HELSINGIN YLIOPISTO
HELSINGFORS UNIVERSITET
UNIVERSITY OF HELSINKI

Nitrogen fixation by *Sphagnum* mosses in a boreal fen ecosystem

Emmi Tuokko

Master's Programme in Ecology and Evolutionary Biology

Faculty of Biological and Environmental Sciences

University of Helsinki

May 2022

Faculty: Faculty of Biological and Environmental Sciences

Degree programme: Master's Programme in Ecology and Evolutionary Biology

Study track: Ecology and Evolutionary Biology

Author: Emmi Tuokko

Title: Nitrogen fixation by *Sphagnum* mosses in a boreal fen ecosystem

Level: Master's thesis

Month and year: May 2022

Number of pages: 34 pages + 4 appendix pages

Keywords: nitrogen fixation, *Sphagnum*, boreal fen, acetylene reduction, diazotroph

Supervisor: Dr. Juha Mikola

Where deposited: HELDA – Digital Repository of the University of Helsinki

Abstract:

Ecosystems in the North are heavily constrained by nitrogen (N) and the main pathway of N for plants is biological N_2 fixation by *Sphagnum* mosses. Mosses fix N with either free-living, associated or symbiotic diazotrophs and convert it to a plant-accessible form. This way N_2 fixation contributes significantly to the level of photosynthesis and carbon sequestration that these ecosystems can maintain. However, diazotrophs are exposed to large fluctuations in abiotic factors and earlier findings have suggested that to affect the rate of N_2 fixed. Only a few studies have focused on boreal *Sphagnum*-dominated fens and thus, I wanted to figure out what environmental factors control N_2 fixation activity in this habitat type. Most of the N_2 fixing bacteria are heterotrophs but also methanotrophs have been shown to participate in N_2 fixation. Therefore, I tested if methane (CH_4) flux was connected to N_2 fixation activity. As N is a necessary nutrient for plants, I also wanted to see whether the variation in N_2 fixation can explain the variation in plant growth and productivity and on the other hand, if the productivity can explain the rate of N_2 fixation by providing more energy. To test these interactions, we established the acetylene reduction assay (ARA) on a fen in Northern Finland to measure, how much N_2 is fixed. We also recorded environmental factors (soil moisture, soil temperature, air temperature, relative humidity and radiation), measured CO_2 and CH_4 fluxes and calculated vascular plant coverage and *Sphagnum* growth from 20 study plots. I expected to find a correlation especially between soil moisture and N_2 fixation because earlier studies have shown it to control the fixation activity the most. I found out that leaf area index (LAI) of vascular plants was explained by N_2 fixation. This is a significant finding because it has not been proved before. It supports the earlier findings about the connection between N_2 fixation and vascular plant photosynthesis. I also discovered that radiation (PAR) and potential gross primary production (GPP1200) explained the variation in N_2 fixation in the first measurement. This shows the dependency of N_2 fixation on the energy that photosynthesis provides. Contrary to my predictions, none of the other factors explained N_2 fixation or were explained by N_2 fixation. Further studies about moss-associated N_2 fixation are needed especially in the light of future changes in climate and N deposition.

Tiedekunta: Bio- ja ympäristötieteellinen tiedekunta

Koulutusohjelma: Ekologian ja evoluutiobiologian maisteriohjelma

Opintosuunta: Ekologia ja evoluutiobiologia

Tekijä: Emmi Tuokko

Työn nimi: Rahkasammalten typensidonta boreaalisessa suoekosysteemissä

Työn laji: Maisterin tutkielma

Kuukausi ja vuosi: 5/2022

Sivumäärä: 34 sivua + 4 sivua liitteitä

Avainsanat: typensidonta, rahkasammal, boreaalinen, suo, ARA, diatsotrofi

Ohjaaja: Dr. Juha Mikola

Säilytyspaikka: HELDA - Helsingin yliopiston digitaalinen arkisto

Tiivistelmä:

Pohjoisen ekosysteemit ovat erittäin typpirajoitteisia, ja kasvit saavat suurimman osan typestä rahkasammalten biologisen typensidonnan kautta. Sammalet sitovat typpeä joko vapaana elävien tai symbionttisten diatsotrofien välityksellä ja muuttavat typen kasvien käytettävissä olevaan muotoon. Tätä kautta typensidonta vaikuttaa merkittävästi koko ekosysteemin fotosynteesin ja hiilensidonnan tasoon. Diatsotrofit ovat kuitenkin alttiita abioottisten tekijöiden suurelle vaihtelulle, ja aikaisemmat tutkimukset ovat todenneet sen vaikuttavan typensidonnan tasoon. Vain harvat näistä tutkimuksista ovat keskittyneet boreaalisiin minerotrofisiin soihin, joilla rahkasammalet dominoivat, joten halusin selvittää, mitkä ympäristötekijät kontrolloivat typensidonnan aktiivisuutta tällaisessa elinympäristössä. Suurin osa typpeä sitovista bakteereista on heterotrofeja, mutta myös metanotrofien on osoitettu osallistuvan typensidontaan. Sen vuoksi halusin tutkia, onko metaanivuo yhteydessä typensidonnan tasoon. Typen ollessa välttämätön ravinne kasveille halusin myös nähdä, voiko vaihtelu typensidonnan tasossa selittää kasvien kasvua ja tuottavuutta, ja toisaalta, voiko tuottavuus selittää typensidonnan vaihtelua tarjoamalla sille lisää energiaa. Testataksemme näitä yhteyksiä mittasimme typensidontaa asetyleenin pelkistysmenetelmällä Halssiaavalla Sodankylässä. Mittasimme myös ympäristötekijöitä (maan kosteus, maan lämpötila, ilman lämpötila, suhteellinen kosteus, säteily), hiilidioksidi- ja metaanivuota, putkilokasvien peittävyyttä ja rahkasammalten kasvua 20 tutkimusruudulta. Odotin löytäväni korrelaation erityisesti typensidonnan ja maan kosteuden väliltä, sillä aikaisemmat tutkimukset ovat todenneet kosteuden olevan merkittävin typensidontaa säätelevä tekijä. Sain selville, että typensidonta selitti ruutujen välistä vaihtelua putkilokasvien lehtipinta-alassa. Tämä on tärkeä löydös, sillä yhteyttä ei olla todistettu aiemmin. Tulos tukee aiempia löydöksiä typensidonnan ja putkilokasvien fotosynteesin yhteydestä toisiinsa. Ensimmäisten mittausten tulokset osoittivat, että säteily ja potentiaalinen bruttoperustuotanto selittivät typensidontaa. Tämä todistaa typensidonnan riippuvuuden fotosynteesin tarjoamaan energiaan. Vastoin odotuksiani muut tekijät eivät selittäneet typensidontaa, eikä typensidonta selittänyt niitä. Lisätutkimukset sammalten typensidonnasta ovat tarpeen erityisesti ilmastonmuutoksen ja lisääntyvän typpilaskeuman valossa.

Table of Contents

1. Introduction

Nitrogen (N) is an essential nutrient for plant growth (Kraiser et al., 2011). However, most ecosystems in the North are heavily constrained by N (Näsholm et al., 1998). This is a consequence of several factors. The level of atmospheric N deposition is very low due to isolated location far away from industrial activities (Van Cleve and Alexander, 1981; Aerts et al., 1992). Cold temperatures restrict biological activity and lead to slower decomposition which favors the development of N limitation (Vitousek & Howarth, 1991). Also, there are only few N fixing vascular plants in higher latitudes, for example *Alnus* spp. and *Dryas* spp., but they are unable to meet the N demand of the whole ecosystem (Lawrence et al., 1967). Therefore, biological N_2 fixation by mosses plays an extremely important role in these ecosystems (Van Cleve & Alexander, 1981; Sorensen & Michelsen, 2011; Leppänen et al., 2015). Many northern ecosystems are characterized by abundance of cryptogams and especially mosses. Besides affecting N availability, they are important components of plant communities in influencing carbon (C) and water cycling (Turetsky et al., 2012). In boreal and arctic peatlands, *Sphagnum* mosses are responsible for most of the plant biomass and peat (Malmer et al., 2003). This dominance is due to special characteristics of *Sphagnum*: They modify the environment unfavorable for other plants and microbes through acidification, waterlogging and the production of recalcitrant organic compounds and cells that are harder to decompose (Verhoeven & Liefveld, 1997).

