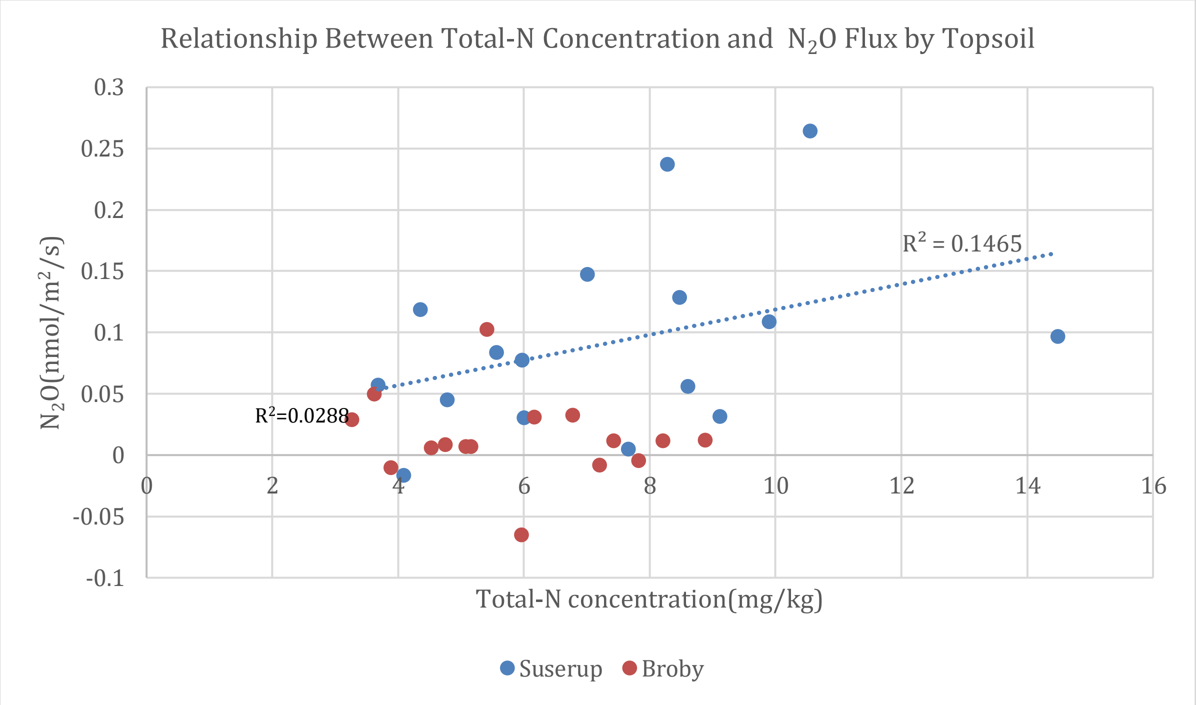

Figure 4.8 The relationship between average N_2O fluxes for December 2023 and topsoil total-N concentration in Suserup and Broby.

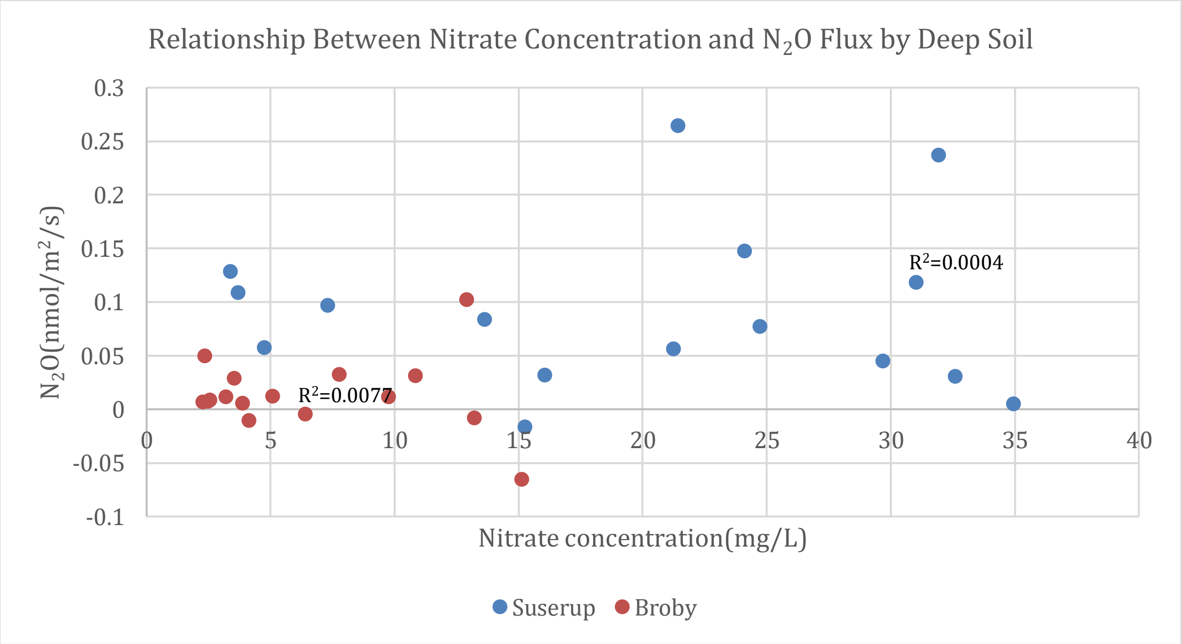

Figure 4.9 The relationship between average N_2O fluxes for December 2023 and deep soil nitrate concentration in Suserup and Broby.

