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Besvarelsesinformationer

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UNIVERSITY OF COPENHAGEN
FACULTY OF SCIENCE

Master Thesis

Investigating Nitrate Leaching in Old-growth Forests

Supervisor: Per Gundersen, Professor, Department of Geosciences and Natural Resource Management

Co-supervisor: Frederik Nygaard Philipsen, PhD-student, Department of Geosciences and Natural Resource Management

Submitted on: 31 May 2023

Name of department: Department of Geosciences and Natural Resource Management

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Title and subtitle: Investigating Nitrate Leaching in Old-growth Forests

Topic description: It is well known that higher nitrogen availability in acidic topsoil promotes the formation and emission of nitrous oxide (N_2O). We have observed high nitrate leaching in several forest reserves in Denmark. In forest reserves where biomass is not harvested, there is no mechanism for removing nitrogen, so excess nitrogen may accumulate in the soil or leach into the groundwater. We visited paired managed and unmanaged forests and carried out gas exchange measurements as well as soil and soil water sampling. This project combines a compilation of data from the literature with fieldwork to analyze the patterning of nitrogen leaching.

Supervisor: Per Gundersen

Co-supervisor: Frederik Nygaard Philipsen

Submitted on: 31 May 2023

Abstract

In recent years, studies have highlighted the issue of nitrogen leaching from unmanaged forests, which contributes to environmental pollution. This study aimed to analyze the patterns of nitrate leaching and N_2O emission by investigating paired managed forest (Broby Vesterskov) and unmanaged forest (Suserup Skov). Gas exchange measurements, as well as soil and soil water quality testing, were conducted. Data were collected and calculated, then analyzed by using ANOVA, t-tests, and other analytical methods. The results show that nitrate leaching continues in unmanaged forests due to elevated nitrogen. Nitrate leaching and N_2O emission showed a seasonal pattern. In forests, the elevation of the terrain affects the amount of N_2O emitted. Nitrate leaching and N_2O release are highly correlated. In addition, we found that levels of nitrate leaching and N_2O emission were relatively low in nearby managed forests compared to unmanaged forests. This is because in managed forests some biomass can be removed by human, which reduces the input of N. These findings highlight the impact of forest management on the nitrogen cycle and environmental pollution.

Keywords: Nitrogen cycle, management plans, nitrate leaching, N_2O emission

Acknowledgments

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Furthermore, I would like to express my gratitude to IGN for generously providing the water sample data, which formed the basis of this research.

Contents

1. Introduction

1.1 Background

For decades, the extensive use of nitrogenous fertilizers such as urea and potassium nitrate in agriculture has caused increasing deposition of nitrogen in natural and semi-natural systems leading to major impacts on the ecosystems (Butterbach-Bahl, Gundersen, 2011, p.100). European natural ecosystems are threatened by excessive nitrogen deposition. The effects on the natural environment of long-term exposure of ecosystems to high atmospheric nitrogen pollutants are serious. Nitrogen pollution poses ecological threats. Nitrogen deposition to the land can lead to severe soil acidification (Lu et al., 2014) and eutrophication of terrestrial and aquatic systems (Butterbach-Bahl, Gundersen, 2011, p. 100).

In Europe, agricultural soils have a considerable amount of nitrate leaching, with even exceeding 50 kg N ha-1 yr-1 in northwestern and southern Germany (Gauger et al., 2011). Compared to agricultural soils, forest soils typically have low nitrate leaching and act as a net sink for the strong greenhouse gas (GHG) methane (CH_4), while emissions of the more potent GHG nitrous oxide (N_2O) are much lower in forests (Gundersen et al., 2012).

It is worth noting that water pollution from nitrate leaching can also act with forest soil systems. Nitrate leaching in forest soils occurs when the availability of inorganic nitrogen (N) exceeds the needs of plants and microorganisms. This situation is often referred to as N saturation. Signs of N saturation in forest soils have been widely reported in Europe. A study conducted by Fowler et al. (2004) illustrates that nitrogen deposition in forests can be 2-3 times higher than in open areas because the forest canopy is an effective sink for atmospheric nitrogen.

In unmanaged forest reserves, biomass is not removed, and no mechanism can remove nitrogen. Thus, excess nitrogen can accumulate in the soil or leach into groundwater and form acidic soils. Nitrogen leaching has been observed in some unmanaged forests in Denmark in previous investigations. Higher nitrogen availability in acidic soils promotes the formation of N_2O.

1.2 Research objectives and thesis structure

In this study, a managed and unmanaged forest will be surveyed for several months and analyzed for nitrogen leakage through three study objects: soil samples, soil water, and for the first time N_2O gas exchange.

This study was based on continued research by Gundersen et al. (2009) and BSc/MSc thesis works by Munk-Nielsen (2018) and Garbu (2020). These previous reports document high N availability and excessive nitrate leaching in the unmanaged forest reserve Suserup. I hypothesized that

H_1: the reserve continues to have nitrate leaching, due to continued elevated N deposition.

H_2: a nearby managed forest will have lower N availability and leaching than the reserve.

H_3: a difference in N availability between managed and unmanaged forests will lead to differences in N_2O emissions, with higher emissions in the unmanaged forest.

The article is divided into six chapters. The first and second chapters are an introduction to the experiment and the theory of the thesis. Experiment and study sites are described in Chapter 3. In Chapters 4 and 5, the results of this study are presented and analyzed. The full thesis is concluded in the last chapter.

2. Theory of experiment

In order to determine the nitrogen leakage in forests, this section provides a theoretical analysis of the nitrogen cycle, nitrogen input, nitrogen output in forests, N_2O emission, and the effects of different management plans on nitrogen leakage, respectively.

2.1 Nitrogen cycling

Nitrogen cycling is the transport, transformation, and turnover of nitrogen between the Earth's atmosphere, biosphere, soil, and hydrosphere. The nitrogen cycle in terrestrial ecosystems is also seen as a process of formal transformation of nitrogen. This process is a complex process involving microorganisms and plants (Butterbach-Bahl, Gundersen, 2011, p.104).

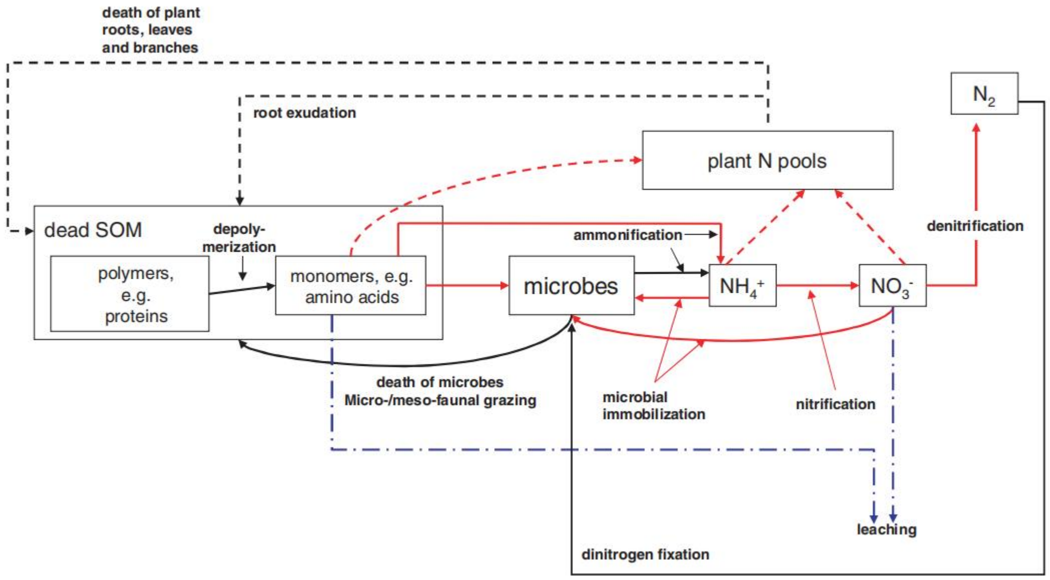

Figure 1 Nitrogen cycle in the natural system (Butterbach-Bahl, Gundersen,2011)

Nitrogen is an indispensable element for plants and animals. However, nitrogen is difficult for plants to use directly because of the strength of the triple bonds in the molecule. Nitrogen input to forest systems is therefore mainly accomplished through nitrogen fixation. N_2 is taken up by plants and incorporated into their tissues where it is converted into ammonium ions. The carcasses and fallen leaves of plants and animals are broken down into organic matter by microorganisms in the soil. The nitrogenous organic matter enters the soil through the mineralization process and is converted into ammonium ions (NH_4^+), which can be reabsorbed by plants. NH_4^+ is converted to NO_3^- by the nitrification process and some NO_3^- is absorbed by the plant. During the process of nitrification, N_2O is produced as a second product. A small part of nitrogen can be taken up by plants as amino acid.

Nitrogen can also be lost from the ecosystem through a process called denitrification, in which bacteria convert nitrate in the soil into nitrogen gas or nitrogen dioxide. Nitrogen goes back to the atmosphere in this way.

In forest soils, decomposition and mineralization, nitrification, microbial immobilization, plant uptake, and dissimilatory nitrate reduction to ammonium play an important role in the nitrogen cycle.

2.2 Nitrogen input

Nitrogen input in forests can come from various sources, including atmospheric deposition, biological nitrogen fixation, and fertilizer application.

The main sources of these compounds are natural and human activities. For example, lightning and the burning of fossil fuels. In forest systems, there are two types of nitrogen deposition: dry deposition and wet deposition. Nitrogen has increased substantially through dry and wet deposition in Europe. A study suggests that in Europe over the last two decades, Nitrogen input increases from 2-6 kg hr^{-1} yr^{-1} to 60 kg hr^{-1} yr^{-1} (Pitcairn, 1995). In forests, nitrogen deposition rises after the trees become mature due to the influence of the closed canopy. After 20 years of tree growth, nitrogen deposition doubles (Gundersen et al., 2009 p.1144-1145).

Biological nitrogen fixation is a process that occurs in nature through the conversion of atmospheric N_2 by specific bacteria into something that plants can take up. In forest systems, free-living bacteria in the soil can accomplish this process. An association and symbiotic relationship between plants and nitrogen-fixing bacteria can also complete this process. In addition, nitrogen fixation by heterotrophic bacteria in the soil and sediment plays an important role in plant litter decomposition. Nitrogen input through this process to terrestrial ecosystems is about 1-5 kg hr^{-1} yr^{-1} (Butterbach-Bahl, Gundersen, 2011, p.102). Biological nitrogen fixation plays a dominant role in the nitrogen input process, with flash nitrogen fixation contributing less to the nitrogen input than biological nitrogen fixation.

Fertilizer is a type of nitrogen input to the forest. Fertilizer application does not usually occur in unmanaged forests. Fertilizer applications can be used as a source of N input to the managed forests or tree plantations and provide a supply of N that does not require conversion.

2.3 N output

In forest systems, the relationship between the amount of nitrogen exported and the amount of nitrogen input is closely related. An increase in nitrogen input leads to an increase in the amount of nitrate in the forest, which in turn leads to an increase in the leaching of nitrate from the forest. Excess nitrogen input can lead to a loss of nitrogen from the forest through leaching (MacDonald et al., 2002).

In forests with nitrogen leaching, there will usually be a threshold for nitrogen input.

Nitrate leaching is usually evident at a nitrogen input of 8-10 kg N ha-1 yr-1. And nitrogen leaching is usually 25 kg N ha-1 yr-1 or more (Butterbach-Bahl, Gundersen, 2011, p. 114). The variability in response to nitrogen deposition is determined by the state of the nitrogen or the availability of nitrogen. In a nitrogen-rich system, the retention of nitrogen input to the system is low and this system is referred to as N-saturated (Butterbach-Bahl, Gundersen, 2011, p. 113). In a forest system, if the loss of nitrogen approaches or exceeds the N input, then the N status of the system is referred to as N saturated. When the C: N ratio is below 25, the soil tends to be saturated with N and leaching rates can be high (Gundersen, 2009) if the N-input exceeds the tree's demand for N.

2.4 N_2O emission

Radiative forcing of N_2O is 298 times stronger than CO_2 on a 100-year timescale (Gundersen et al., 2012). In forest systems, N_2O can be generated through nitrification and denitrification. The rate of N_2O production will be greatest when nitrate is produced in environments with soil C: N ratios below 25. Nitrate leaching is high at this C: N ratio. Therefore, C: N ratios can be used to detect nitrate leaching and N_2O production (Gundersen et al., 2012, p.4005).

In soils, the relationship between CH_4 and N_2O emissions is reciprocal, with N_2O emissions increasing when CH_4 emissions become lower (Ma et al., 2016). Nitrogen deposition, climate change, land use change and forest management as the external drivers to influence N availability, land PH, temperature, and humidity of soil to influence N_2O and CH_4 emission (Gundersen et al., 2012, p.4005).

2.5 Impact of forest management on nitrogen budgets

Land use and land management practices are key factors influencing the nitrogen cycle in the region (Butterbach-Bahl, Gundersen, 2011, p. 109). Nitrogen can be artificially or naturally input or output into the forest system.

Logging is a way to remove nitrogen from the forest by removing organic matter from the forest. Different methods of harvesting also have different effects on the removal of nitrogen from the forest. This is due to the low C: N ratio in the leaves and branches of the trees (Gundersen, Schmidt, & Rasmussen, 2006, p. 28). Therefore, harvesting is a way to remove nitrogen from the forest. Manually removing fallen leaves and fallen tree trunks from the forest also reduces nitrogen leaching by removing biomass from the forest.

3. Methodology

3.1Sites description

The two forests selected for this study represent different forest types, management practices, and soil types in Denmark. The forest called Suserup Skov is not managed and Broby Vesterskov is managed forest. the location of the forests under study is shown in the figure shown below.

Figure 2 Location of study sites

3.1.1 Suserup Skov

Suserup Skov is a forest located on the island of Zealand and covers 19.2 hectares. It is located at 55°22'N, 11°34'E and is 22 meters above sea level (Emborg, et al. 1996). The eastern and northern parts of the forest are connected to an agricultural field that has been abandoned for 20 years. The grassland to the west of the forest was a gravel pit until 1960, and to the south of Suserup Skov is the freshwater lake called Tystrup (Heilmann-Clausen, Jacob, et al. 2023).

Suserup Skov (as well as the nearby Broby Vesterskov) has an average annual precipitation of 635 mm. August and February are the hottest and coldest months of the forest, with average temperatures of 16.7°and 0.8°, respectively (Emborg, et al. 1996). The forest has developed from boulder clay soils with an undulating landscape. The parent soil is mainly a nutrient-rich calcareous glacier with approximately 20% clay. The type of soil is mainly classified as a single soil rich in brown clay (Larsen, Vesterdal, Bentsen, & Larsen, 2019, p. 68). The pH value of the soil was tested to be 4.5, C: N-org ratio was 30.3, and C: N ratio in mineral soil was 15.1. The main vegetation of the forest is Fagus Sylvatica, Fraxinus excelsior, and smaller amounts of Quercus robur and Ulmus glabra (Hannon et al., 2001). Susserup is currently one of the closest places to the primary forest in Denmark（Suserup Skov, n.d.）

Before 1792, Suserup Skov was part of a forested pasture, meadow, and arable land. Between 1792 and 1860, the Suserup Skov was enclosed and managed as woodland. After that, the forest was transformed into a park area for recreation and leisure, and in 1925 the nature reserve became legally protected, and after 1970 the policy of non-interference was implemented in Suserup Skov (Hannon et al., 2001)

Figure 3 Location of Suserup Skov and three zones (Heilmann-Clausen, Jacob, et al. 2023）

As shown in the figure above, to better study Suserup Skov, the forest is divided into three zones. These three zones differ in terms of vegetation type, previous management practices, and topography. Part A has experienced the least human impact in the last 200 years and is also the location of the seepage water survey. Part B was heavily grazed and had human-sown oak before 1792, while part C is close to the lake and can be distinguished by botany (Emborg et al.,1996). We used 4 long-term monitoring plots (ICP-Forest plots) that are situated in the northern half of part A towards an open field now left for natural colonisation.