Sphagnum mosses can fix N_2 from atmosphere with diazotrophic bacteria (Fig. 1) that can be either free-living, associated or symbiotic to mosses (Cleveland, 1999). They convert the atmospheric N_2 into a plant accessible form (Rousk et al., 2016). In peatlands, most of these bacteria are shown to belong to the Alphaproteobacterial class and smaller proportion to the Cyanobacterial phylum, whereas in forests Cyanobacteria account for most of the N_2 fixation (Bragina et al., 2013; Leppänen et al., 2015). This ratio may also vary between peatlands (Carrell et al., 2019). Most of the bacteria are heterotrophic but in younger peatland stages, like fens, and in wet depressions methanotrophs can account for almost half of the N_2 fixed. In general, methanotrophic activity enhances N_2 fixation (Larmola et al., 2014). Diazotrophs may explain the dominance of *Sphagnum* mosses in many peatlands because they provide N to the poor and acidic ecosystem where nutrient recycling and decomposition are otherwise slow because of low temperatures (Larmola et al., 2014; Rousk et al., 2016). They are adapted to the challenging habitat that mosses form, but associations

between mosses and bacteria are mostly loose and this makes bacteria exposed to large fluctuations in abiotic factors (Opelt et al. 2007; Rousk & Michelsen 2017).

Although there are already numerous studies about *Sphagnum* N_2 fixation in northern ecosystems, there is still some level of uncertainty about the factors that have an influence on it (van den Elzen et al., 2020). Therefore, I wanted to study which environmental factors affect the rate of N_2 fixation and if the rate of N_2 fixation further influences productivity, plant growth and methane (CH_4) flux. I was also interested to see if potential primary production and CH_4 flux can explain N_2 fixation activity. In collaboration with another master's student, I measured N_2 fixation, greenhouse gas fluxes, vegetation properties and environmental factors (air and soil temperature, soil moisture, air humidity and short-wave radiation) in 20 study plots placed on a boreal fen in Sodankylä, Northern Finland to answer these questions.

I predicted that soil moisture would be the most important factor to explain the rate of moss-associated N_2 fixation as this has been shown in many previous studies, such as Larmola et al. (2014), Stewart et al. (2014), Rousk & Michelsen (2017) and Rousk (2018). The studies seem to be unanimous about the importance of moisture. The effect of temperature on N_2 fixation instead, is not equally clear. The temperature optimum for nitrogenase is approximately 26 °C (Houlton et al., 2008) and thus, the warming should have a positive impact on the fixation in northern ecosystems, where mean annual temperatures are low. However, the findings from the field seem to be conflicting: for example, Lett & Michelsen (2014) and Rousk & Michelsen (2017) concluded that temperature can promote N_2 fixation whereas Sorensen & Michelsen (2011) did not find any effect in their warming experiment. These previous studies have focused on air temperature but I was also interested to see the influence of soil temperature because I found out that these two temperature values did not correlate in many situations. I predicted that also higher soil temperatures would have a positive impact to N_2 fixation activity according to the optimum temperature of nitrogenase. Following Larmola et al. (2014) and van den Elzen et al. (2017), who discovered that light enhances N_2 fixation, I expected that light would have a positive impact on N_2 fixation at least until a threshold is reached. For the impact of relative humidity inside the chamber I did not have a hypothesis because I did not find any studies about that.

N_2 fixation by moss-associated diazotrophs can provide N to the moss itself but also to vascular plants enhancing their growth and leading to higher rates of photosynthesis (Cleveland, 1999; Berg et al., 2013). The more there is N available for vascular plants, the more they can grow. On the other hand, N_2 fixation needs lots of energy that photosynthesis can provide. Thus, I predicted that there might be a correlation between N_2 fixation and *Sphagnum* growth as well as the leaf area of

vascular plants. For the same reason, I hypothesized that there should be a connection between N_2 fixation activity and the level of gross primary production. Previous studies have also found a connection between N_2 fixation and methane production. Larmola et al. (2014) demonstrated that methane-induced N_2 fixation can explain more than one third of the new N input especially in the wet fen depressions. However, this might not show clearly in my results because the observed methane induction might be at least partly indirect (Larmola et al. 2014). Due to all these potential factors affecting the rate of N_2 fixation, I wanted to test their importance on a northern fen ecosystem and thus expand the general knowledge about N_2 fixation.

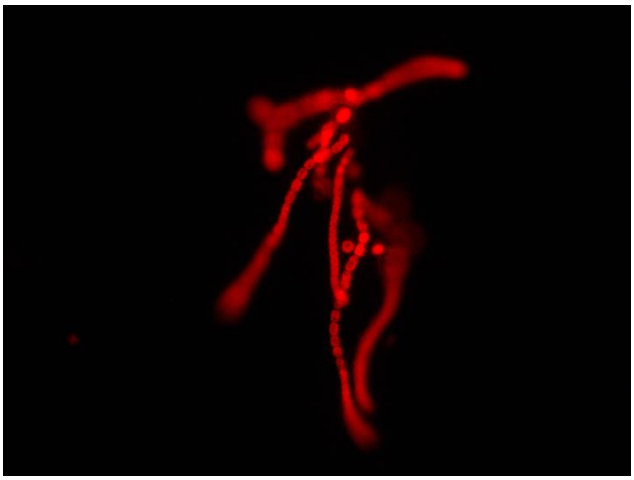


Figure 1. *Sphagnum*-associated cyanobacteria from our moss sample under an UV-fluorescence microscope.

2. Materials and methods

2.1. Study area & setup

The studied area was a boreal fen called Halssiaapa (67°22′N, 26°39′E, 180 m.a.s.l.), located near the Arctic Space Center in Sodankylä in Finnish Lapland. The mean annual air temperature in the area is -0,4 C (1981-2010) and the mean annual precipitation is 527 mm (1981-2010) (Pirinen et al., 2012). In 2021, the thermal growing season lasted approximately from 18.5. to 20.9. (The Finnish Meteorological Institute). The fen consists of wet flarks (high water table with patches of open water and peat) dominated by sedges, lawns (intermediate water table) with graminoids, shrubs and

Sphagnum mosses dominating vegetation and hummock strings (low water table), where also birch trees (*Betula pubescens*) and shrubs like *Betula nana*, *Andromeda polifolia* and *Vaccinium oxycoccos* thrive. The depth of the peat layer varies from < 0.5 m to 3.5 m (Mikola et al. 2022) and there is no continuous permafrost in the soil. Therefore, it is representative of the peatlands in the area.

The setup consisted of 20 study plots, placed on lawn surfaces between strings and flarks on spots, which had a continuous surface of *Sphagnum* mosses and *B. nana* representing the dwarf shrubs (Fig. 2a). Aluminum collars (59×59cm) were installed on the plots in late summer 2020. Vegetation properties, environmental conditions and gas fluxes were tracked throughout the growing season 2021 on the plots. N_2 fixation was measured using an acetylene reduction assay -method (ARA) according to Stewart et al. (1967). I carried out all field work in collaboration with master's student Milja Männikkö, with assistance given by Juha Mikola, Mika Aurela and Tarmo Virtanen.

a)  b)  c) 

Figure 2. a) Overview of the study area with a gas measurement in the forefront. b) Water addition during ARA and c) ARA setup.

2.2. Acetylene reduction assay in the field

Acetylene reduction assay (ARA) and fixation of $^{15}N_2$ are the most common methods used for measuring N_2 fixation. We used the ARA-method in the field plots because it is cheaper and simpler to perform than the ^{15}N -method, but still very sensitive and therefore a generally used method to measure N_2 fixation in the field (Hardy et al. 1973). ARA is based on the fact that

Sphagnum mosses reduce acetylene (C_2H_2) to ethylene (C_2H_4) in a same ratio than they fix N_2 from the atmosphere. The applied acetylene concentrations are so high that they are not significantly decreased by microbial reduction and can thus also be used to tell whether the system is leaking or not. With a correction factor the absolute amount of fixed N_2 can be calculated (Dilworth 1966). No other N_2-fixing plants were present in our study plots so we assumed *Sphagnum* to be responsible of the N_2 fixation measured.

ARA measurements were carried out twice during the summer for each plot: one in July (in the middle of the growing season) and one in August (later stage of the growing season). We used a transparent chamber with an attached fan, placed on the existing aluminum collars. The collars were filled with water to make the system airtight. To produce acetylene gas in the chamber, we embedded two plastic containers with 20 g calcium carbide (CaC_2) to the moss inside the collar, and after closing the chamber, added 36 ml of water to each of the containers through the chamber wall using a syringe and a needle (Fig. 1b & c). Two minutes later we took the first air sample from the chamber using a smaller syringe and a needle and injected the 7-8 ml air sample into a 5-ml airtight Exetainer® vials. The needle holes in the chamber wall were blocked with a piece of tape. The second sample we took after 120 minutes of incubation repeating the same steps.