4.3.3 Effect of gradient

In this part, "gradient" refers to the position of the experimental plots on the slope of the hill. The positions from G1 to G6 were arranged in descending order, with G7 being added in September 2023 and located between G5 and G6. Due to the low-lying location and abundant rainfall, in January 2024, both G6 and G7 became frozen wetlands. As the weather warmed up in February, March, and April 2024, G6 and G7 transformed into waterlogged wetlands. It can be seen from Figure 4.10 that gradient had an impact on N_2O emission. The N_2O emissions of G5, G6 and G7 in the figure were higher than elsewhere.

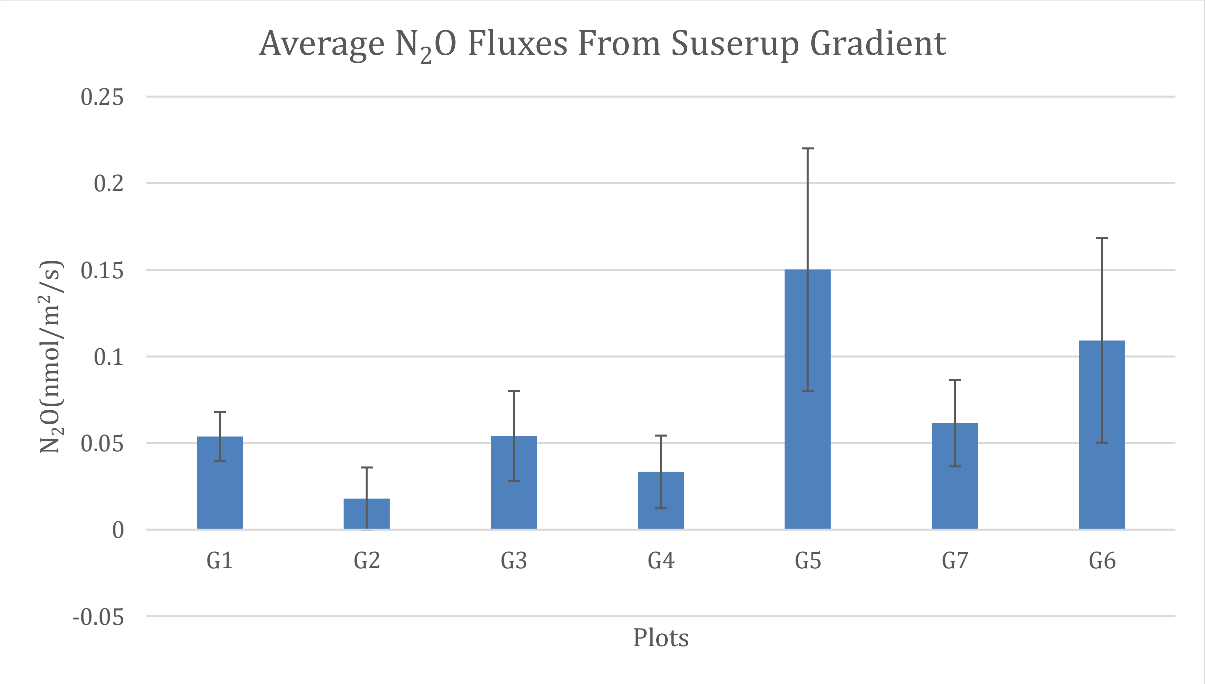

Figure 4.10 The average N_2O fluxes from August 2023 to April 2024 at different gradient plots in Suserup.

To explore the influence of slope was to explore the influence of soil water content (moisture) actually, because the water flows to a lower place, the more water content of the soil at the bottom of the slope. Due to cold weather, gas data were not successfully collected at some plots in some months, we selected the complete data of the month with good condition (December 2023) to show the relationship between N_2O emission and soil water content in Figure 4.11. This was a curve change with a strong correlation (R^2=0.87), when the soil water content was extreme, the N_2O emissions were small, and the maximum fluxes may appear at about 65% of the soil water content.