3.1.2 Broby Vesterskov

Broby Vesterskov, an area of about 4.5 square kilometers, is located in the southeastern part of the Sorø Sønderskov Forest area, about 5 kilometers from Sorø. It is also located to the northeast of Suserup Skov, an approximate distance of 1.7 km. The sampling site was chosen in the northern part of the forest and is shown on the map （Sorø Kommune, n.d.）.

The area consists of approximately 13 hectares of oak. The majority consists of Holm oaks planted between 1917 and 1925. A small part consists of North American red oaks from the 1910s and 1950s. However, the section used in this study is dominated by beech (Sorø Kommune, n.d.). The presence of stumps and branch piles indicates cuttings in recent years. The lower southern part is ditched. We placed sampling plots in the higher-laying northern part that has an edge towards a field. In this way, the forest setting was comparable in the two forests. The soil characteristics of Suserup forest and the area used in Broby Vesterskov are comparable with the C/N ratio (0-10 cm) being 14±2 across both forest but with an insignificant difference in pH (4.5 vs 5.0, Broby vs Suserup) according to an extensive soil survey by Hansen (2020).

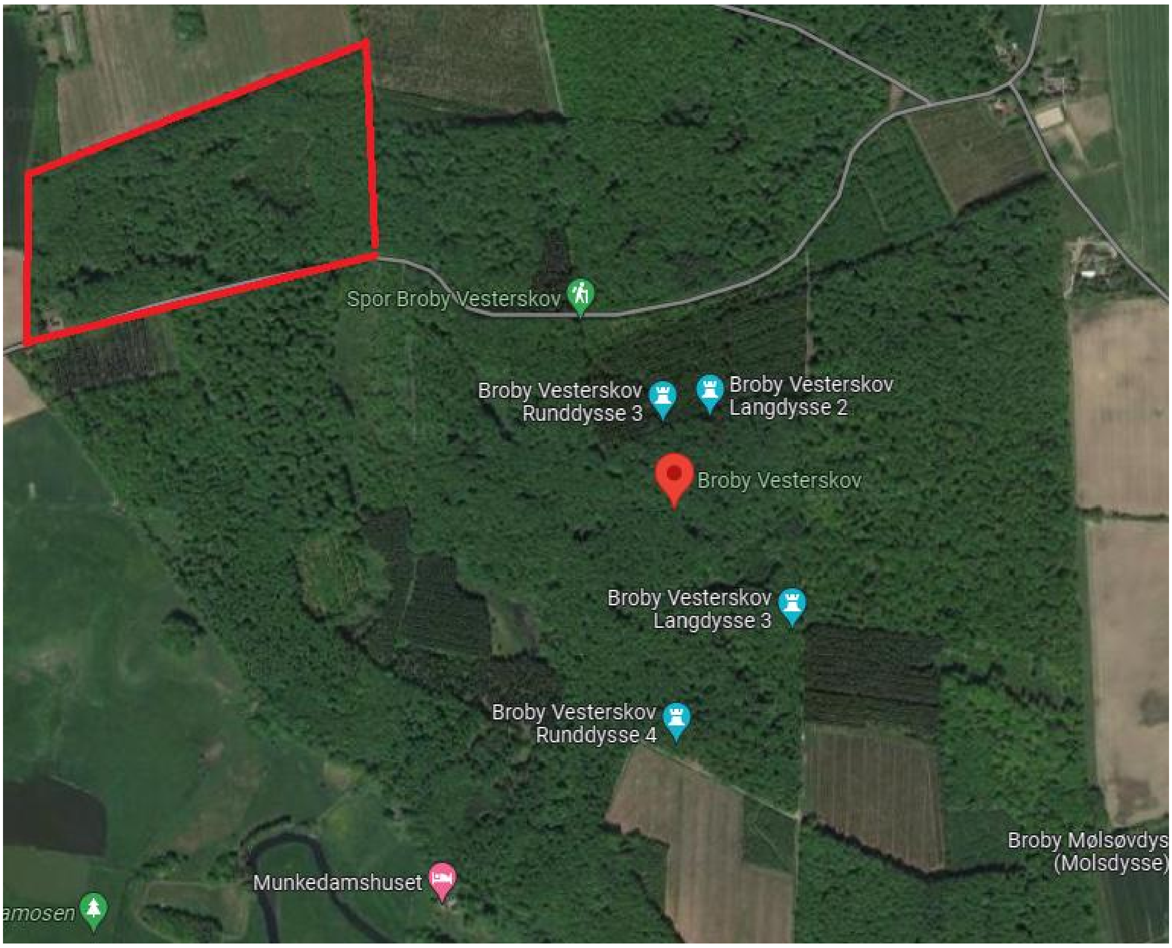

Figure 4 Sampling part of Broby Vesterskov (Google map)

3.2 Experiment design

The study builds on a nitrate survey using extraction of soil samples from Suserup by Munk-Nielsen (2018) and a seepage and nitrate leaching study of Suserup by Garbu (2020). Garbu (2020) analyzed deposition and soil water data from 2003-2018, while Munk-Nielsen (2018) analyzed soil nitrate concentrations at 90 cm depth. The objectives of this study were to collect and follow up on these studies with the latest data, to measure and analyze the emission of N_2O from soil, and to assess the impact of management patterns on nitrate leaching and N_2O emission. Collection of percolated water, evaluate N_2O emission of soil and exam nitrate concentration soil are the three methods used to carry out the experiment.

The study is conducted in two forest areas with different management plans. Four plots in the Suserup Skov are long-term sampling sites for water samples, where monthly water quality surveys are carried out for throughfall and soil water at 90 cm depth. Monthly water chemistry data from 2020-2022 were generously provided by IGN for use in this project. These four plots were also used for soil sampling and soil N_2O gas exchange. Further, 16 survey sites are selected based on study of Munk-Nielsen (2018) for being compared to previous nitrate concentration. To study topographical effects on gas emissions 6 plots are taken on a hillside for N_2O release in relation to slope. Maps below show the sampling point of Suserup Skov.

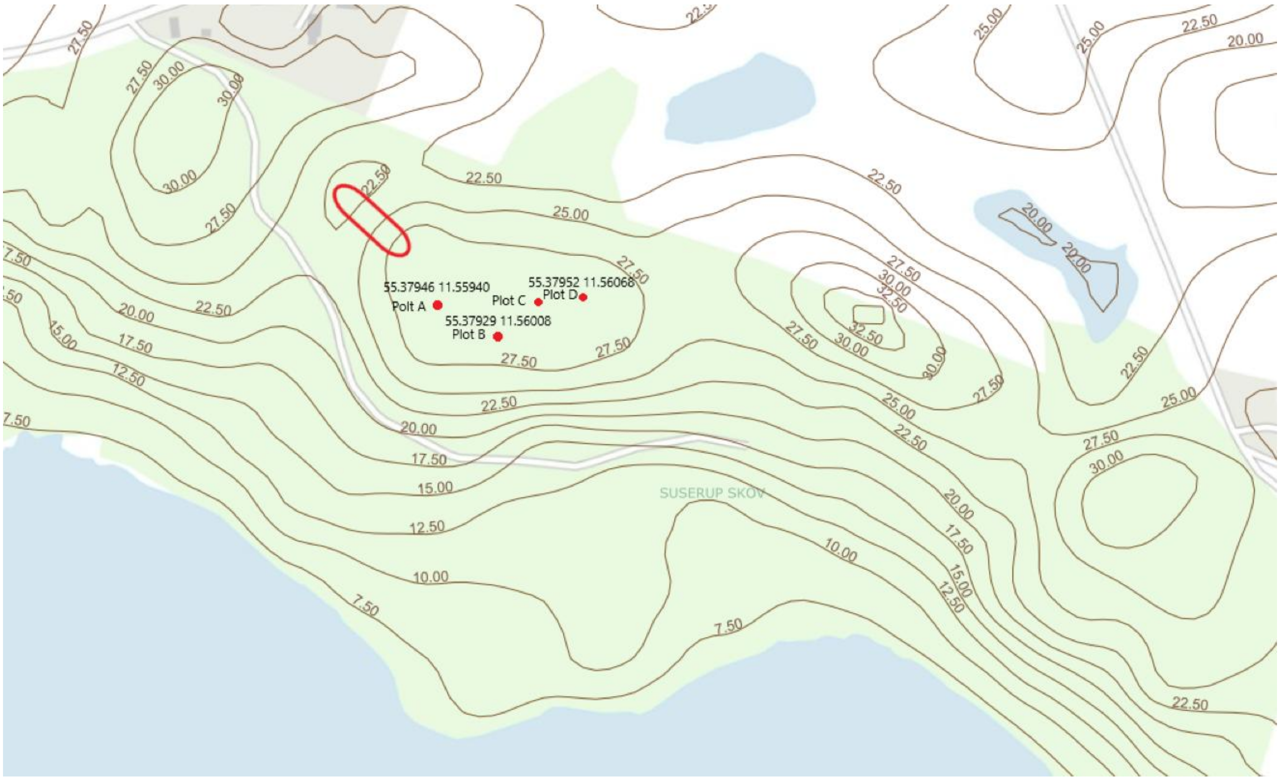

Figure 5 Slope test and long-term monitoring plots in the Suserup Skov. (SDFI kortviewer) The slope test site is circled in red

Figure 6 16 soil sample sampling sites in Suserup Skov

To investigate N_2O and nitrate leaching patterns in managed forests, four points are selected based on similar geographical features to four points in Suserup Skov where long-term water quality monitoring is conducted on. The experimental site in Broby Vesterskov is depicted on the map on the right.

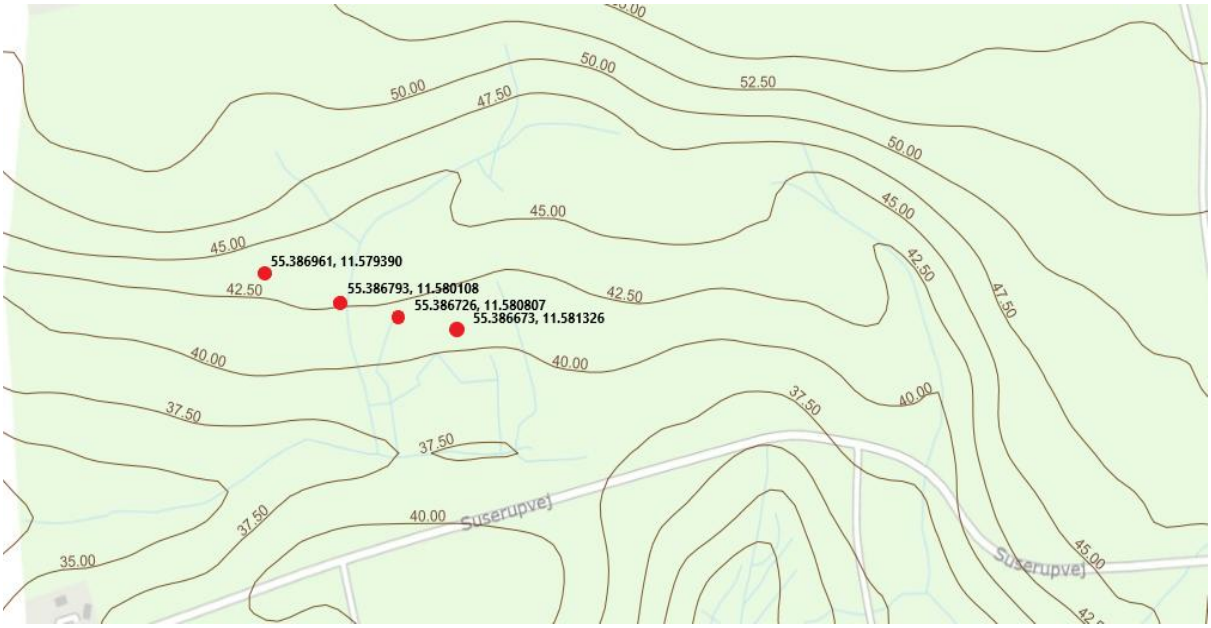

Figure 7 Sampling plots in Broby Vesterskov (SDFI kortviewer)

3.3 Sampling and data collection

3.2.1 Seepage water monitoring

Soil water samples are collected from mineral soil at a depth of 0.9 m using a Teflon suction cup lysimeter. These concentrations are assumed to be representative of leaching concentrations from the root zone. The study consists of four circular subplots, each representing a group of forest stands. Within each circular subplot, four lysimeters are installed, mounted in four directions, for a total of 20 lysimeters, and the seepage water is then collected into each vial. These devices were installed in November 2002 and are used for a long term. Samples are brought to the laboratory as soon as possible after sampling. During transport, the samples are stored in a cool, dry place, out of direct sunlight place. Samples are then stored at +4°C and conductivity and pH values are measured using a pH electrode for two days after sampling. Analysis of Cl^-, NO_3^- and other ions by ion chromatography is performed within one month after sampling to measure their concentrations. To protect the samples from heat and sunlight, all sampling vials are placed in a soil pit (Hansen et al., 2007).

3.2.1 Soil sample collection

In each plot a 3 cm soil auger is hammered down to 90 cm four times to represent a 10 m2 area for survey plots and a 10 m circle for plots also used for gas sampling. Soil from 75-90 cm is bulked to one sample per plot. Soil samples are bagged and labeled and stored in foam boxes with ice packs before being brought into the laboratory for nitrate extraction experiments.

In the laboratory, the pretreatment experiment for nitrate determination in soil samples is divided into two parts:

Figure 8 Picture of soil sampling

a. Soil sample screening: The soil sample is passed through a 2 mm size strainer. About 10 g of the sample is taken and weighed for nitrate extraction, stored in a 50 ml plastic tube, and kept in the refrigerator.

b. Determination of water content in soil: Soil samples are weighed and bagged after the soil was obtained by comparing the difference in quality of the soil samples before and after.

20 mL of 0.1 M KCl solution is added to the soil sample and then shaken in an orbital shaker for one hour to mix it homogeneously with the soil sample. The solution is then centrifuged in a centrifuge and filtered through a Cellotron filter. The nitrate concentration in the solution is analyzed by flow injection analysis (FIA) for the nitrate concentration in the solute. Concentrations from the analysis have been corrected for solute dilution (p.m. P. Gundersen).

3.2.3 Gas exchange measurement

In the gas experiment, N_2O emission is measured in 4 directions in the vicinity of the point where the extraction of the soil sample is carried out. The manual chamber (used for December) and smart chamber (used for January and April) are used for gas collection and trace gas analyzers LI-COR-7810 and LI-COR-7820 are used for gas flux measurements. The chamber is connected to the analyzer by tubes. Therefore, gas can pass through the tube from the chamber into the analyzers（Licor, n.d.）.