To convert the ethylene production to the actual N_2 fixation rate I later calculated a conversion factor using *Sphagnum* samples brought to a laboratory. For the laboratory analysis, I collected three shoots of moss from four points of each plot so that they represented the *Sphagnum* species composition of the plot.

2.3. Laboratory work

I did all the laboratory work in the K. Rousk laboratory at the University of Copenhagen, where I was advised by Dr. Kathrin Rousk and postgraduate student Yinliu Wang. I transferred the air and moss samples to Copenhagen making sure that they were kept in cool all the time.

The air samples from ARA were analyzed for acetylene and ethylene concentration using a gas chromatograph (SRI 310C, FID, SRI Instruments, Torrance, CA, USA). Before the analysis, I transferred 5 ml of the samples to bigger vials (20 ml) and prepared standard vials with different ethylene concentrations (2%, 10%, 20%, 50%). To link moss acetylene reduction and N_2 fixation, 5-6 shoots of every moss sample were incubated for 20 h in glass vials (20 ml) where 10% of the air was replaced with acetylene. I also prepared 3 control vials without mosses and acetylene and 3

control vials without mosses but with acetylene. All samples were kept in a growth chamber with 300 μmol m^{-2} s^{-1} photosynthetic active radiation, 24-h daylight and air temperature of 10 °C. After incubation the samples were run with the gas chromatograph. In addition, we ran six empty standard vials with different ethylene concentrations (1%, 10%, 20%, 50%) and no ethylene (blanc 1 & 2). The same moss samples were then used for ^{15}N fixation measurements: 3 ml of air inside the vials was replaced with ^{15}N-N_2 gas and the vials were incubated for 24 h.

To measure moss dry mass and total N and ^{15}N concentrations, oven-dried paper bags were labelled, weighed empty and then with the incubated *Sphagnum* samples. Five random plots were also chosen to test the natural abundance of ^{15}N and mosses from them were weighed as well. All samples were dried three days in the oven in 70 °C, weighed with paper bags and then cut to a very fine powder with scissors. Of the moss powder, 4-6 mg was transferred to tin cups which were squeezed as small as possible and weighed again. ^{15}N enrichment of the samples was then measured using an Eurovector elemental analyzer (Eurovector, Milan, Italy) coupled to an Isoprime isotope ratio mass spectrometer (Isoprime Ltd., Cheadle Hulme, UK).

2.4. N_2 fixation calculations

Acetylene concentration should not change during ARA, but our results varied a lot suggesting that some chambers leaked during the assays. Therefore, I divided all the acetylene concentrations with the greatest observed acetylene concentration (we supposed that this was closest to the situation without leaking) to get a correction factor that was used to correct the ethylene concentrations (we assumed that the ethylene leakage was similar to that of the acetylene). From GC standard samples I then created a standard curve for ethylene concentrations that together with time and plot area was used to calculate the final ethylene production rate in the field plots. The same procedure was followed with the moss samples in the laboratory except that the plot area was replaced by the dry weight of the moss sample.

I calculated the N_2 fixation rate using the formula created by Zechmeister-Boltenstern (1995) and modified by Liengen (1999) in which I placed the results from acetylene reduction assay (acetylene area) and ^{15}N enrichment.

$$Y = \frac{\text{atom\% } ^{15}\text{N}_{\text{excess}}}{100} \cdot \frac{\text{total N}_{\text{sample}} \cdot 10^9}{t \cdot 28} \cdot \frac{100}{\text{\% } ^{15}\text{N}_{\text{air}}}$$

where Y (nmol N g dw^{-1} h^{-1}) is the amount of N_2 fixed during the experiment, atom % ^{15}N excess is the difference between atom% ^{15}N sample and atom% ^{15}N control, total N is the total amount of N in the sample (g 100 gdw^{-1}), t is the incubation time, 28 is the molecular weight of N_2 (g/mol) and $\%^{15}N_{air}$ is the percentage of N gas in the incubation tubes (Liengen 1999).

By dividing the ethylene produced in the lab conditions by these ^{15}N results I got the conversion factor that I could use to convert the ethylene produced in the field to the actual N_2 fixation rate.

2.5. Vegetation cover, vascular plant leaf area and *Sphagnum* growth

We tracked the growth and phenology of vegetation in the field plots by estimating the areal coverage of moss (*Sphagnum* and other genera separately) and vascular plant species, as well as the average height of every vascular plant species every second week throughout the summer. The areal coverages were always carried out by two people so that the results would be more reliable. The average height was estimated by measuring ten average-sized individuals of every vascular plant species.

In the end of the growing season, we calculated the Leaf Area Index (LAI) of each vascular plant species for each plot by counting every leaf and measuring lengths and widths of 4-10 average-sized leaves of each species from every plot. Afterwards the sizes produced by multiplying the lengths and widths of leaves were corrected for each species using correction coefficients calculated in 2020 by Kristiina Muller from the same field plots. However, our LAI values did not seem reasonable (variation between 1.27-4.12 m^2 m^{-2}) compared to the results in literature and we did not find an explanation for these unusually great values so finally, I estimated the LAI for each species using our coverage-estimations and formulas created for plant functional groups by Räsänen et al. (2020) and Virtanen & Räsänen (2022) (Table 1). Despite different magnitude, LAI values that we calculated in the field and these LAI estimations had a strong positive correlation (Pearson's product-moment correlation test, $r = 0.84$, $p<0.001$).

Table 1. Vascular plant functional groups and LAI-equations used with root mean squared error and adjusted coefficient of determination values. Here, c = coverage and h = height.

Functional group	Equation	RMSE	Adjusted R2
Evergreen shrub	LAI = 0.0166636 + 0.0093295 * c	0.1012685	0.7610
Deciduous shrub	LAI = -0.0233321 + 0.0156296 * c	0.2098186	0.6616
Forb	LAI = -1.886E-02 + 1.126E-03 * c * h	0.1405202	0.7816
Graminoid	LAI = 6.579E-02 + 3.853E-04 * c * h	0.1719353	0.4315
Evergreen dwarf shrub	LAI = 0.017011 + 0.009075 * c	0.1008974	0.7437
Deciduous dwarf shrub	LAI = -0.0200554 + 0.0192717 * c	0.1624301	0.7783
Evergreen tall shrub	LAI = 0.001862 + 0.010324 * c	0.0178658	0.8554
Deciduous tall shrub	LAI = 0.0062262 + 0.0076126 * c	0.061594	0.6145
Betula nana	LAI = 0.0045111 + 0.0077656 * c	0.0612696	0.5953
Salix	LAI = 5.375E-04 + 2.955E-04 * c * h	0.0068206	0.8923

The growth of the *Sphagnum* mosses was measured using a modified cranked wire method (Clymo 1970), in which small bottle brushes were embedded partly to the moss so that the bristles prevent them from moving. By measuring the visible part of the bottle brush (thin metal stem) once a month during the summer we were able to estimate the total growth of the moss.

2.6. Gas fluxes

We measured carbon dioxide (CO_2) and methane (CH_4) fluxes using the closed-chamber method (Witkamp 1969; Alm et al. 1999). This method is commonly used when the interest is in small-scale differences in greenhouse gas fluxes like we have. The greenhouse gas fluxes were measured twice a month throughout the summer with a 50 cm high transparent polycarbonate chamber that was connected to an online gas analyzer Picarro G2401. The analyzer recorded CO, CO_2, CH_4 and H_2O continuously which allowed us to do short, 2-min measurements. We used the same collars than in ARA and filled their grooves with water to prevent leaking. The air inside the chamber was mixed with an attached, battery-driven fan. The volume of the chamber was corrected individually for each plot because it varied depending on collar height and terrain.

CO_2-exchange was always measured in ambient light conditions and after that the chamber was covered with a dark canvas to measure respiration. We aimed to do the measurements in stable light

conditions and thus, most of the measurements were carried out in sunny conditions. We measured gas fluxes also before ARA, but since some ARA measurements were carried out at night, the attached CO_2 flux measurements were not usable as it was too dark. Therefore, CO_2 fluxes from the nearest successful gas measurements were used instead.

CO_2 uptake, or photosynthesis is affected by the amount of radiation, but I was interested in the potential gross primary production (GPP1200) of every plot with the effect of radiation removed. Therefore, MSc student Milja Männikkö first calculated the observed net ecosystem exchange (NEE) and GPP and then removed the effect of radiation by calculating NEE and GPP for PAR 1200 µmol m^{-2} s^{-1}. This was done by fitting a curve to three data points (different light levels: ambient, shaded and dark measurement) and extrapolating the value for PAR 1200 µmol m^{-2} s^{-1}.