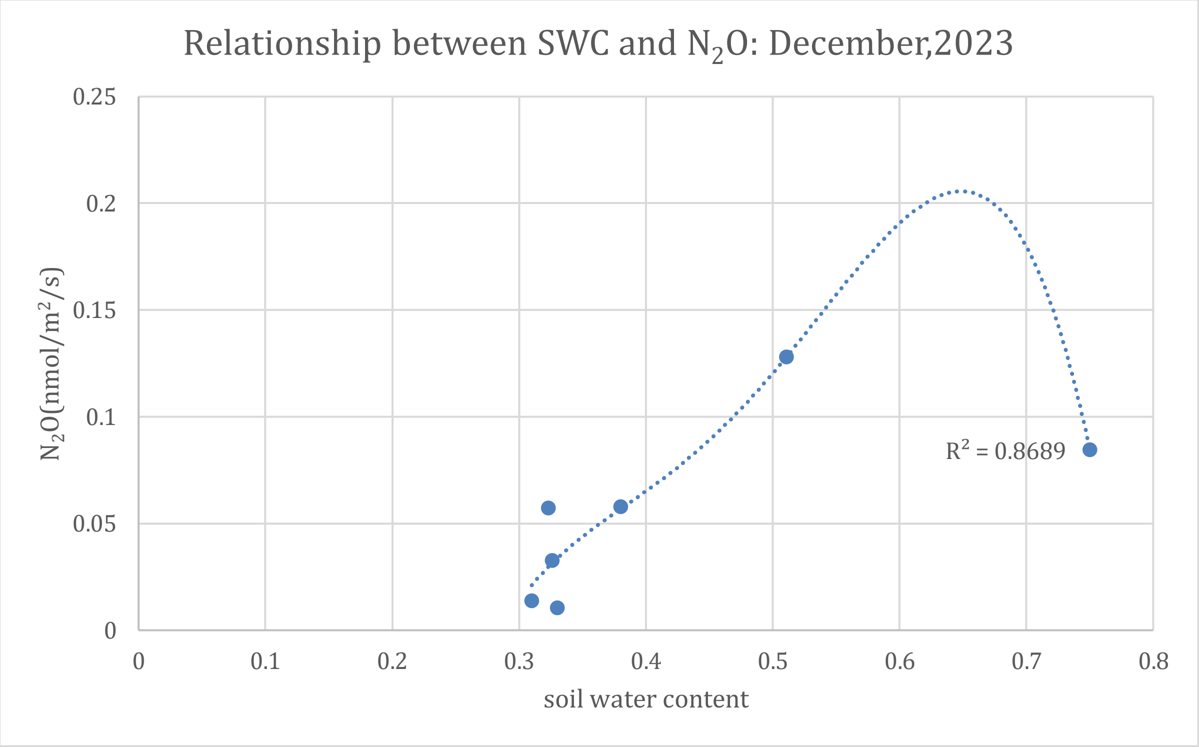

Figure 4.11 The relationship between average N_2O fluxes and soil water content from different gradient plots in Suserup, December, 2023.

4.4 Effect of canopy gaps

Canopy cover in a forest is not uniform, but is formed by a series of canopies that intertwine with each other. Within the canopy, there are gaps or openings that allow sunlight to penetrate into the forest floor, promoting the germination and growth of some plant seeds, and may also alter the distribution of water and nutrients in the soil. Plot C5 in Broby was right in the canopy opening with some brash from a previous thinning left in the area around. It received more sunlight and was wetter than the other plots C1, C2, C3 and C4. From September 2023 to April 2024, the average soil temperature of Broby C5 was 8.8 ± 1.3 ℃, while it was 8.7 ± 1.3 ℃ across C1-4. The average soil moisture was 0.29 ± 0.02 in Broby C5, compared with 0.28 ± 0.03 across C1-4. The soil temperature (P=0.9) and soil moisture (P=0.9) showed no significant differences between these plots.

4.4.1 Nitrate concentration

In the deep soil, the nitrate content at plot C5 was 13.4 mg/L, which was 2.4 times the average of the other four plots. In the topsoil before the respiration experiment, the total-N concentration at C5 was 7.6 mg/kg, which was slightly higher than the average of the others. After the respiration, the total-N concentration at C5 increased significantly to 25.5 mg/kg, much higher than the average of the others (16.6 ± 2.0 mg/kg). Thus, the soil at C5 produced approximately 1.7 times more net N mineralization than the average of the others. These results (Table 4.2) suggested that the soil properties and ecological processes at C5 were different from those at the other plots and may have been affected by different environmental conditions or anthropogenic disturbances.

	Nitrate in deep soil (mg/L)	Total-N in topsoil (mg/kg)		Net N mineralization (mg/kg/day)	CO_2/net N mineralization
		Before respiration	After respiration		
Average of C1-C4	5.6 ± 1.2	6.2 ± 1.4	16.6 ± 2.0	0.55 ± 0.04	0.56 ± 0.15
C5	13.4	7.6	25.5	0.95	0.34

Table 4.2 Comparison of soil properties at different sites. C5 was in the canopy opening, C1-C4 were under canopy shelter, all in Broby forest. During January 2024.

4.4.2 N_2O fluxes

From September 2023 to April 2024 in the Broby forest, the average N_2O emission flux at plot C5 reached 0.028 ± 0.008 nmol/m^2/s, nearly matching the average N_2O emission in Suserup. However, the average N2O emissions at plots C1-4 were only 0.018 ± 0.005 nmol/m^2/s. The following Figure 4.12 illustrated that from September to December 2023, the average emissions at C5 remained consistently higher than those at the other four plots. However, starting in January 2024, the average emissions at C5 sharply declined, dropping below the average emissions at the other four plots. Additionally, it even experienced negative fluxes in March. There was no significant difference of N_2O fluxes between the plots (P=0.3).