Figure 9 picture of gas exchange measurement

During the experiment, a circular column with a plastic surround is first placed at the point to be measured and compacted with a sandbag, then the chamber connected to the analyzer is placed on the column and made sure that it is well fitted. The next step is to control the smart chamber via an iPad to get it running and the analyzer to start recording the gas flux. Final, N_2O flux can be calculated by R.

The manual chamber requires manual measurement of column height and soil temperature and moisture. With the smart chamber, the temperature and humidity of the soil are measured automatically by inserting the instrument's own probes into the soil.

Keep holding the breath before the measurement to reduce the impact of breathing on the air in the chamber. In addition, avoid moving around the measurement plot before and during the measurement to minimize any deviation in gas emission caused by squeezing the soil.

3.3 Data quality assurance

To ensure the accuracy of the data, The Standard Method (APHA, 1992) was implemented to determine the accuracy of the measured data. According to the principle of conservation of charge, the number of major anions in seepage water is equal to the number of cations. By following the standard method, the anion cation balance in throughfall and seepage water can be checked to determine if the data is acceptable.

Because organic anions (weak acid anions) are not measured in lab, the cation concentration may be slightly greater than the anion concentration (personal communication, P. Gundersen), as shown below:

$$\begin{aligned} &\mathrm{H^{+} + B^{3+} + Na^{+} + Mg^{2+} + Al^{3+} + P^{5+} + S^{2+} + K^{+} + Ca^{2+}} \\ &\qquad \geq \mathrm{CO_3^{2-}/HCO_3^{-} + Cl^{-} + NO_3^{-} + SO_4^{2-} + NH_4^{-}} \end{aligned}$$

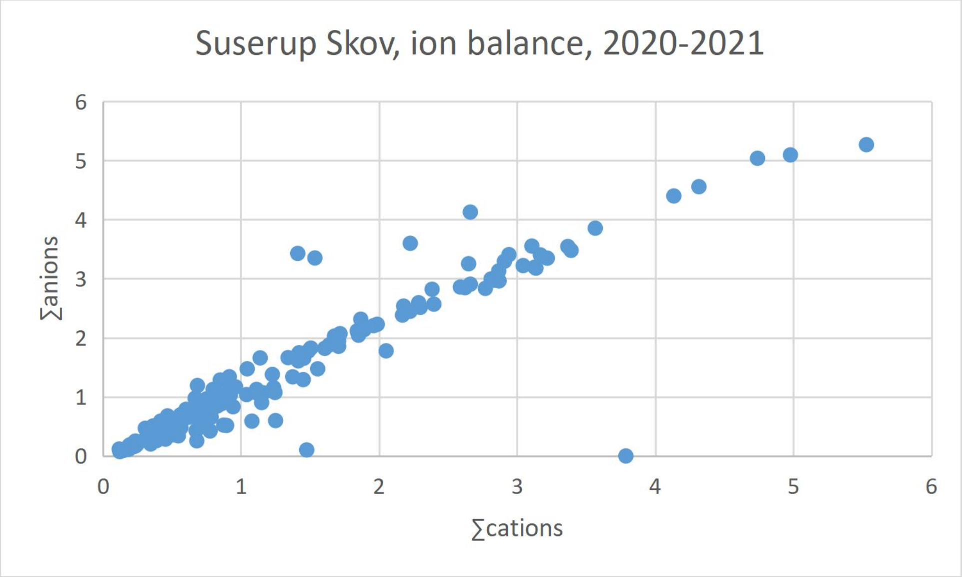

Figure 10 Charge balance regressions, Suserup Skov (2020-2021)

The linear regression was done for ion balance and is shown above in Figure 10. The value of R^2 indicates that the linear regression model fits relatively well, but there are also some data that fit poorly, which means that some data need to be considered for their plausibility.

Based on the principle of electrical neutrality, an equation of charge balance can be used as a criterion to judge whether the seepage water data is satisfactory

:

$$E = \frac{\sum cations - \sum anions}{\sum cations + \sum anions} \times 100\% \quad \text{(El Baba et al., 2020)}$$

Data with a margin of error at or below 10 percent is deemed acceptable, where data outside this range can be taken for a secondary evaluation.

Upon review of the data, all the test points were off by ion concentration due to missing data on one or more ions. In this case, all test points were retained. While data with complete ion tests but which would have caused serious ion concentration deviations should be removed, but no observation fell for these criteria.

One throughfall data was removed due to high P concentrations was detected, implying that bird excrement influenced the data.

3.4 Data calculation

3.4.1 NO_3^- flux in seepage water

In the Suserup Forest, this study conducts monthly measurements of the sample water data, including bulk precipitation outside the forest, throughfall and seepage water at depths of 90cm. To accurately assess the nitrate content in the seepage water at a depth of 90cm, this paper employs the chloride budget method for calculating and analyzing soil water data.

In the Suserup Forest, there are four sampling sites (sample nr 1-5, 6-10, 11-15, and gap), consisting of a total of 20 funnels, used for water quality measurements of throughfall. In addition, four other sampling sites (sample nr 21-23, 24-26, 27-30, and 31-36), comprising a total of 20 sub-sampling points, are employed for water quality testing of seepage water at a depth of 90cm. Moreover, three external forest sampling sites (1,2,3) are utilized to measure throughfall amounts. The throughfall amount is calculated as the average of the recorded water amounts in the 20 funnel pools, with the formula expressed as follows:

$$throughfall(mm) = \frac{\sum water\ collected\ in\ funnel(m^3)}{20 \times 1000 \times funnel\ area(m^2)}$$

Precipitation is usually expressed in millimeters (American Meteorological Society，2009). To estimate the input of nitrogen compounds, including NO_3^-, NH_3^+, and TN, into the soil, the measured concentrations of these substances in water samples (expressed in mg/L) can be converted to areal density in kg/ha by accounting for throughfall amounts. This conversion allows for a more comprehensive understanding of the input of these elements into the soil system. The formula is expressed as:

$$Areal\ Density(kg/ha) = concentration(mg/L) \times pp(mm)$$

The chloride mass balance method was employed to ascertain the percolation of substances at a depth of 90 cm. By utilizing this method, a comprehensive understanding of the leaching processes taking place within the soil profile can be achieved, while also reveal on the underlying mechanisms governing nutrient and solute transport. Consequently, this approach enables the determination of the amount of soil elements leached (Garbu, 2020).

$$Percolation\ rate = \frac{Cl_G(kg/ha)}{Cl_J(mg/L)} \times 100$$

In the aforementioned formula, Cl_G (kg ha-1) represents the deposition amount of chloride ions penetrating through the soil, while the Cl_J (mg/L) represents the concentration of chloride ions detected at a depth of 90 cm, which corresponds to the leaching concentration. By incorporating these parameters into the formula, the leaching processes and the behavior of chloride ions within the soil profile can be better understood.

After obtaining the percolation rate, the amount of nitrate leakage can be calculated by uniting the NO^{3-} concentration of seepage water at a depth of 90cm, represents as $NO^{3-}{}_J$ in the formula shown below.

$$Nitrate_{out}(kg/ha) = \frac{NO_{3\,J}^{-} \times Percolation\ rate}{100}$$

Since $NO_3^-{}_J$ is measured one time a month, $Nitrate_{out}$ stands for monthly nitrate leaching amount for Suserup Skov. The annual leaching amount for nitrate in seepage water can be calculated by summing the data for every month. The data from September to December in 2020 and 2021 is missing due to drought, which is deemed to be 0 leakage from soil water. The formula for annual leakage is described as:

$$Nitrate_{out-y}(kg/ha) = \sum Nitrate_{out}(kg/ha)$$

3.4.2 N_2O flux

In this study, we utilized LI-COR-7810 and LI-COR-7820 with two different chamber type (manual chamber and LI-COR smart chamber) to measure CO_2, CH_4 and N_2O fluxes, here only the N_2O fluxes are reported. Different R codes were used for the calculation of Fluxes from the detected N_2O concentration, but the basic principle for measurement and calculation was the same. Normally, N_2O flux can be determined by the equation:

$$\mathrm{F(t)} = \frac{\Delta \mathrm{c_{dry}}}{\Delta \mathrm{t}} \times \frac{\mathrm{VP(1-W)}}{\mathrm{ART}}$$

In the equation presented, the following variables are defined:

F: flux of N_2O (μmol m^{-2} s^{-1})

c_{dry}: dry N_2O concentration difference of N_2O from the end to the end beginning of the measurement (μmol mol^{-1})

t: measurement time (s)

P: internal pressure of the chamber (kPa)

V: gas throughput (L)

W: concentration of H_2O (mol mol^{-1})

A: collar area (m2)

R: universal gas constant (8.314 L kPa K^{-1} mol^{-1})

T: chamber temperature (K = 273.15 + T°C)

The amount of nitrogen dioxide diffusing out of the soil per unit of time is then calculated using this equation. In practice, the effect of the chambers on the flux of gases needs to be taken into account. In order to modify the conditions for the passage of gases within and without the chambers, a few factors need to be studied.

Firstly, as the N_2O concentration in the chamber increases, it causes a difference with the outside N_2O concentration, which affects the rate at which N_2O diffuses out of the soil. Differences in air pressure, temperature and humidity also affect the rate of diffusion of N_2O from the soil. It is important to note that in the first 10-30 seconds after the gas is drawn into the chamber, the gas in the chamber is not fully stabilized and this part is called the dead band, which needs to be removed from the calculation.

To correct the deviation brought by these factors and calculate the flux of N_2O, the r-code produced by Klaus Steenberg Larsen and Karelle Rheault is used to calculate the flux of nitrogen dioxide. Two different types of chambers, each with its own set of R codes, are used in this study. Data from the manual chamber requires manual selection of the start and end time points of the measurement, whereas data from the smart chamber does not.

The Hutchinson and Mosier model (hm) is used to calculate the non-linear N_2O increments over the test time, while the Linear model (lm) is utilized for determining the linear gas increments. A linear graph representing gas increments is consolidated to ascertain the model's application. In most cases, the model exhibiting a higher R^2 fit is employed to determine N_2O flux. For data with low N_2O fluxes, variations in background N_2O values significantly influence flux calculations (personal communication, J. Christiansen). The hm model can be easily influenced by background at comparatively low fluxes, while the lm model exhibits greater stability at low gas fluxes. Consequently, the choice of model should consider not only the degree of fitting but also the magnitude of the flux (personal conversation, J. Christiansen). For data with very little increase in flux, the flux is treated as 0 considering the effect of background values. For data showing a small increase in flux and significant fluctuations at the start, the effect of background values is taken into account and is therefore treated as zero. Example of N_2O flux model selection shown below.

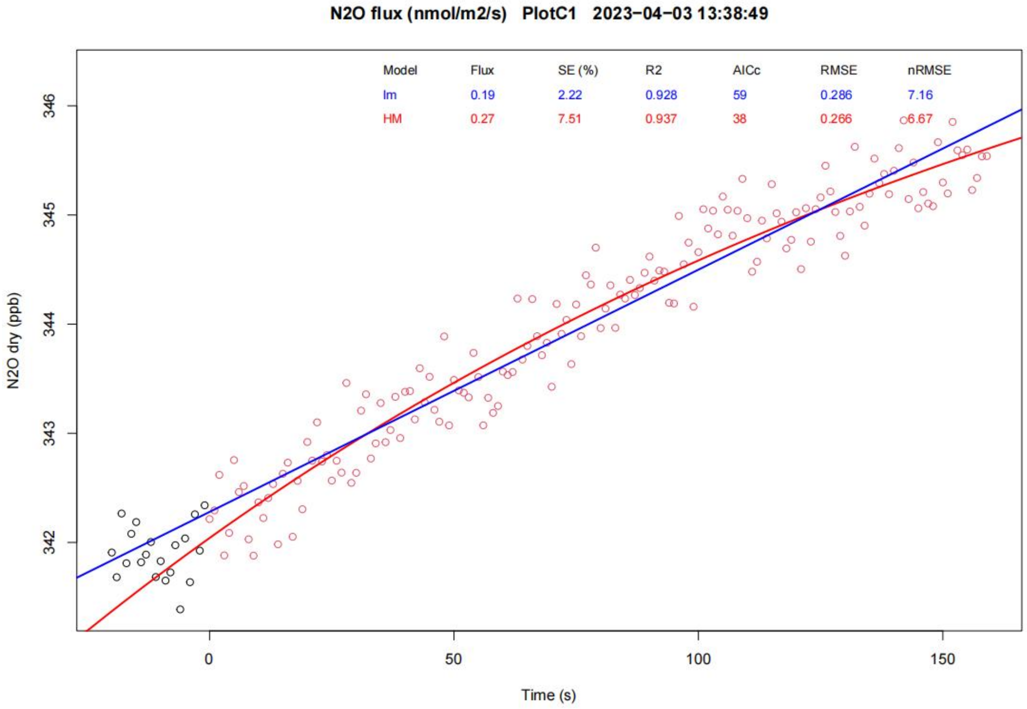

Figure 11 N_2O flux (nmol/m^2/s) PlotC1 2023−04−03

The hm model was chosen above because the background N_2O values have little effect on it and the hm model fit (R^2) is better than the lm model.