CH_4 flux does not depend on light conditions, so I used an average of light and dark measurements as data for statistical analyzes. Plot 19 showed unusually high CH_4 emissions in light measurement during the first ARA-survey compared to the dark measurement and all the other measurements. I therefore deduced it to be an error, probably caused by a methane bubble released as a result of excessive pressure, and only used the dark measurement for this plot.

2.7. Environmental conditions

During ARA and greenhouse gas measurements we recorded environmental conditions continuously inside and around the chamber with multiple probes attached to Titan S8 (Madgetech, USA). Air temperature and humidity inside the chamber were recorded with Vaisala HMP110, soil temperature at 10 cm depth (10 cm away from the collar) with PIMZOS PFH14109 (Pt100, Class A), soil moisture with Delta-T ML3 (also 10 cm away from the collar) and short-wave radiation with Kipp & Zonen SP Lite2 that was placed on top of the chamber. In addition, air temperature was measured every half an hour at one meter height in the middle of the plots. For statistical analyzes, I always calculated the average of every environmental factor during the 2-h (ARA) and 2-min gas measurements. To transform the short-wave radiation to photosynthetically active radiation (PAR), I multiplied all radiation results by two.

2.8. Statistical analysis

I carried out statistical analyzes using R (version 1.4.1106). I used linear models to see whether variation in CH_4, GPP1200, *Sphagnum* growth or LAI can be explained by variation in N_2 fixation among the study plots and if environmental factors, laboratory ^{15}N-N_2 fixation and gas fluxes can explain the variation in the rate of N_2 fixation in the plots. I transformed N_2 fixation values to logarithms because this way the AIC-value for every model was lowest. The rough p-value limit for significant results was 0.05, but also p-values smaller than 0.1 are reported.

To see the connections between N_2 fixation and environmental variables, methane flux, laboratory ^{15}N-N_2 fixation and gross primary production, I first plotted correlations of all variables to see if some of the variables were linked to each other. I then ran separate models for the July and August data. In these models, N_2 fixation was the response variable and all others were explanatory variables. I excluded the least significant explanatory variables to find the best model (lowest AIC). I also paid attention to multiple R^2 value because it tells how much of the variation of the response variable can be explained by explanatory variables. I ensured the reliability of the results with several residual plots, which showed if the residuals were normally distributed and homoscedastic (Fig. 3).

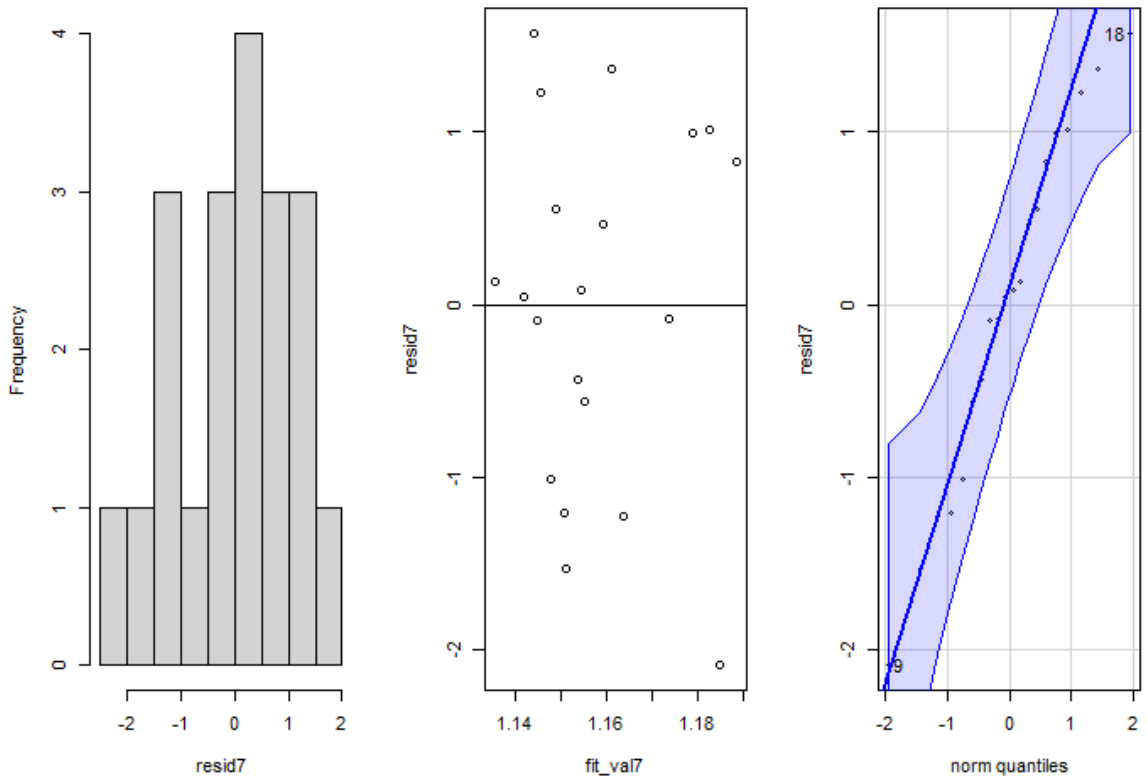


Figure 3. Examples of residual plots that I used to ensure the normality of data and therefore, the reliability of statistical results. The histogram (left) and qqPlot (right) show that residuals are normally distributed (the blue area shows a 95% confidence interval) and the scatter plot (middle) shows that the residual spread is homoscedastic. These example residuals are from the linear model, which tested the effect N_2 fixation on *Sphagnum* growth.

To test if N_2 fixation can explain any of the variables among the plots, I ran separate models for every response variable. For GPP1200 and CH_4 -models I used month-specific values but for *Sphagnum* growth I used the total growth of mosses and a mean of the two N_2 fixation measurements. For LAI -models I used estimated LAI-values and again mean values of the N_2 fixation.

3. Results

3.1. Variation of N_2 fixation, gas fluxes and vegetation properties among the study plots

Based on ARA measurements, the N_2 fixation rate varied between 0.68 and 30.02 ng N m^{-2} h^{-1} among the study plots (Appendix 1; Fig. A). Some of the measurements failed due to too high leakage (plots 3, 10 & 11 in July, and 11 & 14 in August) and were excluded from the calculations. The average (±SE) N_2 fixation for all the plots was 8.97 ± 1.53 ng N m^{-2} h^{-1} in July and 9.83 ± 1.83 ng N m^{-2} h^{-1} in August. The results from July and August did not correlate (Pearson's product-moment correlation test; r=x, p=y).

In June, GPP1200 varied from -1334.92 to -645.72 mmol m^{-2} d^{-1} (Appendix 1; Fig. B) and CH_4 flux from 7.22 to 71.17 mmol m^{-2} d^{-1} (Appendix 1; Fig.C) among the plots. In August, GPP1200 varied between -1089.45 and -567.46 mmol m^{-2} d^{-1} and CH_4 flux between 3.53 and 33.95 mmol m^{-2} d^{-1}. The mean GPP1200 (±SE) was somewhat larger in June (-824.80 ± 30.97 mmol m^{-2} d^{-1}) than in August (-771.97 ± 28.34 mmol m^{-2} d^{-1}) as was also the case for CH_4 flux that was 22.23 ± 3.66 mmol m^{-2} d^{-1} in June and 10.14 ± 1.72 mmol m^{-2} d^{-1} in August. GPP1200 measurements from the two ARA days correlated weakly (r = x, p = 0.064), but did not correlate with other measurements done during the summer (Pearson's product-moment correlation test; r=x, p > 0.05). There was no correlation between CH_4 measurements from the ARA days or from other measurement days.

Estimated leaf area index varied from 0.20 to 0.49 m^2 m^{-2} (Appendix 1; Fig. D) and the total *Sphagnum* growth from 0.37 to 1.93 cm (Appendix 1; Fig. E) among the study plots.

3.2. Associations between explanatory variables

In the data collected in July, many of the environmental variables (PAR, soil temperature, soil moisture, air temperature, relative humidity) correlated with each other (Fig. 4a). This is inevitable because we did measurements in both day- and nighttime, and temperature and radiation both follow the cycle of the sun. Air and soil temperatures were also related to each other and to relative humidity. However, for some reason these links were not as pronounced in August even though PAR and temperature were connected here too (Fig. 4b). GPP1200 correlated with PAR in July and soil temperature in August. CH_4 was also connected to PAR and soil temperature in July but did not show any significant interactions in August. GPP1200, ^{15}N measurements and CH_4 fluxes did not correlate in either of the months.