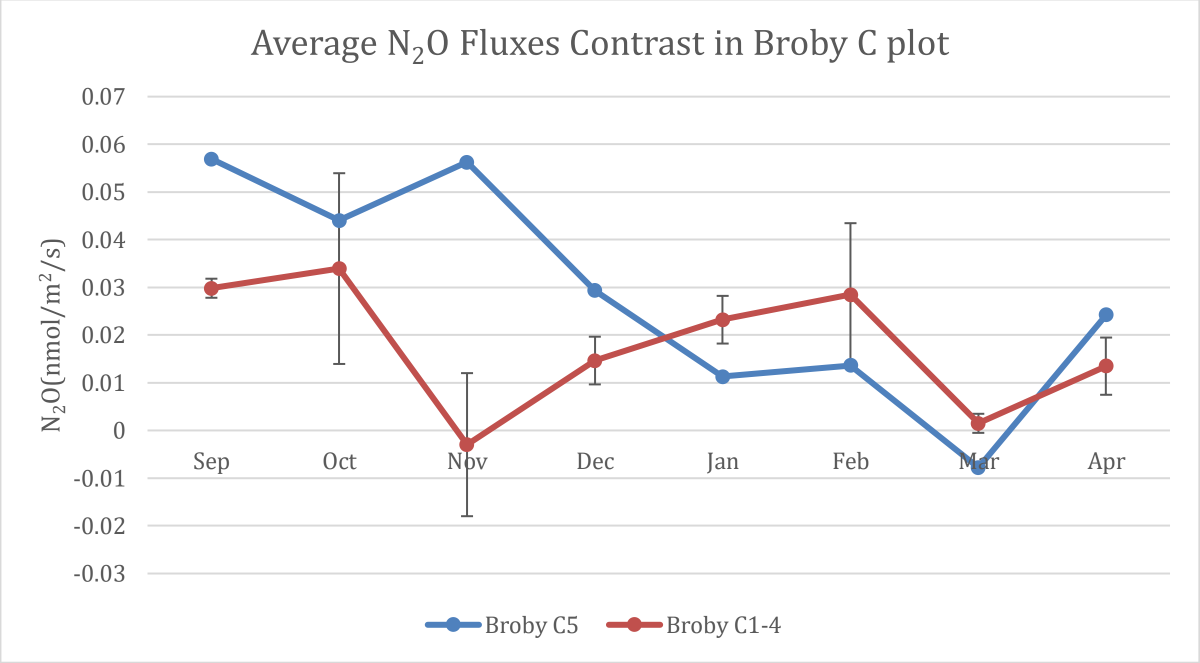

Figure 4.12 The average N_2O fluxes from September 2023 to April 2024 at plot C in Broby.

5. Discussion

5.1 Difference in soil properties

Broby soil had a lower pH and was more acidic than Suserup soil. When plants grow, they need to take up positive charged base cations or metal ions from the forest soil in exchange plants release protons: $R-COOH+Me^{+} \leftrightarrow R-COOMe+H^{+}$ In Broby, biomass was removed regularly, so the reaction was constantly shifted to the right side, producing more hydrogen ions, which may explain a lower pH in Broby (P. Gundersen, personal conversation). In Suserup, all produced biomass remain to decay in the forest and the protons are consumed again.

When examining the data on the basic properties of the soil in the two forests, it can be observed that the value of PO_4-P in 0.1M H_2SO_4 in Suserup was much higher than that in Broby which means that the content of phosphorus (P) in Suserup top soil was high. Soil pH value considered to be the "master variable" of soil chemistry, so the effect of pH on P dissolution was mainly considered. Many prominent scientists have done remarkable and meaningful work on soil P chemistry (Chad J. Penn & James J. Camberato, 2019), and they believe that the dynamic change of P solubility can be simplified to the rough but comprehensive diagram shown in Figure5.1.

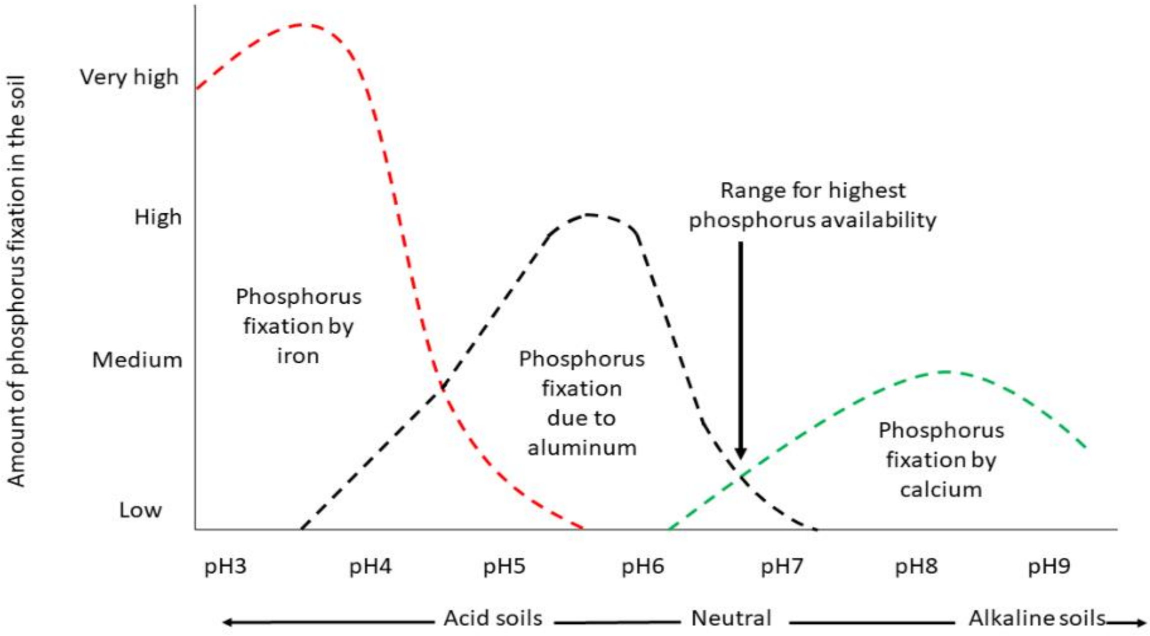

Figure 5.1 General qualitative representation of soil phosphorus availability as impacted by pH. Redrawn from Price (Chad J. Penn & James J. Camberato, 2019).