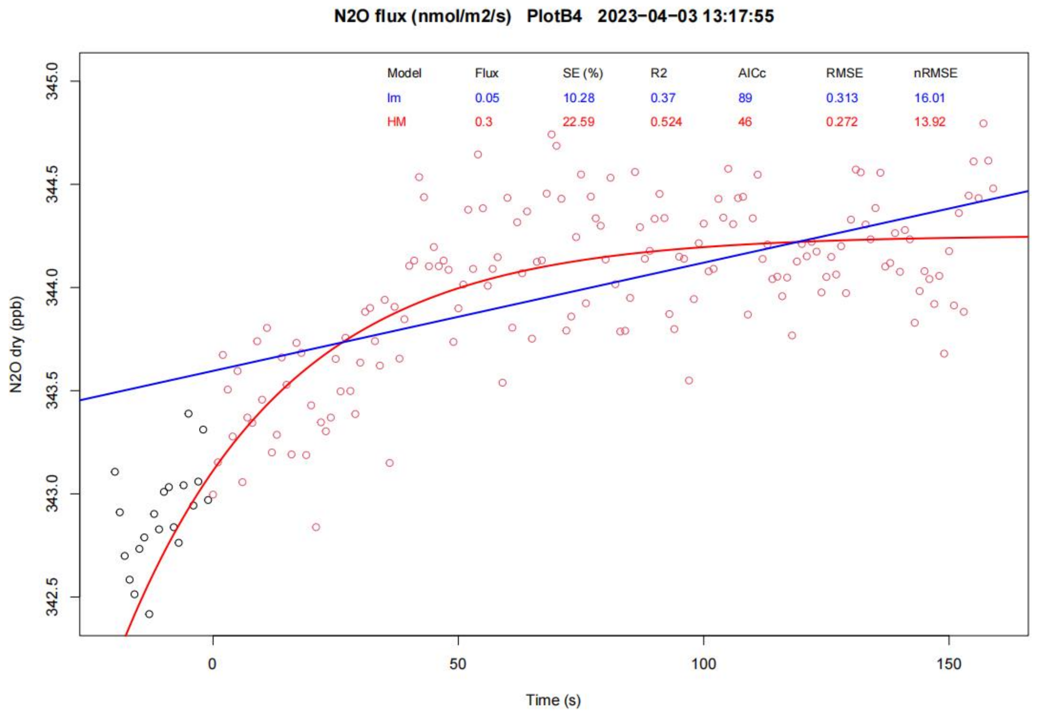

Figure 12 N_2O flux (nmol/m^2/s) PlotB4 2023−04−03

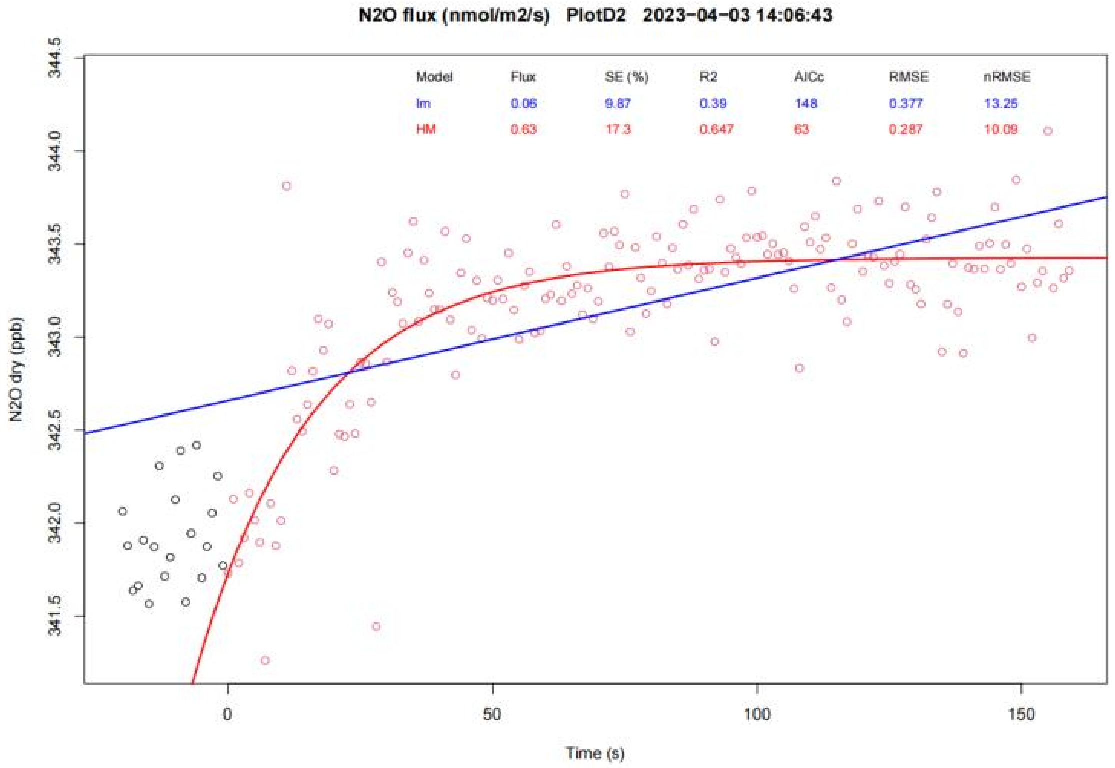

Figure 13 N_2O flux (nmol/m^2/s) PlotD2 2023−04−03

In the first graph, the lm model is more stable with respect to the background effects than the hm model. In the latter graph, the N_2O concentrations are variable as this is mainly background of measurement. This is considered to be a 0 flux since there is no concentration increment with time passing in this case.

3.4.3 NO_3^- concentration in subsoil

In Suserup Skov, twenty sampling plots are selected in unmanaged site and four sampling plots in managed site for soil sample collection. The soil samples are collected for analysis in the laboratory so that the wet and dry weights of the soil samples, including the containers, can be measured and the nitrate concentration in the solution replaced by the potassium chloride solution is recorded in ppm. To quantify nitrate in tested soil water, the nitrate concentration in 0.1 M KCl 20 ml extraction solution is converted to the nitrate concentration in the soil.

Soil water content is determined by subtracting the net wet weight from the net dry weight.

$$\theta(\%) = \frac{DW(g) - TW(g)}{WW(g) - TW(g)}$$

Where DW is dry weight with container, WW is wet weight with container and TW is tare weight. Nitrate concentration of soil water can be expressed as the formula below:

$$C_x(ppm) = \frac{(WW_{KCl}(g) \times \theta(\%) + 20(g)) \times C_{KCl}(ppm)}{WW_{KCl}(g) \times \theta(\%)}$$

Where C_x is concentration of Nitrate in measured soil water, WW_{KCl} is weight of tested soil and C_{kcl} is concentration of KCl extraction solution.

4. Results

4.1 Nitrogen variation

In the Suserup Skov, from 2020 to 2021, the precipitation, throughfall and seepage fluxes are 760 mm, 471 mm and 186 mm, respectively, where the seepage water flux is calculated by the chloride budget method.

4.1.1 Variation of nitrogen input and output trend

The graphs below show the nitrate concentrations in throughfall and seepage water measured at the measurement sites in 2020-2021. High nitrate concentrations in throughfall were observed in June 2020 and July 2021. For nitrate concentration of seepage water, some measurement points did not collect seepage water during the drying period, the nitrate concentration is 0 during this time. The different test plots are presented in different colors in the scatter plot, with the highest nitrate concentration measured in January at plot D.

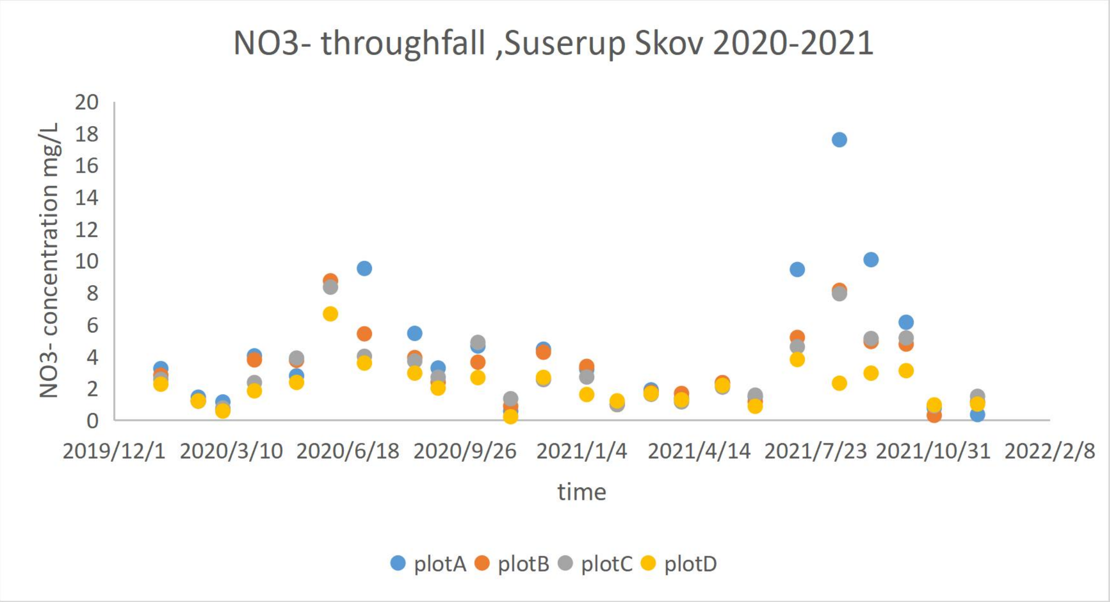

Figure 14 Variation of NO_3^- concentration of throughfall in Suserup Skov with time (2020-2021)

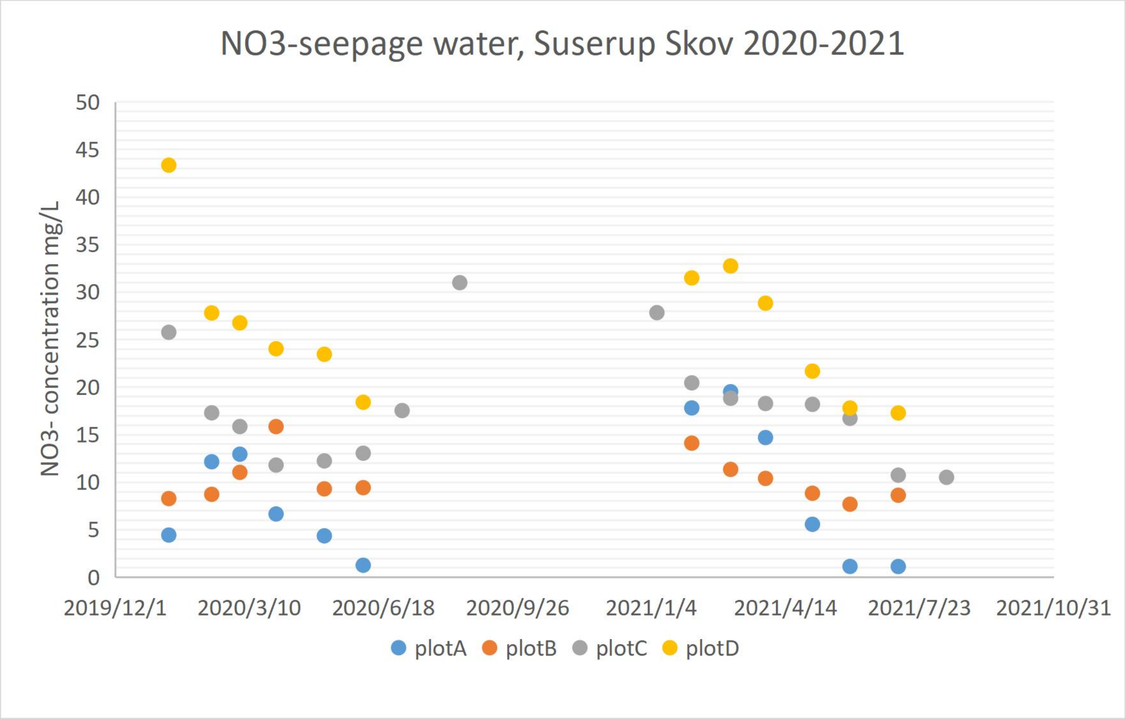

Figure 15 Variation of NO_3^- concentration of seepage water in Suserup Skov with time

We obtained the N-input and N-output in the unmanaged part of Suserup Skov for the period 2020-2021 by calculation (see 3.4.1). The next graph (figure 16) shows the trend of N-input and N-output during this period. The orange line shows the trend of nitrogen dioxide input, while the blue line stands for the trend of nitrate output.

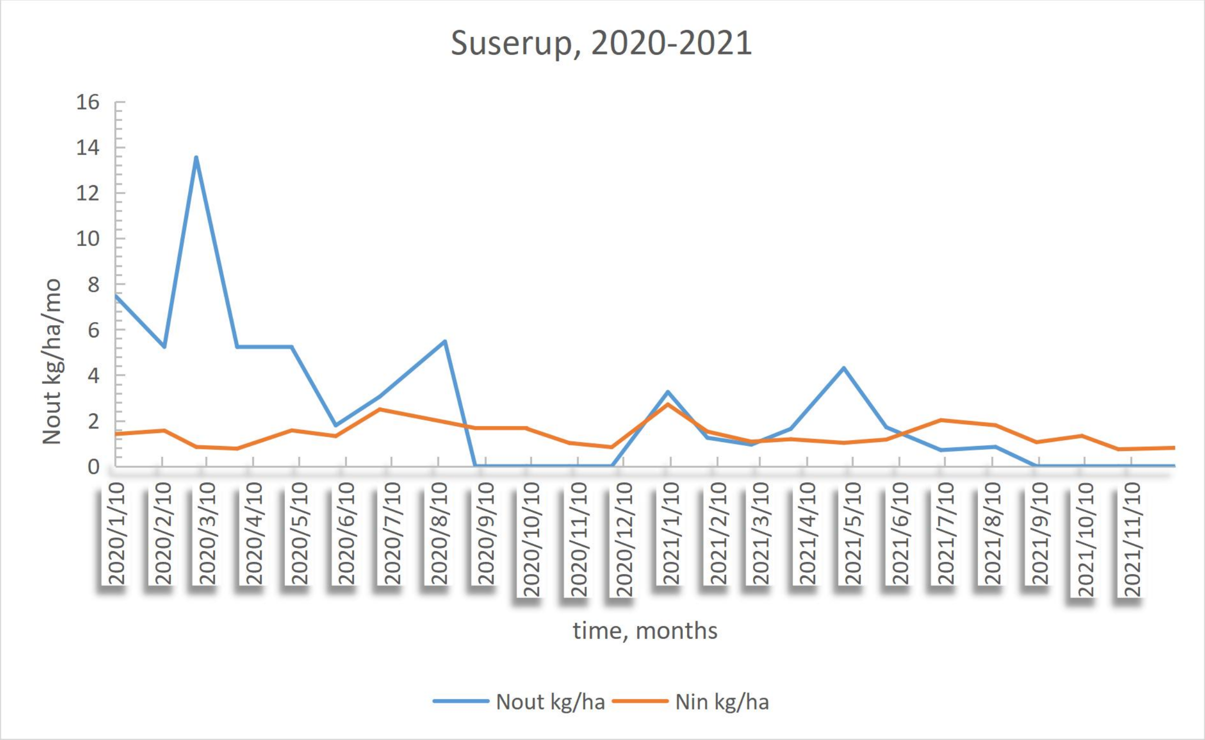

Figure 16 N-out and N-in change with months (2020-2021)

As can be seen from the graph, input of nitrogen has a relatively stable trend, with its highest value of 3.3 kg ha-1 mo-1 in January 2021 and its lowest value of 0.8 kg ha-1 mo-1 in March 2020. In contrast, N-output shows a significant fluctuating trend compared to N-input, peaking in March 2020 at 13.5 kg ha-1 mo-1. There is no N-leakage from September to December in both 2020 and 2021, which is attributed to the influence of drought. Overall, in the absence scenario of drought in the seepage water, N-output is greater than N-input, except for the period from July to August 2021.

The following chart illustrates the difference between nitrogen input and output in the soil of Suserup Skov during 2020 to 2021. It is evident from the chart that the difference in monthly nitrogen input and output is marginal statistically significant, with a P-value of 0.1. This suggests that the relationship between N-input and N-output is relatively strong. The figure shows that nitrogen output is higher than its input, with an average monthly input of 1.4 kg ha-1 mo-1 and an average monthly output of 2.6 kg ha-1 mo-1. Moreover, the difference between them is 1.2 kg ha-1 mo-1.

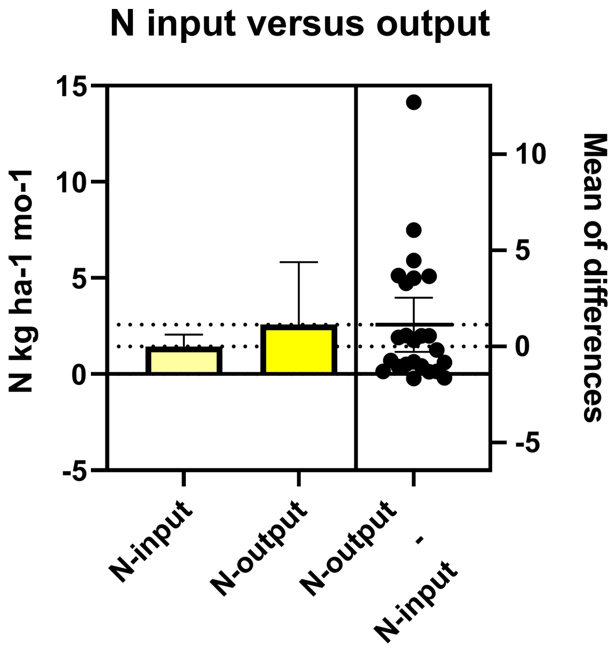

Figure 17 Differences in the input and output of nitrogen (2020-2021)

4.1.2 Data on a yearly and monthly basis

To better study nitrogen input and leaching pattern, monthly data and annual data are evaluated and studied.