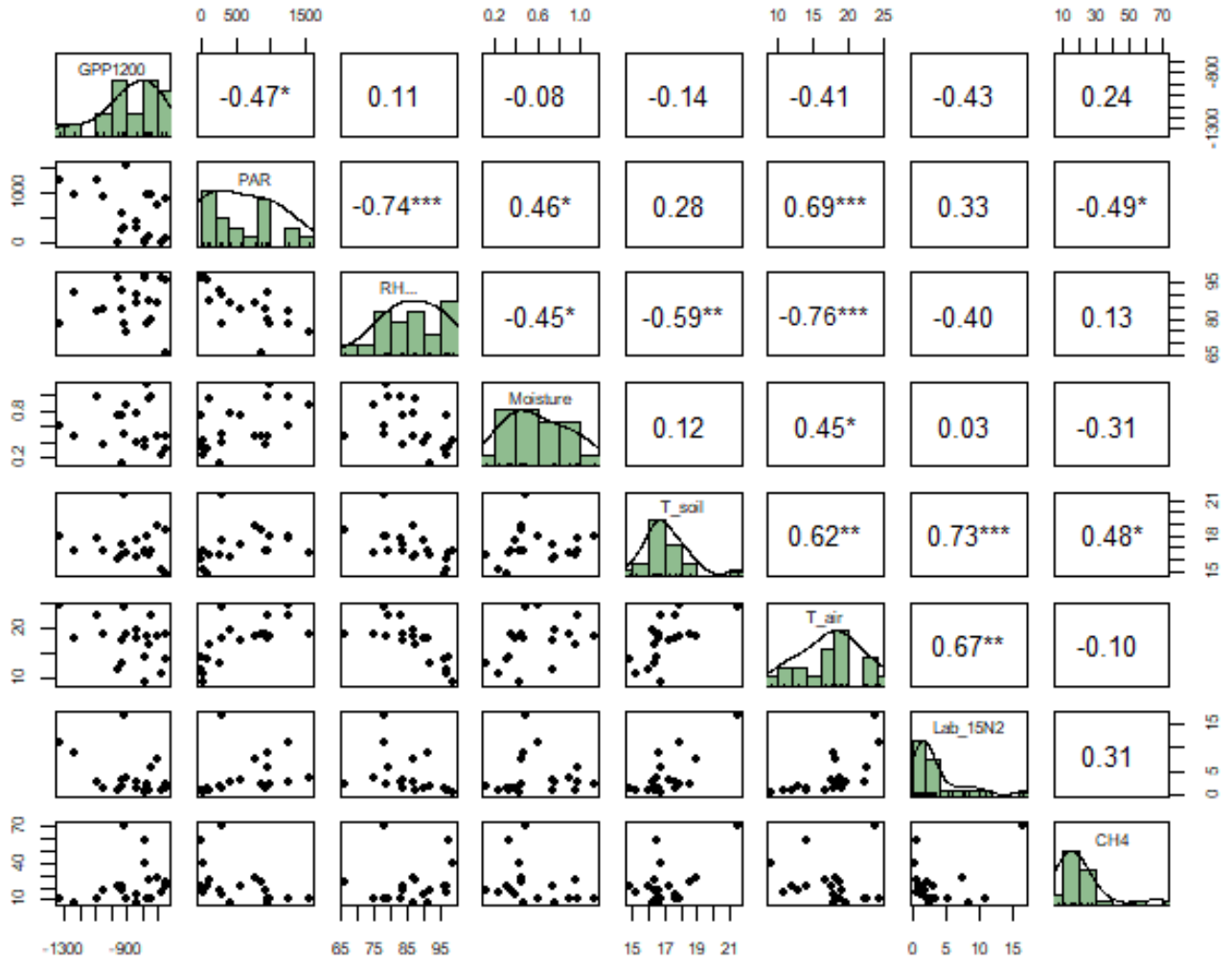


Figure 4. a) Associations between explanatory variables (GPP1200, PAR, relative humidity, soil moisture, soil temperature, air temperature, 15N measurements and CH4) from July illustrated as Pearson's correlation values and scatterplots (GPP1200 = potential gross primary production (mmol m^{-2} d^{-1}), PAR = radiation (µmol m^{-2} s^{-1}), RH = relative humidity (%), Moisture = soil moisture (%), T_soil = soil temperature (°C), T_air = air temperature (°C), Lab_15N2 = ^{15}N-N_2 fixation (nmol g^{-1} h^{-1}), CH4 = CH_4 flux (mmol m^{-2} d^{-1})).

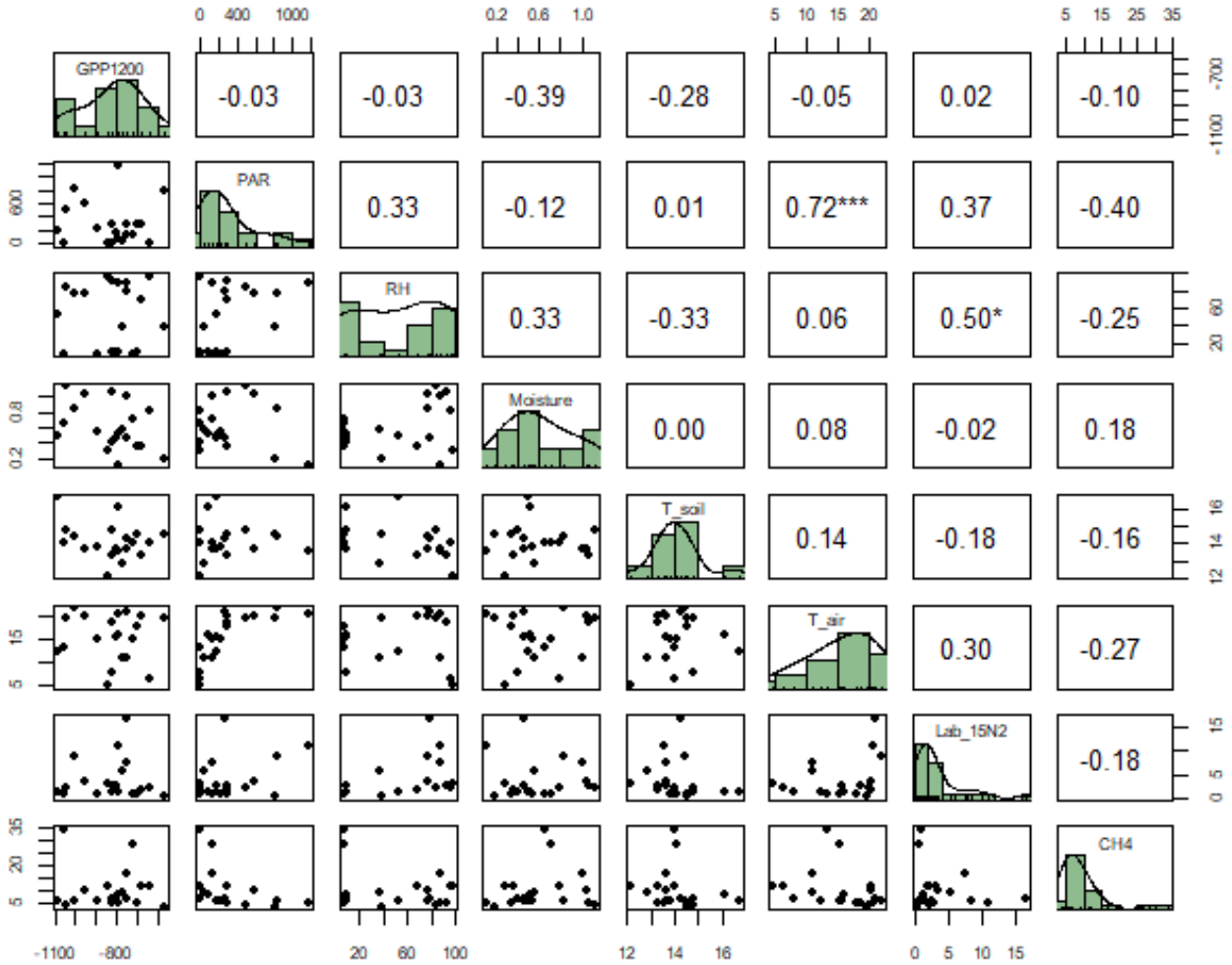


Figure 4. b) Associations between explanatory variables (GPP1200, PAR, relative humidity, soil moisture, soil temperature, air temperature, 15N measurements and CH4) from August illustrated as Pearson's correlation values and scatterplots (GPP1200 = potential gross primary production (mmol m^{-2} d^{-1}), PAR = radiation (µmol m^{-2} s^{-1}), RH = relative humidity (%), Moisture = soil moisture (%), T_soil = soil temperature (°C), T_air = air temperature (°C), Lab_15N2 = ^{15}N-N_2 fixation (nmol g^{-1} h^{-1}), CH4 = CH_4 flux (mmol m^{-2} d^{-1})).