The soil pH of Suserup was 4.07±0.08 while the pH of Broby was 3.84 ± 0.06. From the figure above, it was clear that Suserup soil had a lower degree of P fixation by Al, and Fe minerals, therefore the dissolved P content in Suserup soil was higher. Again, also the constant removal of biomass at Broby may reduce the available P pool compared to in Suserup where P taken up by plants eventually will be released by decomposition.

5.2 Impact of managements

The top soil (10 cm deep) collected in the experiment contains natural organic matter from leaves, grass, and bark, which becomes organic soil. The deep soil (90 cm deep) was more mineralized soil (Jason James et al., 2015).

Nitrate concentration from deep soils under unmanaged forest were approximately 3 times higher than in managed forest. There were significant differences in both between and within forests, suggesting that managements do affect N leaching from deep soil ($P<0.0001$). However, total-N concentration from the topsoil was 1.28 times higher than in managed forest. There was only a marginally significant difference for total-N concentration between the two forests, suggesting that managements do affect nitrate leaching from topsoil, but not strongly ($P<0.01$).

A study in the United States (N. Whitney & D. Zabowski, 2004) has shown that large amounts of carbon and nitrogen in all soils are stored in the subsurface layer, even below 1.0 m, so that sampling only the surface layer (<0.5 m) misses out on all the biologically available N at greater depths. Leaching is the primary mechanism of N transport from the soil surface to the deep, and more mobile forms of N (NO_3- and to a smaller extent NH_4+) are leached to the deep layer of soil. Also, because the increase in pH with soil depth alters the mineral surface charge balance, this variable surface charge allows retention of NO_3-, NH_4+, and other organic matters by adsorption to the mineral matters surface. So total N increases with the soil depth of sampling, nitrogen from deep soil is actively cycled in forest ecosystems (Jason James et al., 2015).

5.2.1 By biomass

In managed forests biomass was regularly removed to reduce inputs of nitrogen sources, plants were taken away, therefore less leaching. Whereas in unmanaged forests, biomass nitrogen inputs were high and cannot be removed even though artificial nitrogen inputs from farms were reduced. In the deep soil, nitrate continued to accumulate in unmanaged forests, much higher than in managed forests. In the top soil, on the other hand, the difference was not significant because the two forests had similar soil types, and surface nitrate leaching was in a short-term state with no long-term accumulation.

5.2.2 By C/N ratio

In the respiration experiment, the CO_2 production rate of Suserup top soil was slightly higher than that of Broby top soil, and there was a significant difference between the two forests ($p=0.046$). It may be that soil conditions and environmental factors were relatively similar and there was no great difference in the relevant microbial communities, resulting in similar organic matter decomposition rates (M. M. Rahman et al., 2017). The C/N ratio should be viewed as a proxy for the availability of N. At high C/N ratios, N immobilization dominates, but net mineralization increases as the C/N ratio decreases (P. Gundersen et al., 2012). There was no correlation between the net N mineralization and C/N ratio by each plot in Suserup and Broby (ANNEX I). However, we observed that the average C/N ratio in Suserup was slightly lower than Broby's, while the average net mineralization in Suserup was found to be slightly higher in the experiment. When the C/N ratio of the soil all lower than 25, the nitrate is formed and available, net nitrification will occur, and the rate of nitrification is greater than the rate of denitrification within a certain period, so the nitrate nitrogen concentration in the soil will increase, and the N_2O flux is expected to be the highest (P. Gundersen et al., 2012).

5.2.3 By soil temperature

Looking at the monthly average N_2O fluxes from August 2023 to April 2024, it was clear that in both forests, the warmer month of August had obviously higher N_2O emissions than the other months. N_2O - Temperature relationships were made for both forests (ANNEX II), and the results showed a positive correlation between these variables ($R^2 = 0.45$ in Suserup, $R^2 = 0.34$ in Broby). However, there were no strong correlations, probably because the effect of warming on N_2O is not a simple proportional change, but a complicated process including e.g. interaction with soil moisture and N availability.

Elevated soil temperatures affect many processes, higher temperatures promote the mineralization of soil organic nitrogen and increase the input of nutrients, thus providing reaction substrates for soil denitrification, leading to an increase in N supply (Huai Yang et al., 2017). However, it also stimulates plant growth and N uptake, which may offset some of the N_2O emissions. Mean while, soil temperature increases are relatively small, so the impacts from warming are also likely to be small (Feike A. Dijkstra et al., 2012).

5.2.4 By soil moisture

We set up sampling plots G1-G7 in the sloping area of Suserup, mainly to explore the effect of a soil moisture gradient on N_2O emission. The results showed that plot G5-7 at the bottom of the slope had more water in the soil (ANNEX III) and had higher N_2O emissions. Soil water content was the controlling factor. In (P. Gundersen et al., 2012) experiment, the N_2O flux showed a clear pattern, with the lowest flux rate at the both extremes of soil water content and the maximum flux rates at the intermediate water content (60%, Figure 5.2). We took the complete data of December 2023 to draw the relationship of N_2O-soil moisture (R^2 = 0.87, Figure 4.11), in which N_2O may reach the maximum flux in around 65% soil moisture.