The data on the amount of precipitation received on the ground and nitrate input in the area of Suserup Skov were recorded in the study. In general, the N-output reached its highest value in March at 7.3 kg ha-1 mo-1, while during this period the level of rainfall was not so high, with 80 mm. The drought conditions of the seepage result in zero data for nitrate leaching. Despite these period and July, nitrate leaching exceeds nitrate input values in other months.

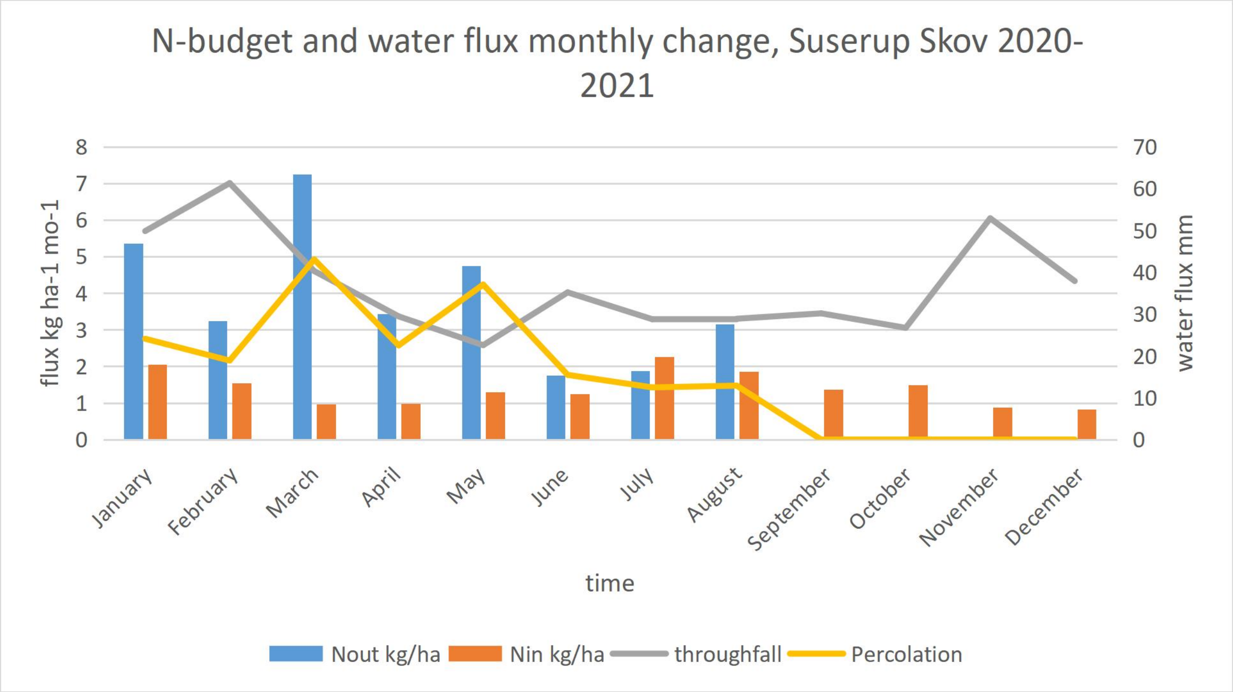

Figure 18 N-out, N-in seepage water flux and throughfall monthly change, Suserup Skov 2020-2021

Based on the following chart, it illustrates that the nitrogen output in 2020 was more than three times greater than the nitrate output in 2021, with a value of 47.0 kg ha-1 yr-1. The difference between the nitrate input and output is larger in 2020 than in 2021, about 29.2 kg ha-1 yr-1. In terms of N-input, the data for 2020 and 2021 are 17.0 kg ha-1 yr-1 and 16.4 kg ha-1 yr-1.

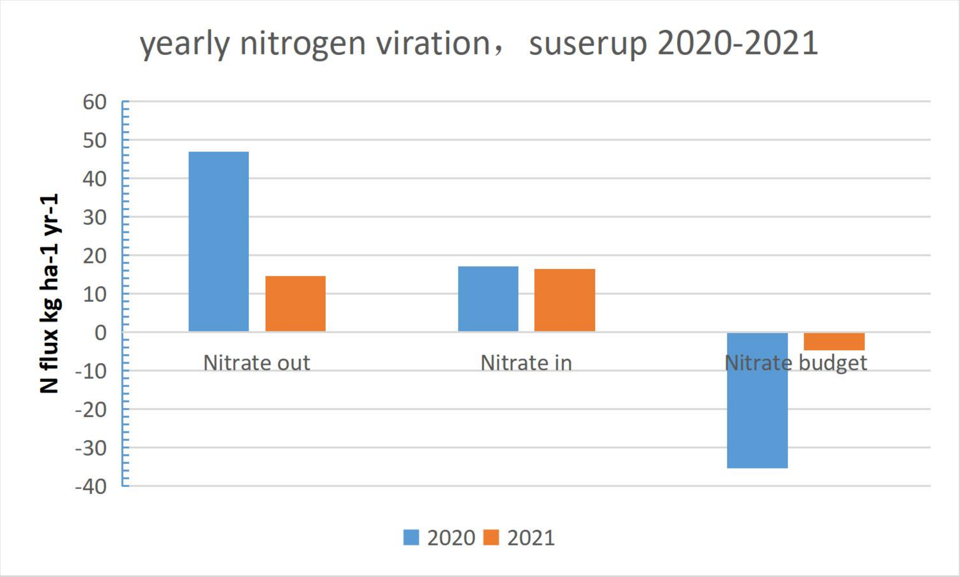

Figure 19 N budget of Suserup Skov in 2021 and 2022

4.2 Nitrate concentration measured by soil sample collection

This section analyzes and compares the concentrations of nitrate at different locations in Suserup Skov under different management plans.

4.2.1 nitrate concentration at unmanaged sites

In water quality testing above, the flux of NO_3^- is detected. Flux refers to the amount of NO_3^- that flows through a spatial area per unit time (Zhou W, Song L. 2005). Since the four plots with monitoring represents a small part of Suserup Skov, we did a cross forest survey about nitrate concentration of Suserup Skov. In this part, the concentration of NO_3^- of 16 plots is tested, which gives a better representation of the pollution distribution.

The concentration of nitrate in these 16 plots shown below unites with figure 20 provide nitrate pollution distribution of unmanaged part of Suserup Skov. Data quality is not infected, even though some sampling could not meet the 90 cm depth (personal communication, P. Gundersen). In order to check the stability of the experiment a replication (n= 4) was done. A standard deviation of 0.36 implies a good stability of the experiment (mean= 1.23). The mean concentration of nitrate was 10.0 mg/l at all surveyed sites (See Annex I).

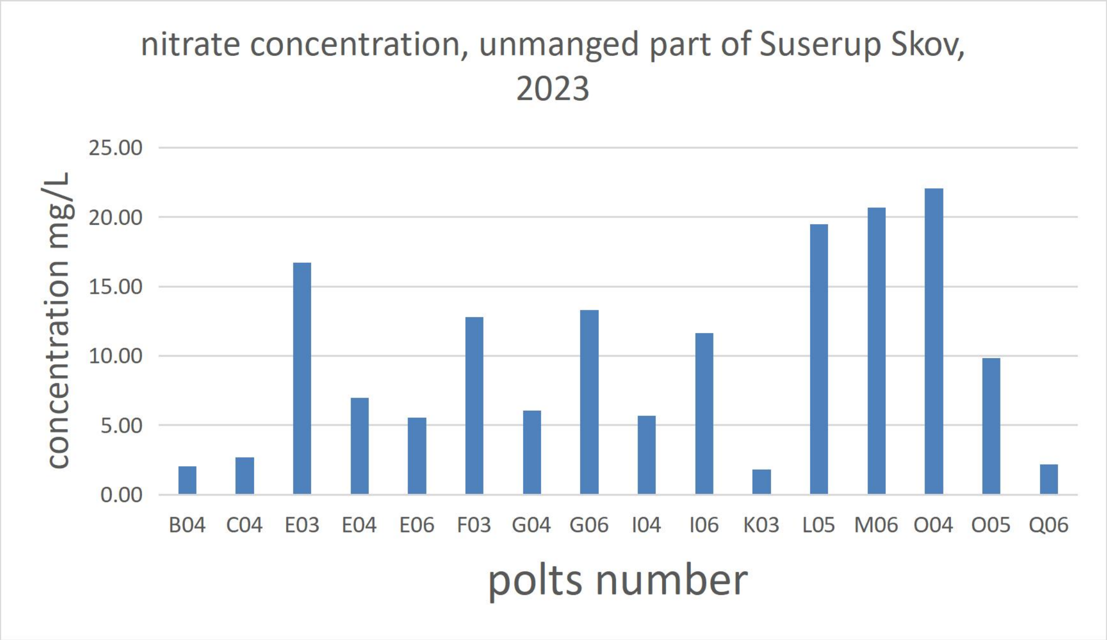

Figure 20 Nitrate concentration cross over Suserup Skov in March 2023

4.2.2 NO_3^- concentration under different management plans

To analyze further nitrate distribution pattern in old-growth forest, four sites are selected for comparison in each of the forests with similar natural conditions and different management practices.

The following graphs show the plots measured in Suserup Skov and Broby Vesterskov. The mean concentration of NO_3^- of unmanaged part is 20.3 mg/L. In contrast, the unmanaged part has a much lower average NO_3^- concentration of 4.8 mg/L. The highest NO_3^- concentration detected in the managed forest is 9.7 mg/L, while the concentration detected in the unmanaged forest is more than 5 times higher, which can reach up to 47.2 mg/L.

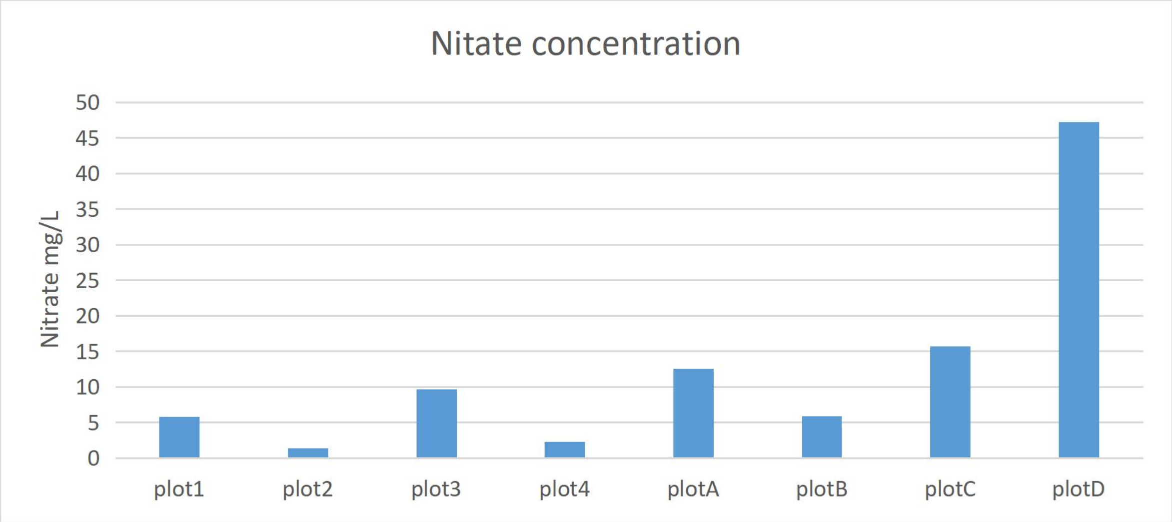

Figure 21 nitrate concentration of Suserup Skov and Broby Vesterskov in March 2023

4.3 N_2O flux

In this section, the presence or absence of N_2O release from the soil is investigated. By checking the N_2O flux emission from soil, nitrogen cycle in forest soil can be better studied.

4.3.1 N_2O emission change with month

N_2O releases from the soil are changing with time, with lowest in January and February at 0.013 µmol m^{-2} $s^{-1}$and 0.019 µmol m^{-2} s^{-1} respectively. In January, a negative N_2O flux is detected at plot A, which means that the soil absorbs N_2O from the air in cold weather. In April, the average N_2O flux reached 0.123 µmol m^{-2} s^{-1}, which is more than six times higher than in January. The p-value of 0.02 in the ANOVA test means that there is a significant difference in the amount of N_2O release in the different months. The average soil temperatures are 2.4, 2.5, 7.2 in December, January. and April, respectively. In April, the N_2O release from waterlogged lowland plot G6 reaches 0.498 µmol m^{-2} s^{-1}, which is the highest N_2O release value detected (Annex Ⅱ). Microbial activity is more vigorous under warm conditions and increased nitrification is coming with intensive microbial activity, which is closely associated with the higher release of N_2O during warmer months (M Ariani et al 2021).

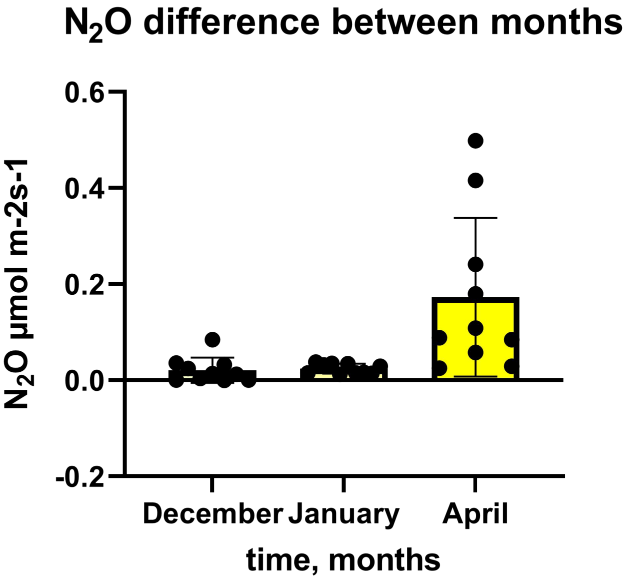

Figure 22 N_2O emissions change with months of Suserup Skov

4.3.2 N_2O emission change with manage plan and topography

The meaning of gradients in this experiment refers to the position of the test plots on the hillside. The positions of G1 to G6 are arranged in a descending order. In April, the location of G6 became a wetland (although no visual water table) due to the low terrain and abundant rain.