3.3. Variables explaining N_2 fixation rate in the field plots

In a linear regression model of the effects of environmental variables (PAR, humidity, soil moisture, air temperature, soil temperature), GPP1200, CH_4 and laboratory ^{15}N-N_2 fixation on the logarithm of July field plot N_2 fixation (Fig. 5; Appendix 2; Fig. A), PAR and GPP1200 appeared as statistically significant explanatory variables (Table 2). However, relative humidity and soil moisture were not far away being significant, which indicates that they might also explain N_2

fixation. The model that included these four explanatory variables explained 60.6% of the variation of N_2 fixation.

Table 2. Results of the best linear model (lowest AIC) where the effect of environmental variables (PAR, humidity, soil moisture, air temperature, soil temperature), GPP1200, CH_4 and laboratory ^{15}N-N_2 fixation on the logarithm field plot N_2 fixation from July were tested. The original model can be found from appendix (Fig. A). Here, the first column shows the slope of the regression line.

July	Estimate	Std. Error	t value	Pr (>|t|)
Intercept	-2.412	2.824	-0.854	0.410
PAR	0.003	0.001	3.859	0.002
Relative humidity	0.067	0.033	2.031	0.065
Soil moisture	-1.124	0.589	-1.909	0.080
GPP	0.003	0.001	2.473	0.029

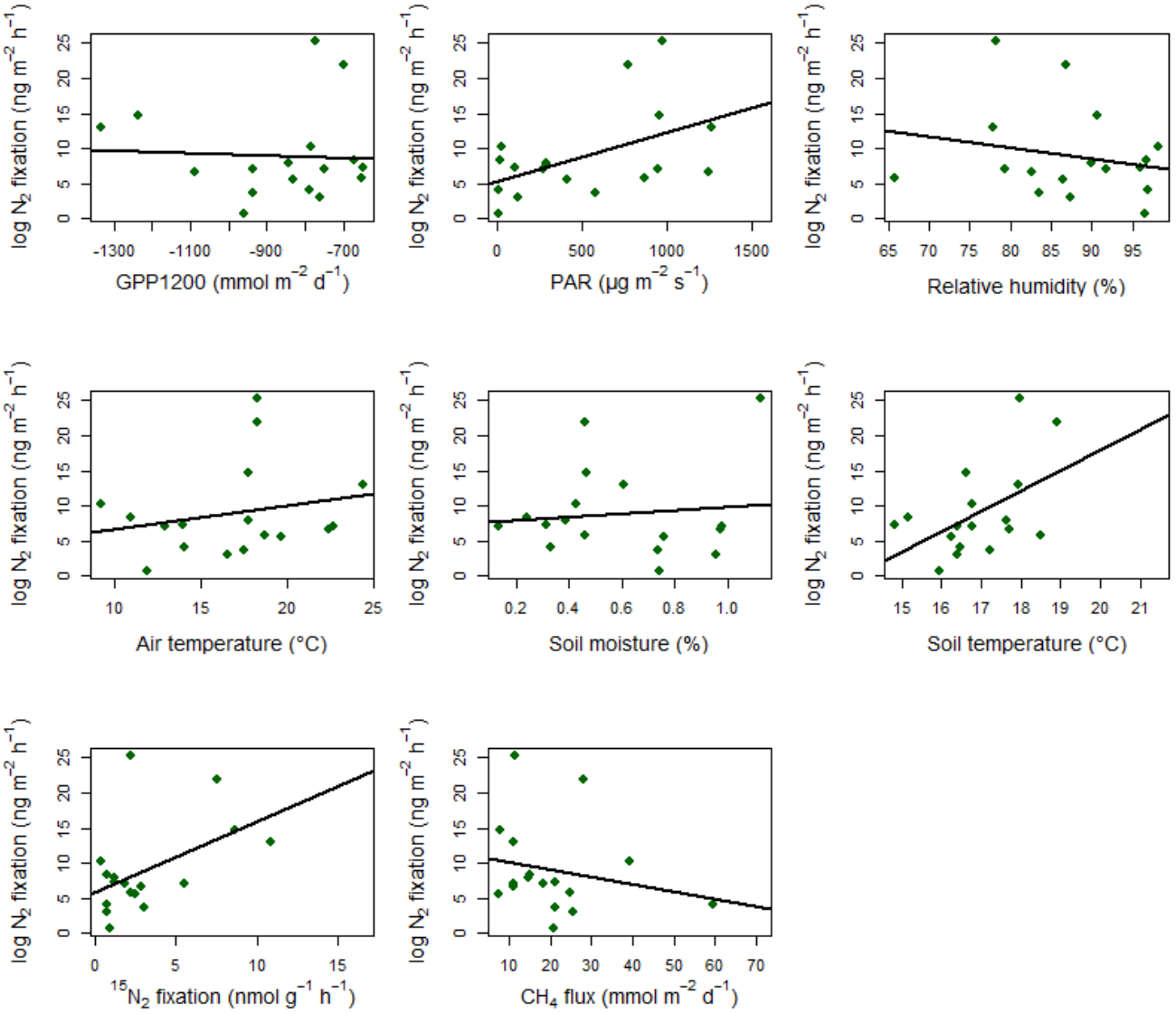


Figure 5. Explanatory variables plotted towards the logarithm of N_2 fixation in July. Black lines are the regression lines from the linear model.

The results of the corresponding model for August were totally different. There, none of the explanatory variables was significant (Fig. 6, Table 3; Appendix 2; Fig. B). The best model included air and soil temperature, GPP1200, ^{15}N-N_2 fixation and CH_4.

Table 3. Results of the best linear model (lowest AIC) where the effect of environmental variables (PAR, humidity, soil moisture, air temperature, soil temperature), GPP1200, CH_4 and laboratory

^{15}N-N_2 fixation on the logarithm field plot N_2 fixation from August were tested. The original model can be found from appendix (Fig. B). Here, the first column shows the slope of the regression line.

August	Estimate	Std. Error	t value	Pr (>|t|)
Intercept	2.608	3.239	0.805	0.436
Air temperature	-0.021	0.041	-0.511	0.618
Soil temperature	-0.175	0.223	-0.785	0.448
GPP	-0.003	0.002	-1.646	0.126
$^{15}N_2$ fixation	0.078	0.047	1.651	0.125
CH_4 flux	-0.030	0.024	-1.243	0.238