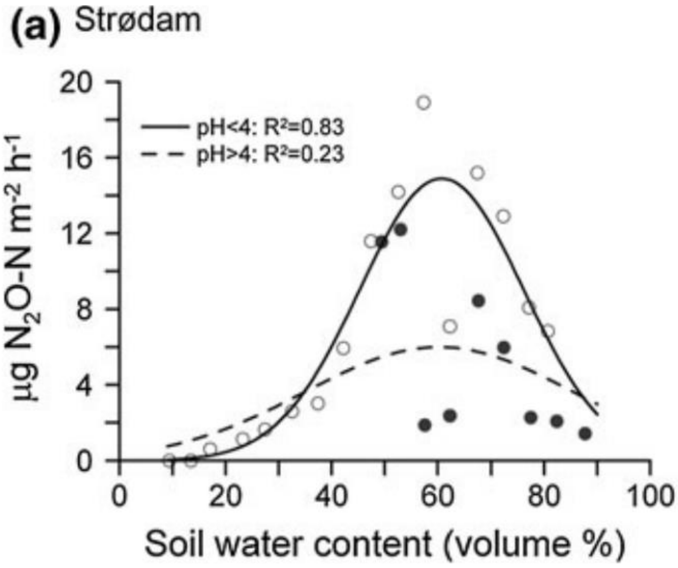

Figure 5.2 The relationship of N_2O-N and soil water content (P. Gundersen et al., 2012).

Higher soil moisture can create anaerobic conditions favorable for denitrification and N_2O emissions. but too high soil moisture would limit the diffusion of gases in the soil. Some sampling plots even showed negative N_2O emissions in winter, possibly because the excessively low soil temperature and moisture inhibited the denitrification process, resulting in the inability of NH_4+ or NO_3- to convert to N_2O (Huai Yang et al., 2017).

5.2.5 By inorganic N

Both forests, especially unmanaged forests, contain large amounts of dead organic matter that can be mineralized and used for denitrification (Huai Yang et al., 2017). In general, the more inorganic N, the more N_2O emissions. However, this relationship was not widespread in our study, with only a weak positive correlation in Suserup top soil. This can be attributed to the fact that we collected samples in the cold December, the lower temperature made the denitrification dynamics of the soil not obvious or the soil sampling frequency was too low (Huai Yang et al., 2017). A higher sampling frequency may be required to reveal the effect of inorganic nitrogen in N_2O flux.

5.2.6 By canopy gaps

Broby plot C5 was under the opening of the canopy, where other plots C1-4 were not, therefore the effect of canopy gap could be analysed. The nitrate concentration and N_2O emissions were higher in C5 compared with the average of C1-4, this could be due to the lower uptake by vegetation and the priming effect of leaf and fine root litter under the canopy opening(Huai Yang et al., 2017). And during the monitoring period, C5 had a little bit higher soil temperature and moisture than the average of C1-4. Many characteristics of the understory microenvironments could be changed due to the canopy gap. The most obvious effect is on the light condition under the opening of the canopy. The soil absorbs more solar radiation, which causes the soil temperature to rise and changes the soil moisture status (Huai Yang et al., 2017).

The N_2O flux in the canopy opening was slightly higher than that under canopy cover, but there was no significant difference ($P=0.3$). Firstly, due to less transpiration of branches and leaves under the canopy opening, it was easy to form an anaerobic environment with high soil moisture, promoting the soil denitrification process. Additionally, with less canopy shielding, rainwater can reach the soil directly to increase soil water availability and wet atmospheric N deposition (Huai Yang et al., 2017). However, our monitoring period in Danish forests was mainly in autumn and winter, with low temperatures and little rainfall, and no visible canopy gaps after all leaves loss, which may explain our observations.

5.2.7 By edge effect

In addition, there are many other factors that may have some influences. In previous (Wang Songqing, 2023) experiment, the plots we selected in Suserup proved to be rich in nitrate

(ANNEX IV). These plots were located on the edge of the forest with the high altitude, so the edge effect could play a role. While the effect of the edge may be quite substantially affected by the surrounding matrix. Specifically, the increase in nitrate concentration and N-leaching could lead to higher amounts of nitrogen being deposited in the edge, compared to the central region of the forest, which increases deposition at the edge. This is because the edge of the forest may have higher fertility and nutrient availability, which can promote the growth of vegetation that requires more nitrogen. A little higher N-leaching may result in rapid and lush growth of early successional plants (Norbertas Noreika et al., 2012).

5.3 Limits of study

The timing disadvantage is that monitoring runs from August 2023 to April 2024, mainly focusing on the cold autumn and winter months, requiring more testing from the warmer summer months. The spatial disadvantage is that the sample plots used to compare different managements are relatively small. More sample plots are needed to increase the reliability and representativeness of the experiments.

6.Conclusion

Due to people's concern about forest nitrogen leaching, based on previous studies, this thesis studied whether the N leaching conditions of Danish forests under different managements were different and their influencing factors through up to nine months monitoring.

Through soil sampling and experiments, we found that soils from unmanaged forest Suserup Skov had higher nitrate leaching, also exhibited higher pH and phosphorus availability, and a lower C/N ratio, resulting in higher net N mineralization, compared to managed forest Broby Vesterskov.

Influenced by soil properties, management models and environmental conditions, the N_2O emissions of the two forests were significantly different, with the average N_2O emissions of Suserup Skov being 5.6 times higher than that of Broby Vesterskov. In addition, the study observed higher N_2O emission fluxes during warmer months, indicating the presence of a temperature-dependent pattern. Through the study of gradient effect, it was found that soil water content was also the key factor affecting N_2O emission, and the maximum emission occurred at the moderate moisture level (65%). Additionally, although it was observed that Suserup Skov with higher nitrate leaching had higher N_2O emissions, there was a positive but not strong correlation between the variances in our study. At the same time, canopy gaps effect and edge effect also increase N leaching.