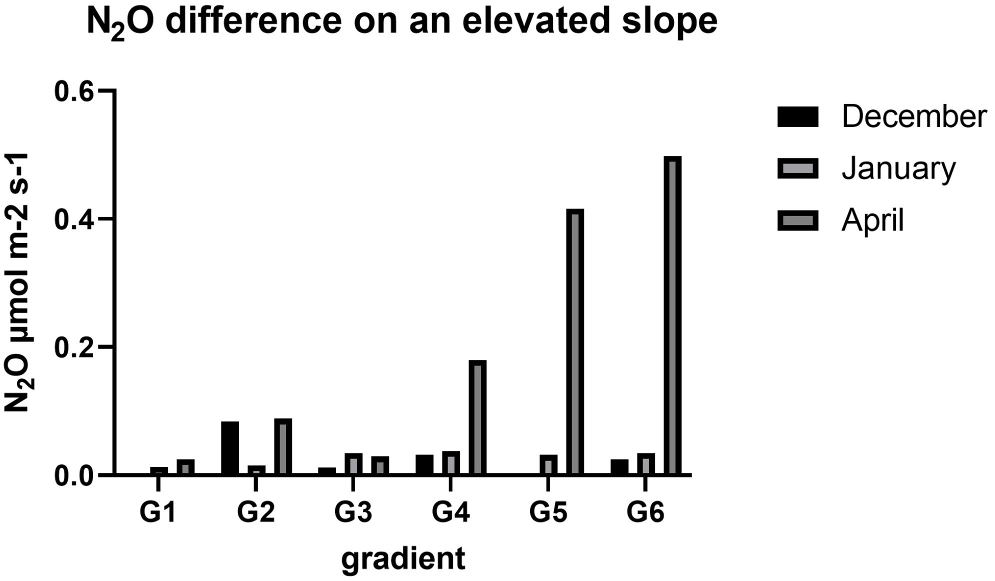

Figure 23 N_2O emissions differ in positions of slope and months

From the chart above, the release of N_2O from the soil increases with increasing gradient, i.e., the lower the terrain the greater the N_2O emissions, however the fluxes are restricted by low temperatures in Dec. and January. The N_2O flux reach its peak at G6 in April. From one-way ANOVA test, p-value shows that the difference over the slope is significant ($P=0.04<0.05$), which mean slope is the factor influence N_2O emission. The lower the terrain, the stronger the nitrification in the soil. The mean N_2O release for the three test months is positively correlated with slope (Annex III).

Like things did for soil sample, comparison of N_2O flux from Suserup Skov and Broby Vesterskov is done to better study N_2O emission soil nitrogen cycle and in forests under different management plans. Figure 24 shows the N_2O flux varies with different manage plans. Plot 1 – plot 4 are test plots from the managed forest, while plot A -plot D are from the unmanaged forest. In April, average N_2O concentrations differed between managed and unmanaged forests, 0.055 µmol m^{-2} s^{-1} and 0.123 µmol m^{-2} s^{-1} respectively.

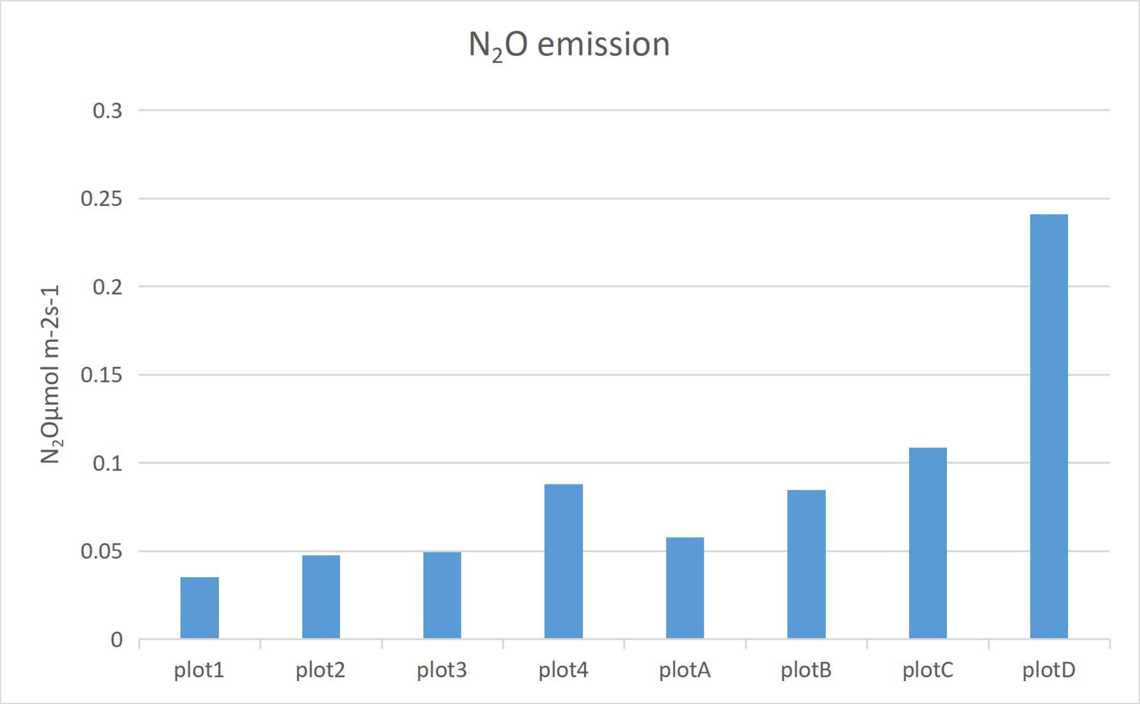

Figure 24 N_2O emissions in Broby Vesterskov and Suserup Skov

5. Discussion

5.1 N-input and N-output change in recent years

In Suserup Skov, the N-input is 17.1 kg $ha^{-1}yr^{-1}$ in 2020 and 16.4 kg $ha^{-1}yr^{-1}$ in 2021. These two data are lower compared to the low nitrogen input in the Suserup Skov from 2003 to 2019(14–62 kg $ha^{-1}yr^{-1}$) (Garbu, 2020). The mean N-input data for 2020 and 2021 are also lower than estimated N-input data for 2002-2005 conducted by Gundersen (2009). Although N-input for recent two years is not high compared to other forests (Gundersen 2009), it is still above the threshold for N leaching at 8-10 kg $ha^{-1}yr^{-1}$ add to explain Suserup Forest is still in a state of nitrogen leaching.

The amount of water penetrating the canopy may affect the amount of nitrogen input, with an average penetration of 598 mm between 2002 and 2005 (Gundersen, et al., 2009) while the average penetration of 444 mm is seen between 2020 and 2021 when nitrogen input is relatively low. N-input shows an increase trend with yearly throughfall water flux. Therefore, the low N input in the last two years may be due to drought in forest. At the level of monthly throughfall precipitation and nitrogen input, there is no significant relationship between them.

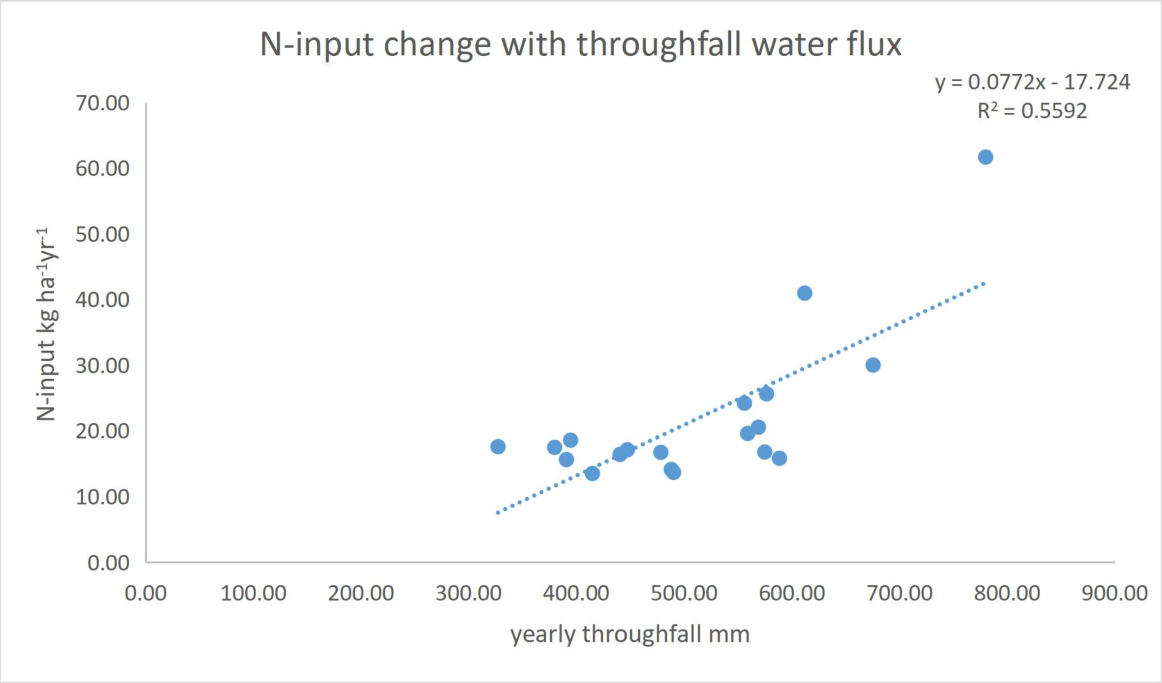

Figure 24 Annual relationship between nitrogen input and water flux (2003-2021)

For N-output data in Suserup Skov, nitrogen leaching is estimated to be 47 kg ha^{-1}yr^{-1} in 2020 and 15 kg ha-1yr-1 in 2021. The nitrogen leaching data for 2020 exceeds the threshold for forests where nitrogen leaching is typically present (25 kg ha-1 yr-1). This value is also higher than the average value of leaching in Suserup Skov from 2003-2019 (45 kg ha-1yr-1, Garbu,2020), but the average value of nitrogen leaching in 2021 and 2022 (31 kg ha-1yr-1) is lower than the average value of the previous 17 years of study.

Combining the yearly data for N-output and throughfall water flux data from 2003 to 2021 (Garbu), a linear regression relationship is obtained (figure 25). The fit between N-output and percolation flux was good, revealing a positive correlation (R^2=0.89). The mean N-output and seepage water flux in Suserup Skov for 2002-2005 are 48 kg ha-1yr-1 and 290 mm, respectively (Gundersen, et al., 2009). This data set (48 kg ha-1yr-1, 290mm) is well fitted in the linear regression curve. A weak positive correlation

(R^2=0.02 Annex Ⅳ) was observed in the relationship between monthly nitrogen output and throughfall water flux (2020-2021). The difference in seepage water flux and throughfall explains the difference in nitrogen leaching in 2020 and 2021.

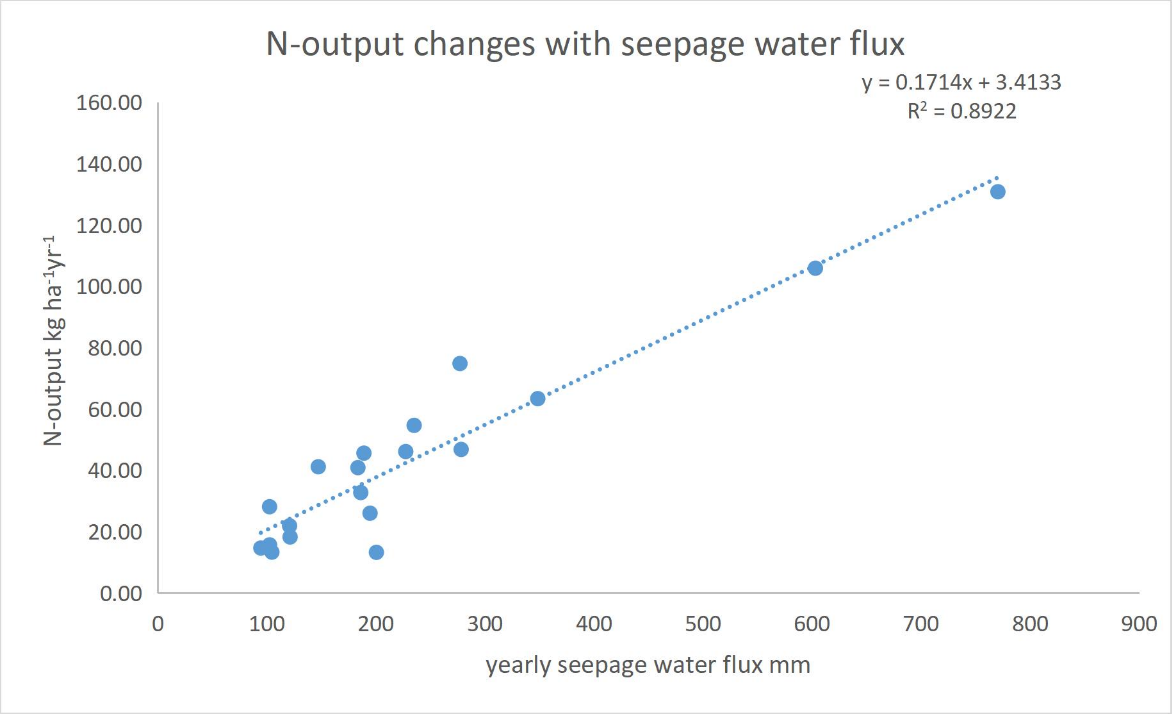

Figure 25 Yearly relationship of N-output and seepage water flux (2003-2021)

According to the definition of nitrogen saturation, this forest is in a state of nitrogen leaching when the amount of nitrate input to the soil is higher than the amount utilized. The C: N ratio of mineral soil in Suserup Skov is lower than 25, while the C: N ratio of the organic layer is higher than 25. Therefore, the C: N ratio of mineral soils is more representative of N leaching in the forest. It also verifies the conclusion reached by Gundersen in 2009. Nitrate leaching above 1 mg/L in seepage water is considered to be elevated.

As shown in the figure below, N-out and N-in do does not have much to do with each other at the monthly level (R^2= 0.02). The months of drought have an impact on the results of the analysis. The the relationship of N-in and N-out at the annual level also failed to show strong correlation (R^2=0.23) (Garbu, 2020). The relationship between nitrogen deposition and leaching on a monthly basis is strongly influenced by other factors, and on an annual basis nitrogen deposition and nitrogen leaching show some relationship but are strongly influenced by other factors.

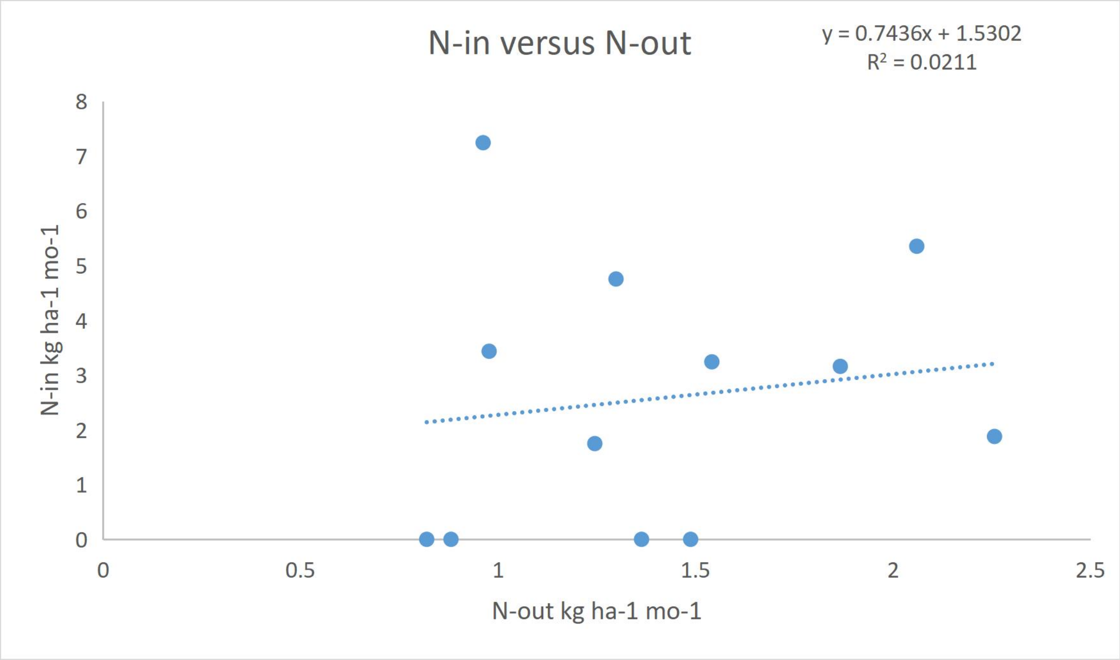

Figure 26 Monthly relationship of N-input and N-output (2020-2021)

The nitrate concentration of throughfall is lower than that of seepage water, which is due to the loss of water during leaching process. The seepage water test indicates that the average nitrate level measured in Suserup Skov from 2020-2021 is 11.4 mg/L. The difference between the two years is not significant, 11.0 mg/L and 11.9 mg/L. Previous studies have shown that the nitrate concentration from 2003-2017 is 14.7 mg/L. In contrast, the concentration of nitrate in soil water has decreased in the last two years. Nitrate leaching in Suserup Skov is considered to be elevated in the last 19 years, since the nitrate concentration is above the threshold of 1mg/L. The concentration of soil water obtained by collecting soil samples at a depth of 90 cm was 20.3 mg/L, which is higher than the nitrate concentration of seepage water obtained from the forest. This may be due to the difference in the year and time of measurement. Soil samples were collected during the unusual dry winter season when seepage water flux was zero. Soil water could not be collected by automatic sampling system during this dry season.