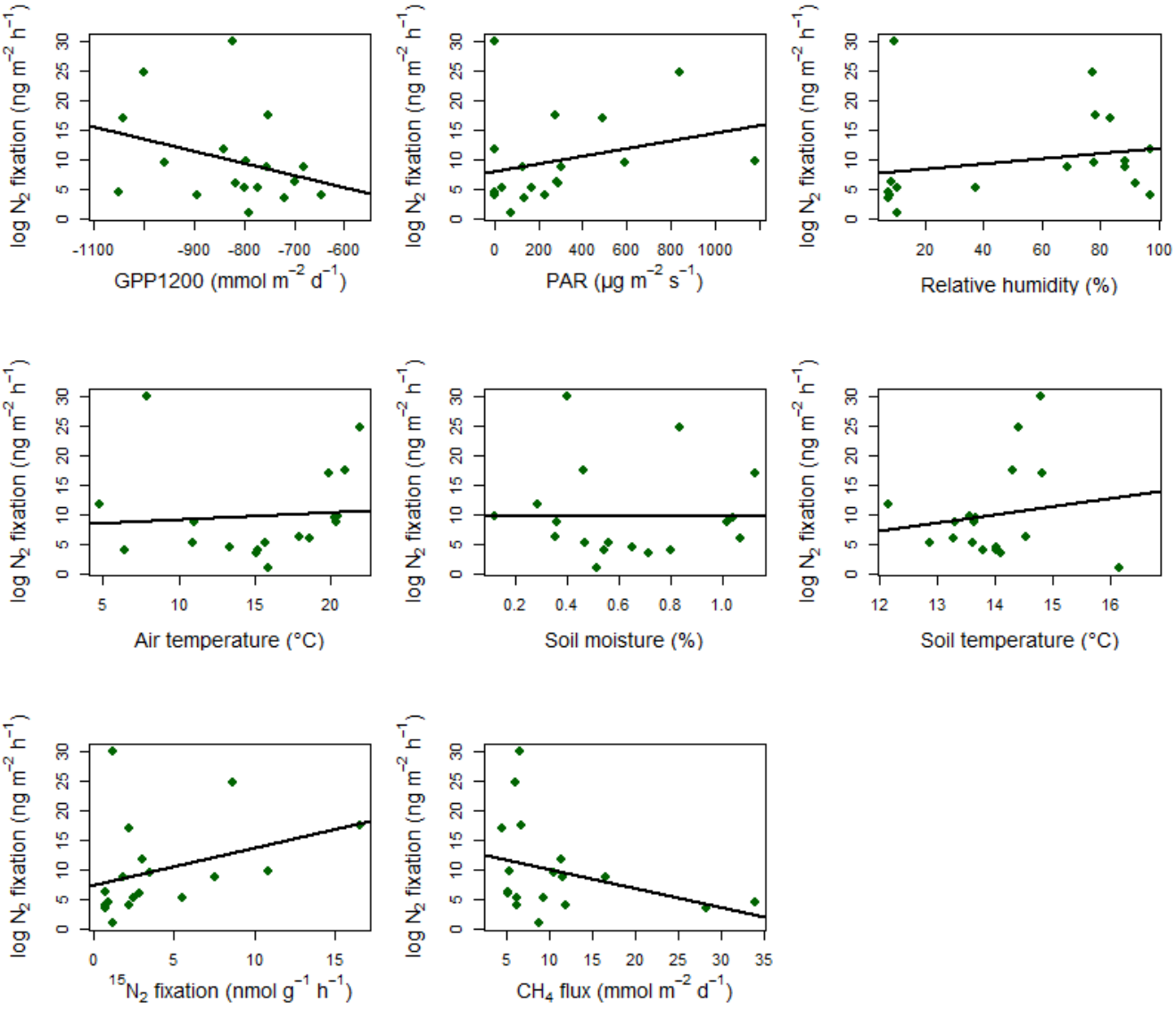


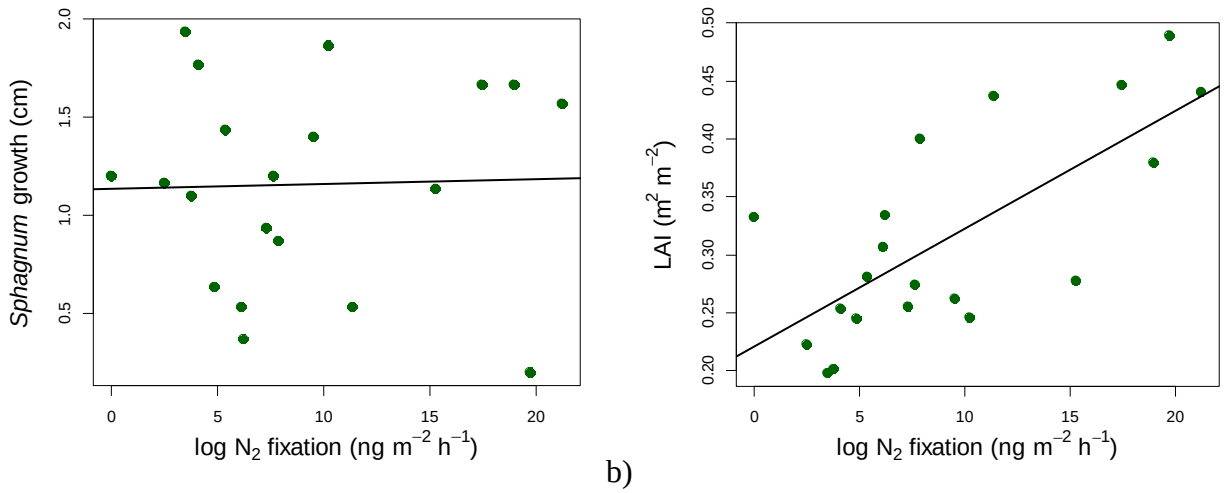
Figure 6. Explanatory variables plotted towards the logarithm of N_2 fixation in August. Black lines are the regression lines from the linear model.

3.4. Effects of N_2 fixation on plant growth in field plots

Mean July and August N_2 fixation did not explain *Sphagnum* growth in field plots (Table 4, Fig. 7a). Instead, N_2 fixation had a clear positive effect on vascular plant LAI and explained 61% of the variation in LAI among the plots (Table 4, Fig. 7b).

Table 4. Results of the linear models where the logarithm of N_2 fixation was explanatory variable and *Sphagnum* growth and LAI response variables. Here, the first column shows the slope of the regression line.

Sphagnum growth	Estimate	Std. Error	t value	Pr (>|t|)
Intercept	1.123	0.241	4.662	<0.001
N_2 fixation	0.003	0.021	0.161	0.873
LAI	Estimate	Std. Error	t value	Pr (>|t|)
Intercept	0.199	0.026	7.666	<0.001
N_2 fixation	0.012	0.002	5.163	<0.001



a) b)

Figure 7. Interactions between a) *Sphagnum* growth and the logarithm of N_2 fixation and b) LAI and the logarithm of N_2 fixation plotted with regression lines.

4. Discussion

In this study I wanted to see if different environmental factors, methane flux or potential gross primary production (GPP1200) affect N_2 fixation by *Sphagnum* mosses and on the other hand, whether the rate of N_2 fixation can explain GPP1200, CH_4 flux and plant growth. The results were not unambiguous: different factors explained N_2 fixation rate in July and in August. In July, photosynthetically active radiation (PAR) and GPP1200 were the significant explanatory factors with the relative humidity and soil moisture also showing some tendency to explain N_2 fixation. In August instead, none of the factors explained N_2 fixation. I found out that the rate of N_2 fixation explained the vascular plant leaf area in the plots. However, N_2 fixation did not explain primary production, methane flux or *Sphagnum* growth.

4.1. Abiotic factors and N_2 fixation

I expected to find connections between the variation of N_2 fixation and variation of abiotic factors among my study plots. My hypothesis about soil moisture was based on several studies (Granhall & Selander, 1973; Larmola et al., 2014; Rousk & Michelsen 2017; Rousk, 2018) that found it to be the most important factor influencing the rate of N_2 fixation. However, I did not find a clear connection between soil moisture and N_2 fixation and there might be several reasons for that. Many of the earlier studies compared N_2 fixation between wet flarks and hummocks. My study plots were located at intermediate lawn surfaces where the differences in water table level might have been relatively small, and this can explain why the connection between soil moisture and N_2 fixation was not visible. Van den Elzen et al. (2020) also found that not all *Sphagnum* species were equally effective N fixers in wet conditions. *Sphagnum magellanicum* showed relatively low N_2 fixation rates despite growing at equally wet conditions than *S. fallax* that instead, showed the highest rates of N_2 fixation. Therefore, also *Sphagnum* species could explain the contrasting results compared to previous studies.

Especially now when anthropogenic climate change is threatening life on Earth (IPCC, 2022), temperature variation is an interesting variable to compare with everything happening in nature. In

this study, however, I did not find any correlation between temperature variation and N_2 fixation rates. This is not extraordinary because earlier warming studies demonstrating the effect of climate change have shown contradictory results. Carrell et al. (2019) investigated the effect of warming to microbial communities of *Sphagnum* moss and found a significant decrease in the taxonomic diversity of microbes resulting in decreased N_2 fixation activity. After only two years of warming the diazotroph community had shifted from a mixed community of Cyanobacteria and Alphaproteobacteria to a dominance of Cyanobacteria. Stewart et al. (2014) instead stated in their review of several studies that temperature is one of the factors that promote N_2 fixation. Also, Rousk & Michelsen (2017) concluded rising temperatures to promote N_2 fixation, according to the results of their warming experiment. Earlier, Sorensen & Michelsen (2011) had not found any warming-induced effects at the same area. These contrasting results indicate that temperature is not the most pronounced factor affecting N_2 fixation activity.

My hypothesis about the correlation between light variation and N_2 fixation activity was supported partly since PAR explained N_2 fixation in July but not in August. Both July and August ARA-measurements were also carried out in night so there was a large variation in light conditions between measurements. However, it is surprising that this correlation was only visible in July even though the light difference between day and night was more pronounced in August. Previous studies have shown that N_2 activity can be 3-10 times higher in light compared to dark conditions (Larmola et al., 2014; van den Elzen et., 2017). One explanation for this could be the connection between light and photosynthesis, and again, photosynthesis and N_2 fixation. Thus, the effect of light on N_2 fixation is likely to be indirect (Larmola et al., 2014). Van den Elzen et al. (2017) tested the effect of light in laboratory and found that N_2 fixation was almost 10 times higher in light conditions (150 µmol m^2 s^{-1} PAR) than in dark conditions. Larmola et al. (2014) instead, performed their experiment in the field in prevailing light conditions and dark conditions and found light to increase N_2 fixation threefold. The large difference between these studies might indicate that there is a threshold in the amount of light after which the light does not enhance the fixation activity anymore. 150 µmol m^2 s^{-1} PAR that van den Elzen et al. used in their light treatment is relatively low radiation compared to sunny days when PAR can get up to 1500 µmol m^2 s^{-1} like in some of our measurements. This threshold hypothesis might also explain why light did not explain N_2 fixation rates in August in my study.