In summary, managed forests had less nitrate leaching and N_2O emissions, and were better in N leaching control than unmanaged forests. Future research should focus on long-term and larger monitoring locations and incorporate other variables to further elucidate N dynamics. After that, try to find the forest management models that effectively reduce N leaching and maintain a good balanced N cycle.

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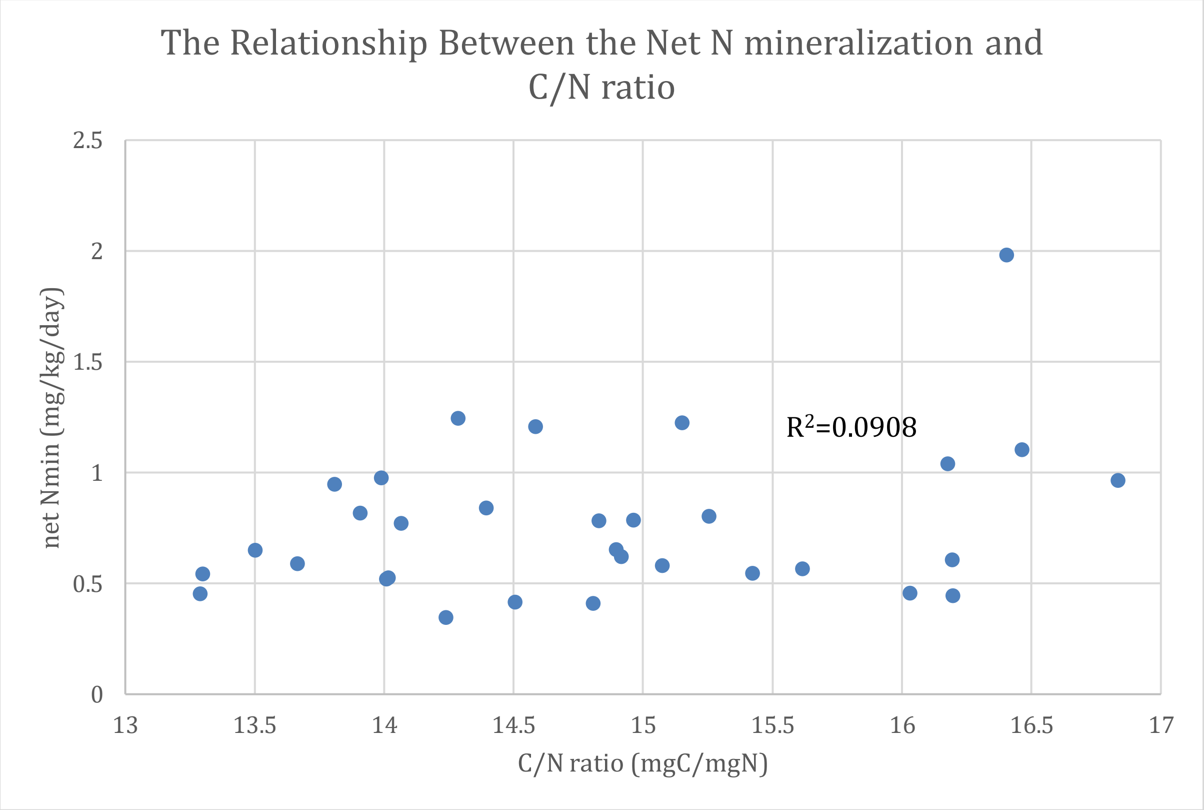
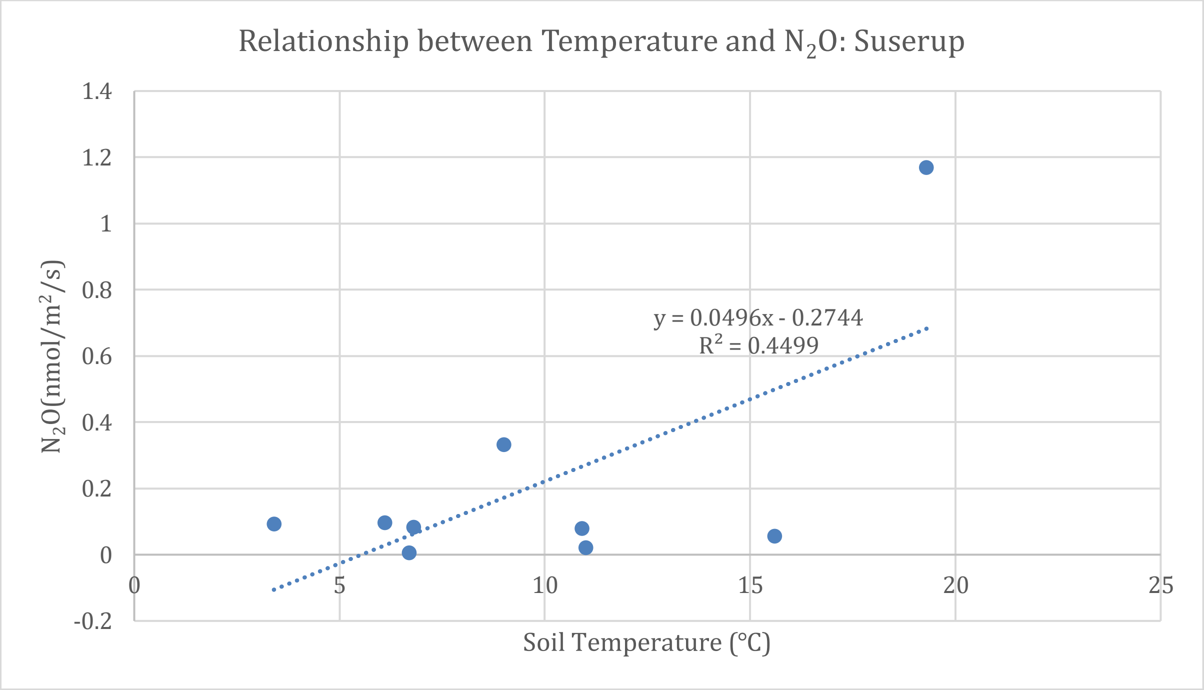
ANNEX I