Therefore, these four plots' concentrations may not be representative of the overall level of nitrate concentrations in soil water in the Suserup Skov.

From the nitrate concentrations examined at 16 soil sample extraction points that represent the entire forest, it is found that the nitrate concentrations at the four points where soil water was sampled were higher than the average level of nitrate concentrations in the forest. Comparatively large concentration differences are observed at some test sites, which is because distribution and concentration of nitrate at different sites can be significantly affected by water transport in the soil.

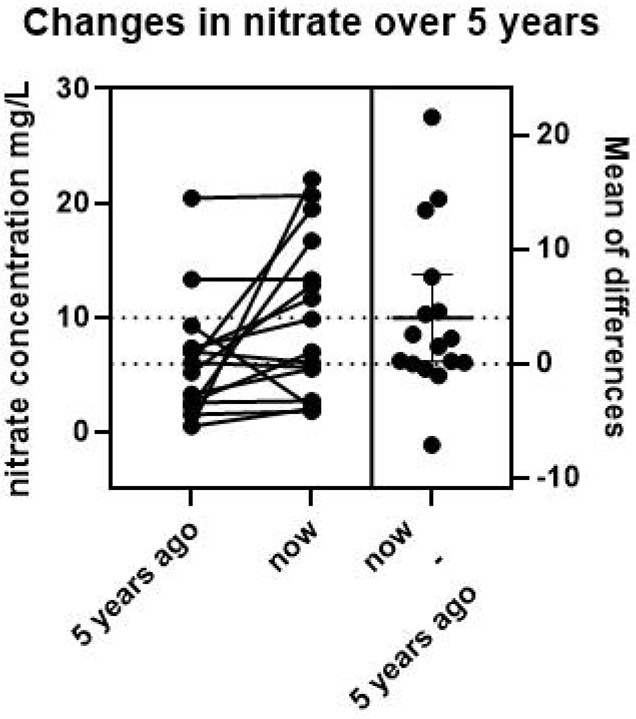

Figure 27 Nitrate concentration changes of Suserup Skov over 5 years

By comparing studies from five years ago, A p-value of 0.04 in paired t-test represents a significant change over 5 years in nitration concentration of 16 plots survey of Suserup (Munk-Nielsen, 2018). This means nitrate concentrations in the Suserup Skov are significantly higher than data from five years ago. In Figure 28, data points further from the 1:1 line indicate high deviations over five years. In addition to the variation of nitrate over time, the drought of the seepage water during this winter may also be responsible for the elevated nitrate concentration.

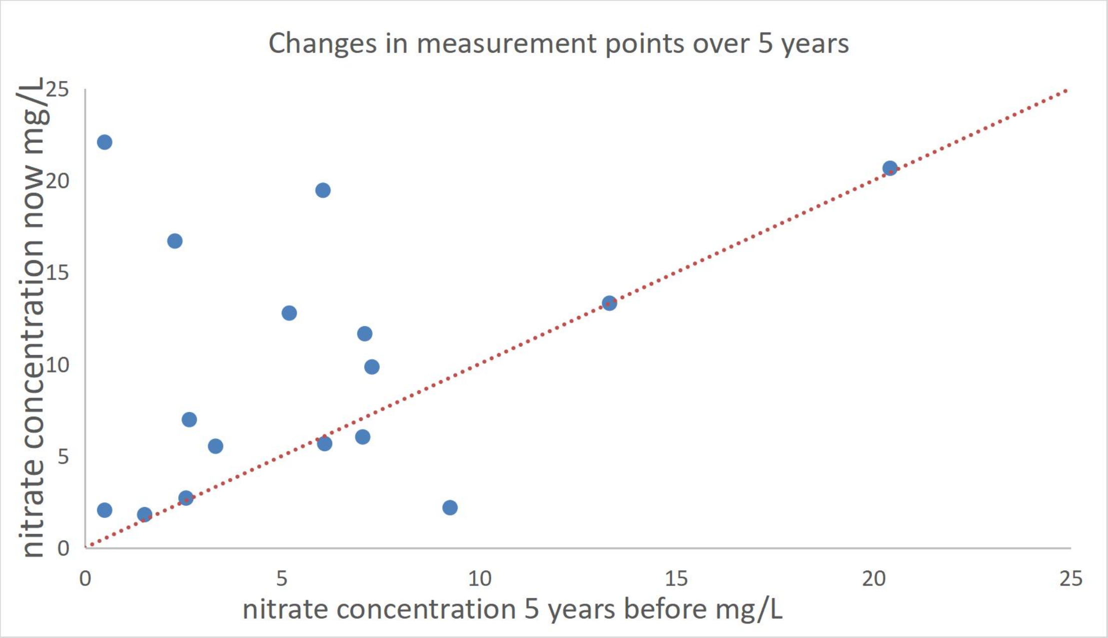

Figure 28 Relationships between nitrate concentration (2023) before and after 5 years (2018)

Overall, nitrogen leaching has continued to occur in Suserup Skov in recent years. The increase in nitrogen input is related to the increase in throughfall, while nitrogen leaching and seepage water flux show a stronger positive correlation. The nitrate concentration in soil water is higher than five years ago, showing an elevated status of nitrate.

5.2 N_2O emission

In Suserup Skov, the amount of N_2O released in the soil varied greatly depending on month in which the test is conducted (see 4.3.1). The relationship between mean temperature and mean N_2O emission for each measurement of Suserup Skov shows a strong fit between temperature and N_2O release ($R^2 = 0.99$, however with only n=3) (see Annex Ⅴ). N_2O release increases with increasing temperature. The study by Gundersen (Gundersen et al.,2012) also confirms that increasing temperature brings an increase in N_2O release. The increase in temperature leads to stronger microbial activity in the soil and subsequently makes stronger nitrification and denitrification processes. Since the release of N_2O is related to the activity of microbial in the soil, it is expected that there will be a threshold for the increase in temperature, above which the microbial activity diminishes.

The N_2O emission is not only related to the temperature but also connected to the N deposition and soil humidity. For the relationship between different month and N_2O variation in the fit is not very good and more data are needed (R^2=0.26 Annex Ⅵ). Moreover, the relationship between soil water content and N_2O emission is higher in the slope experiment (R^2 = 0.65 Annex Ⅵ). Therefore, this leads to the conclusion that the lower the terrain the higher the water content and the higher the N_2O release.

For the relationship between N_2O emission and N-output, R^2=0.98 indicates a high degree of fitting. The higher N-output, the higher N_2O emission.

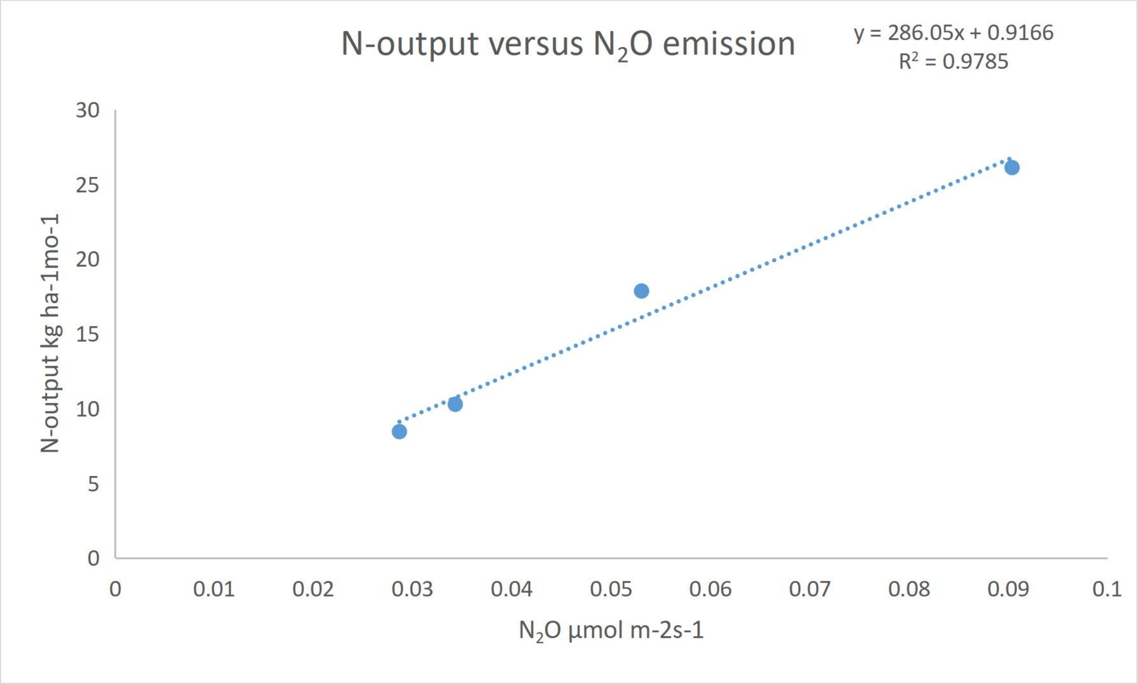

Figure 29 Relationship of N-output and N_2O emission of at the 4 monitoring plots in Suserup Skov

As expected in the results of Gundersen's study (2012), soil water content, temperature and N deposition caused an increase in N_2O release. Besides, N_2O release is related to the amount of nitrate leaching.

5.3 management plans affecting nitrate output and N_2O emission

Although there were differences in N_2O concentrations between management models, the differences were insignificant, with only 30.1 percent of the variation in the data being explained by differences in management models. (R^2=30.1, p=0.16) The graph still indicates a relationship between N_2O emission and manage plans, which unmanaged forest tend to produce more N_2O in soil, which deserves further study.

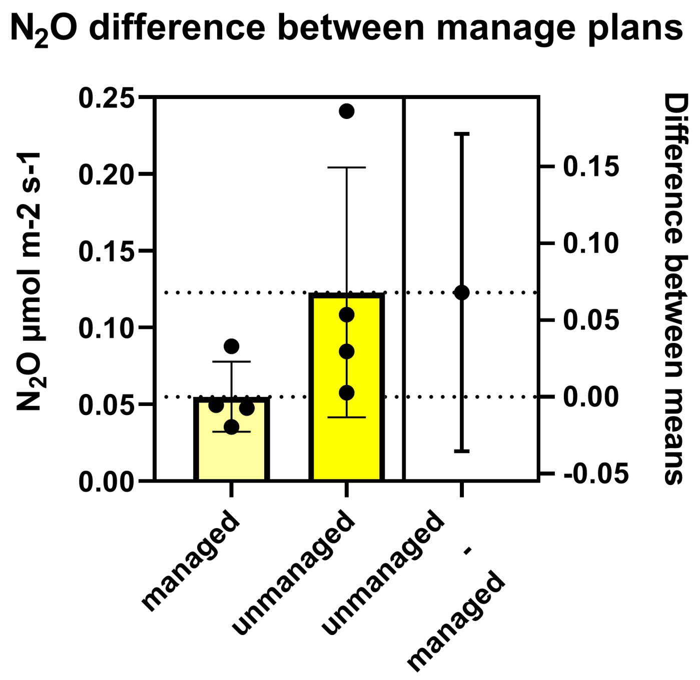

Figure 30 Difference of N_2O emissions with different management plans

The concentration of NO_3^- in the unmanaged forest was four times higher than in the managed forest. However, NO_3^- concentrations show there are no significant difference with different management plans (Unpaired t-test for difference in nitrate concentration between unmanaged and managed shows p=0.15). Though the test result shows the difference is not significant, but a fairly small p value could indicate that different management plans are affecting the emission of N_2O.

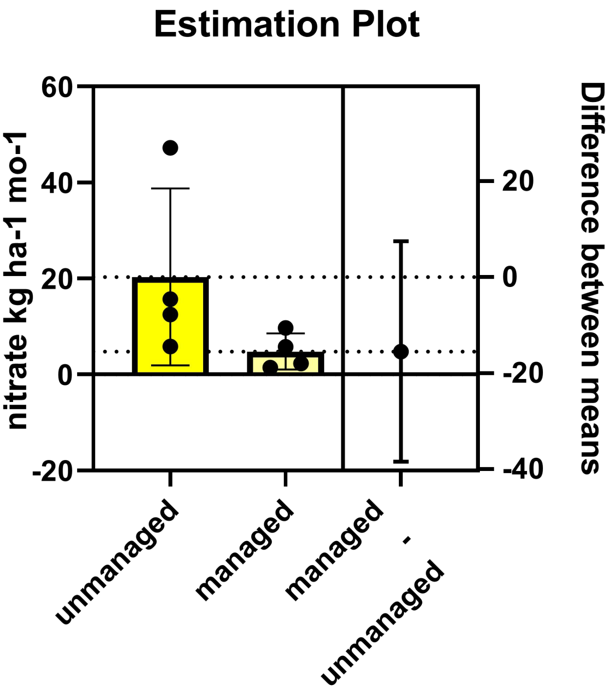

Figure 31 Difference of nitrate concentrations with different management plans

In managed forests, biomass is removed manually and regularly, which reduces the input of nitrogen sources. For Suserup Skov, the nitrogen input to the forest from the farm next to the 20 years of abandonment is becoming less and less. However, the nitrogen input from biomass is higher in Suserup Skov than in Broby Vesterskov because there is no means to remove biomass from the forest. Under similar temperature and soil moisture conditions, higher N deposition resulted in higher N_2O release and nitrate leaching.

To sum up, the soil nitrate concentration and N_2O emission in unmanaged forests are higher compared to those in managed forests. Different management plans affect N deposition and N_2O emission. Relation between nitrogen leaching and N_2O emission shows a positive correlation, i.e., nitrogen leaching increased with N_2O release.

5.4 limitations of study

a. The experiments were only carried out in December, January and April due to time constraints for N_2O release testing. The effect of temperature on N_2O needs to be further investigated as tests under warmer months are missing.

b. Another limitation of this study is relatively small sample plots for comparing different management plans. More sampling plots would enhance the robustness of our study.

c. The experiment did not study the N_2O emission from slope in managed forests. A slope test in an unmanaged forest can provide a better understanding of the effect of terrain on N_2O emissions. More experiments on relationship between terrain and N_2O emission would lead to a better understanding of the patterning of N_2O emission.

6. Conclusion

This thesis is a study of nitrogen leaching in old-growth forests in Denmark and contributes to forest environmental protection.

By comparing the nitrate leaching patterns with those from 2003 to 2019 in the Suserup Skov, we find that both nitrogen input (17 kg $ha^{-1}yr^{-1}$) and nitrogen leaching (31 kg $ha^{-1}yr^{-1}$) have decreased. These values still exceed the thresholds considered to be nitrogen leaching in forests. It is worth noting that nitrogen input is related to throughfall water flux, while nitrogen input is related to seepage water flux. In comparison to the nitrate concentrations in the soil samples five years ago, the nitrate concentrations measured in this experiment are higher than the overall concentrations five years ago and exceed the threshold for N-elevated. This means that the Suserup Skov is still in a nitrogen leaching status. A significant positive relationship is found between nitrate leaching and N_2O emission. This means that the release of N_2O is influenced by the leaching of nitrogen. N_2O emission is also influenced by terrain, which is partly due to water content. More research is needed to analyze how terrain affects N_2O emission. The difference in management plans has a considerable impact on nitrogen leaching. N_2O emission and nitrate leaching are less in managed forests, which means that managed forests can effectively address the problem of nitrogen leaching. This provides a reliable solution to the problem of nitrogen leaching from forests.

In the future, Studies with long-term and more place measurements of N_2O emission could be carried out to investigate the effect of seasonal variations on N_2O emission.

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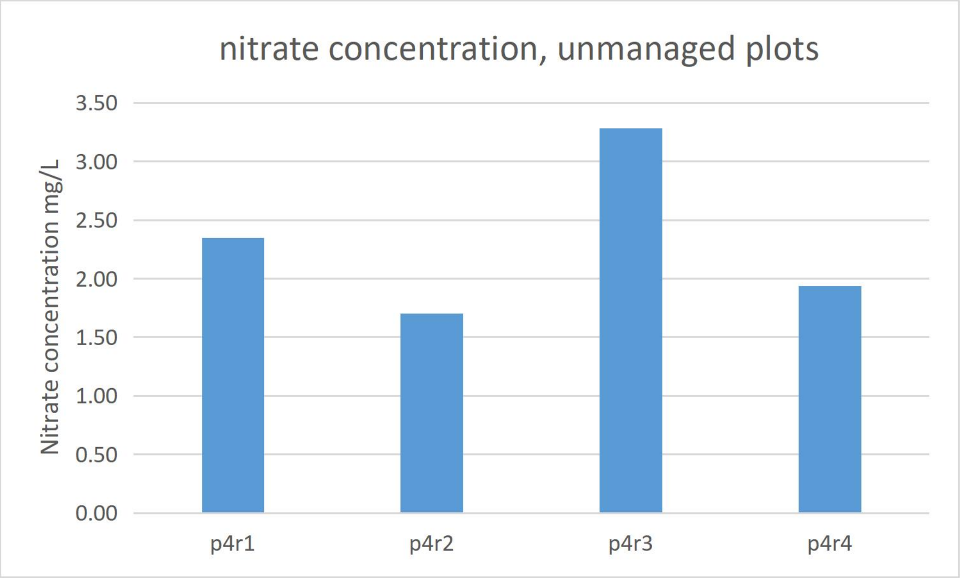
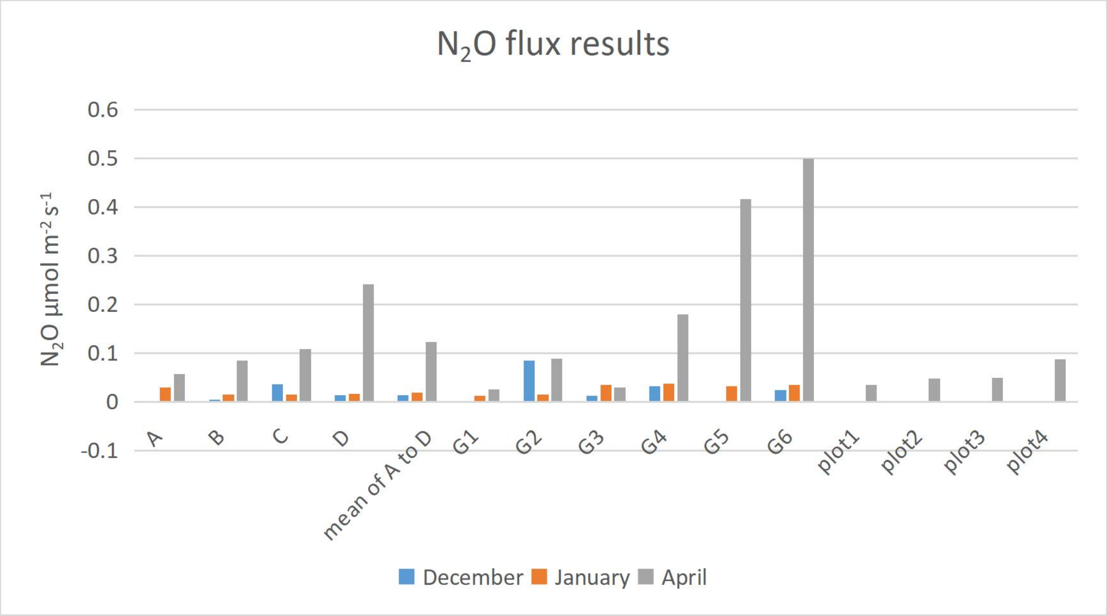
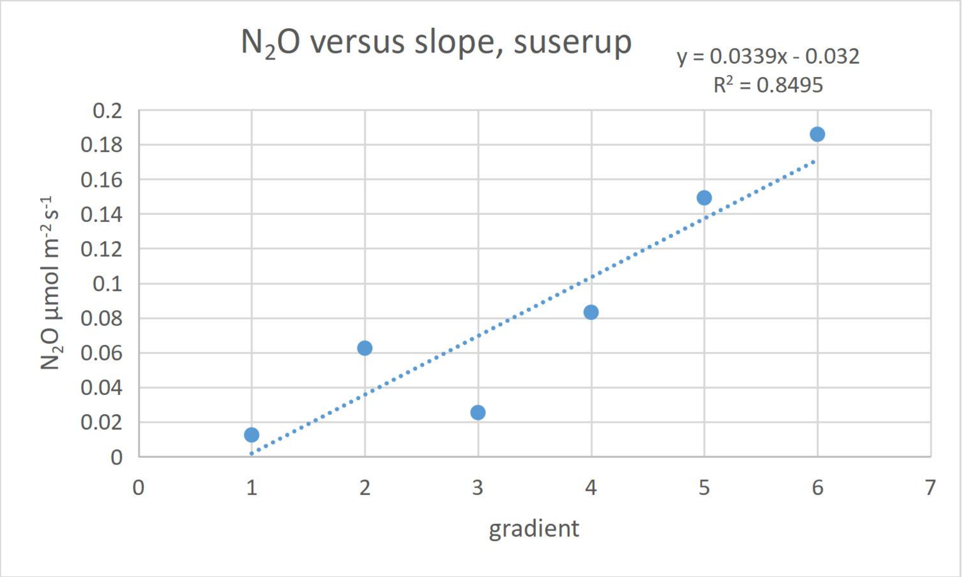
Annex Ⅰ



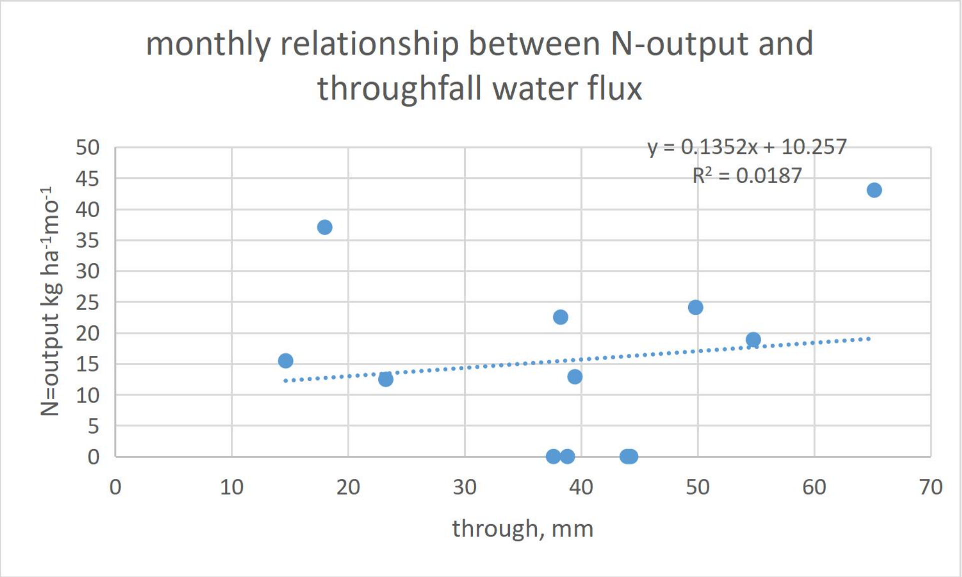
Annex Ⅱ



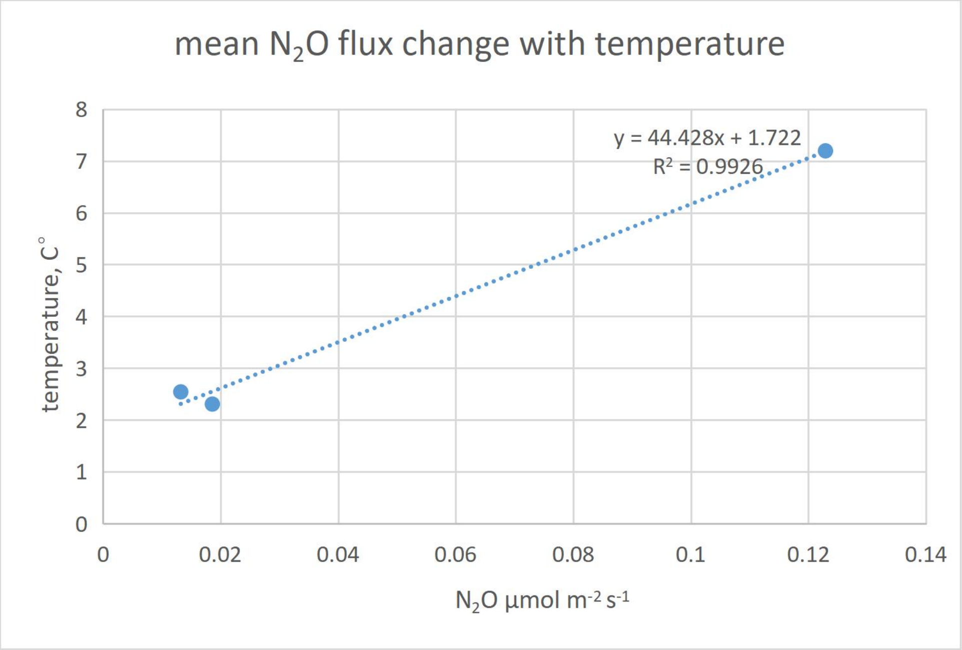
Annex Ⅲ



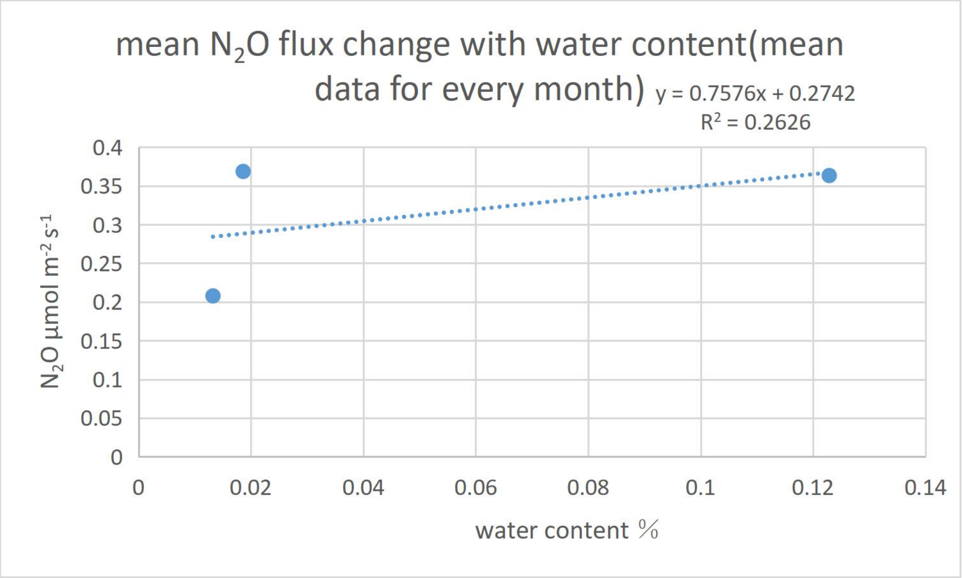
Annex Ⅳ

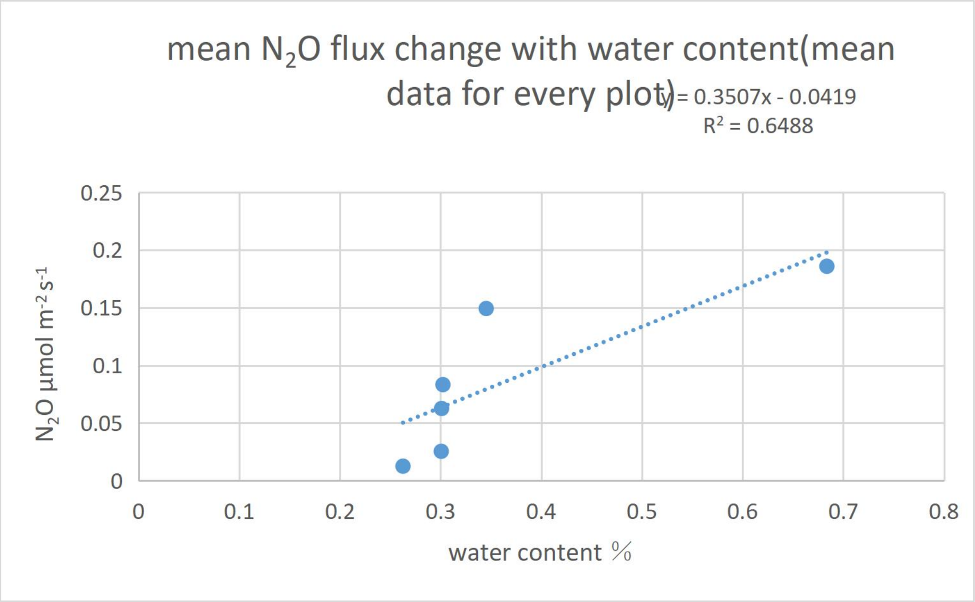


Annex Ⅴ



Annex Ⅵ




mean N₂O flux change with water content(mean data for every plot)
y = 0.3507x - 0.0419
R² = 0.6488
N₂O µmol m⁻² s⁻¹
0.25
0.2
0.15
0.1
0.05
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
water content ％