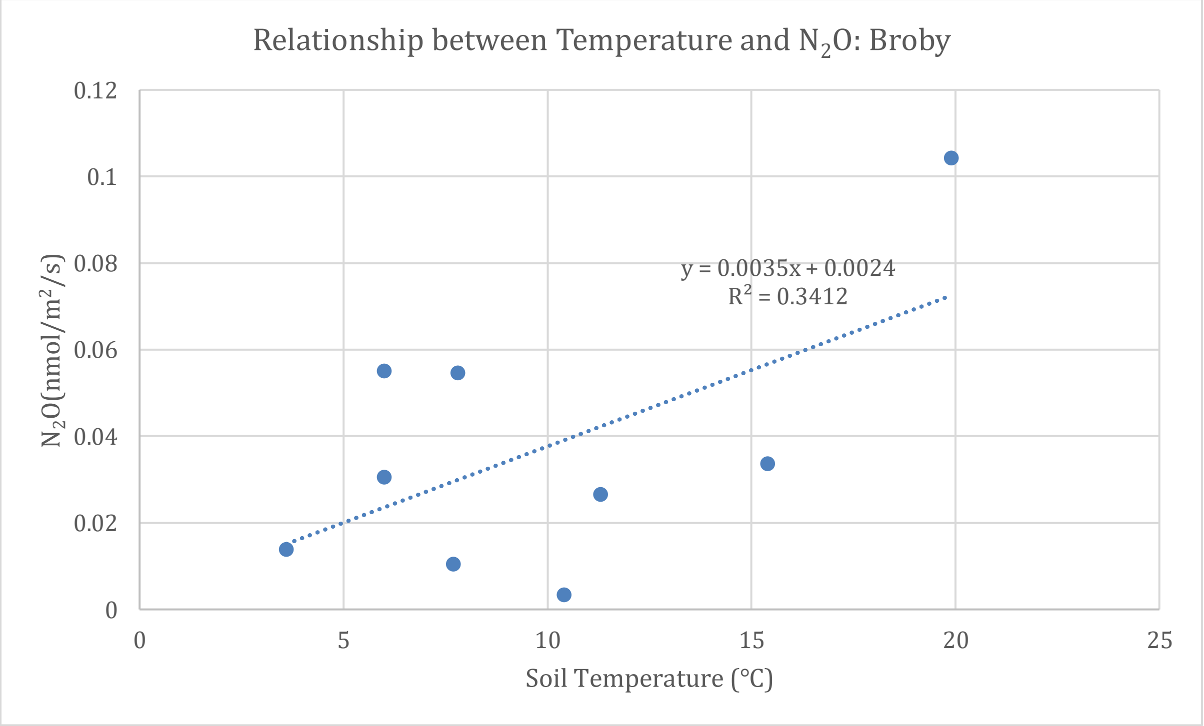
The relationship between the bet N mineralization and C/N ratio in two forests, sampled in December 2023.



ANNEX II

The relationships between average N_2O and temperature in Suserup and Broby from August 2023 to April 2024.





ANNEX III

The soil water content/moisture table for G1-G7 for each month, from Aug 2023 to April 2024. “\” means did not be detected successfully.

Moisture	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr
G1	0.267	0.149	0	0.238	0.326	0.33	0.286	0.29	0.31
G2	0.363	\	0.171	0.397	0.33	0.353	0.394	0.33	0.344
G3	0.329	0.168	0.179	0.074	0.38	0.28	0.42	0.36	0.47
G4	0.369	0.129	0.07	0.326	0.31	0.342	0.36	0.26	0.33
G5	0.385	0.192	0.194	0.38	0.323	0.31	0.39	0.47	0.43
G6	0.95	\	0.332	0.752	0.75	0.654	\	0.755	1
G7	\	\	0.439	0.554	0.511	0.647	\	0.72	0.75

The soil temperature table for G1-G7 for each month, from Aug 2023 to April 2024. “\” means did not be detected successfully.

Temperature	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr
G1	19.9	16	10.9	5.4	3.4	6.1	8.7	6.5	10.8
G2	19.5	\	11.1	5.3	3.3	6.2	8.5	6.5	11
G3	19.5	15.8	11.2	5.6	3.4	6.1	8.1	6.6	11.4
G4	19.3	16.2	11.2	5.7	3.3	6	8.5	6.6	11.8
G5	19.1	16	11.1	5.9	3.3	5.9	8.8	6.6	11.3
G6	18.9	\	11.1	6.6	3.3	5	\	6.5	10.7
G7	\	\	11.1	6.4	3.2	5.7	\	6.6	10.7

ANNEX IV

The soil sampling plots in Suserup Skov and the nitrate concentration in different plots in March 2023.