4.2. Gas fluxes, primary productivity and plant growth

N_2 fixation needs lots of energy (16 moles of ATP per mole of N_2) and thus depends on photosynthesis that can provide the energy (Houlton et al., 2008; Igarashi & Seefeldt, 2008). On the other hand, enhanced *Sphagnum* N_2 fixation can promote production of vascular plants by producing N for their use (Vitousek & Howarth, 1991; van den Elzen et al., 2020). In this study, GPP1200 explained N_2 fixation activity in July but not in August. N_2 fixation did not explain GPP1200 in either month. This asymmetric result might be due to statistical models used: When N_2 fixation was the response variable, there were several explanatory variables whereas in the other model N_2 fixation was the only explaining factor. Number of explanatory variables and their potential correlations may influence the outcome of the model. Contrary to my predictions, neither CH_4 flux explained N_2 fixation rates or the other way round. This is not unusual because Leppänen et al. (2015) also found no effect when they experimentally added CH_4 to *Sphagnum* to see if it affects the N_2 fixation. Larmola et al. (2014) & Vile et al. (2014) in turn, concluded that because methanotrophs oxidize CH_4 to CO_2, which can be utilized for photosynthesis, methanotrophic activity promotes N_2 fixation. Therefore, N_2 fixation may mitigate methane fluxes from peatlands (Vile et al., 2014). My study area was a fen, suggesting that methanotrophic N_2 fixation plays an important role there (Larmola et al. 2014). However, previous studies have found that acetylene has a methanotroph-inhibiting effect which can lead to underestimated results when ARA is used (Larmola et al., 2014; Vile et al., 2014). Therefore, the methanotrophic N_2 fixation might not be visible in our results.

Sphagnum growth was not explained by N_2 fixation unlike I predicted. However, this finding is in line with van den Elzen et al. (2020) who also did not find a correlation between N_2 fixation and *Sphagnum* growth and stated that water availability might be more important factor regulating the growth. This might be due to diazotrophs that use fixed N for the production of their cell structures before it becomes available for *Sphagnum* (van den Elzen et al., 2017). Berg et al. (2013) instead, proved that N_2 fixation influences positively the formation of new *Sphagnum* biomass i.e., growth. A possible explanation for these contrasting findings might be the species-specific traits of *Sphagnum*. It has been shown that there is a trade-off between *Sphagnum* growth and production of decay resisting compounds and that these characteristics vary among species (Bengtsson et al., 2016; van den Elzen et al., 2020). In accordance with my hypothesis, vascular plant leaf area was explained by the rate of N_2 fixation. I did not find any corresponding studies about this connection although it has been recognized that moss-associated N_2 fixation benefits also vascular plants, and thus, contributes to primary production (Vitousek & Howarth, 1991; van den Elzen et al., 2020). In

the light of this, the association between GPP1200 and N_2 fixation that I found, makes perfect sense.

Many of the explanatory variables I have used in this study are unavoidably correlated with each other which makes the interpretation of the results of statistical analyses difficult. Abiotic factors like radiation and temperatures as well as their effects on greenhouse gas fluxes and other processes are interconnected. This should be taken into consideration when interpreting the results.

4.3. Other possible explaining factors of N_2 fixation rate

As many ecological processes, also biological N_2 fixation is affected by numerous factors, and it is impossible to test all of them in the same study. However, several studies have investigated the effect of different factors like *Sphagnum* species, diazotroph community, litter input and phosphorus availability in addition to the ones that I measured. Also, habitat and topography have been suggested to explain differences in N_2 fixation rates (Stewart et al., 2014; Leppänen et al., 2015; van den Elzen et al., 2020). They are both composed of many different factors. Van den Elzen et al. (2020) noticed the rate of N_2 fixation to be lower in open bogs compared to mire margin areas. In general, it has been shown that N_2 fixation activity is higher in wetter habitat (Granhall & Selander, 1973; Larmola et al., 2014). However, these same studies that emphasized the role of habitat also noticed that there was a difference in the fixation between different *Sphagnum* species and Leppänen et al. (2015) further found that differences between species were significant in certain habitats only. *Sphagnum* species produce different amounts of decomposition-inhibiting metabolites that impede N_2 fixation. Therefore, higher decomposition rates often lead to higher growth rates and N_2 fixation activity whereas species that can resist decomposition grow slower and fix less N_2 (Van den Elzen et al., 2020). *Sphagnum* species are adapted to different kind of environmental conditions (Bengtsson et al., 2016) and this has an influence in their microbiome as well (Carrell et al., 2019). There are contrasting findings whether the diazotrophic community composition is varying between species or between individuals. Leppänen et al. (2015) found that the community structure was independent of *Sphagnum* species and therefore, did not explain N_2 fixation, whereas Bragina et al. (2013) proved microbiome to vary between species when they compared *S. fallax* and *S. magellanicum*. However, depending on *Sphagnum* species or not, microbial diversity is important, because it seems to explain the N_2 fixation activity (Carrell et al., 2019). According to Hsu & Buckley (2009) the effects of diazotrophic community structure can exceed the role of soil characteristics.

Other possible explaining factors for N_2 fixation suggested by literature are phosphorus (P) availability and litter. Phosphorus have been noticed to enhance diazotrophic activity especially in ecosystems that are P limited (Houlton et al., 2008; van den Elzen et al., 2017; van den Elzen et al., 2020). The effect of litter to N_2 fixation can be either positive (Sorensen & Michelsen, 2011) or negative (Rousk & Michelsen, 2017) depending on the plant species where the litter is from. Birch litter has been shown to promote N_2 fixation probably due to increased P availability in the soil (Rinnan et al., 2008). Willow litter instead led to higher N input to the soil and inhibited N_2 fixation (Rousk & Michelsen, 2017). Measuring the factors mentioned above would have improved my study by adding more information. For example, we did not identify *Sphagnum* species from our study plots because it requires strong expertise that we did not have. However, this would be an important thing to take into account in future studies.

4.4. Impact of the project

The connection between *Sphagnum*-associated N_2 fixation and LAI is a significant finding because it has not been proved before. It shows that N_2 fixation can enhance vascular plant growth and this way promote the primary production of the whole ecosystem. This was also partly supported by my findings about the connection between GPP1200 and N_2 fixation. The role of *Sphagnum* mosses as ecosystem engineers in northern peatlands has been known to be important because of their ability to provide nutrients for vascular plants and balance water table (Malmer et al., 2003). They form most of the peat due to slow decomposition and thus, store vast amounts of carbon to the soil forming a globally important C sink (Carrell et al., 2019). *Sphagnum* mosses also stabilize the ecosystem making it more resilient to disturbances (Turetsky et al., 2012). However, Hedwall et al. (2017) proved that vegetation community composition is likely to change in northern peatlands in the future due to the climate change and increasing N deposition. This might have drastic effects on nitrogenase of *Sphagnum* mosses (Larmola et al., 2014). Elevated levels of atmospheric deposition can inhibit N_2 fixation rate (Gundale et al., 2012) and as already discussed, rising temperatures might affect that as well. Carrell et al. (2019) showed that the microbial diversity of *Sphagnum* will decrease due to warming and thus, make them more susceptible to future changes. In addition, climate change can influence several other factors like precipitation that may have indirect effects on N_2 fixation. Climate change will also have indirect effects on N_2 fixation. The change in the vegetation community composition, for example shrub expansion (Hedwall et al, 2017), will lead to increased litter input that can have varying effects on N cycle as discussed (Sorensen & Michelsen,

2011; Rousk & Michelsen, 2017). As N_2 fixation is also tightly linked to C cycling by affecting the rate of primary production and decomposition, it is even more important to understand the dynamics of the N_2 fixation (Vitousek & Howarth, 1991; Gundale et al., 2012; van den Elzen et al., 2020).

My N_2 fixation results appeared to be significantly lower than the ones reported in literature. If the results from other fen studies are converted to the same unit, the daily N_2 fixation rates (during growing season) vary vastly. Van den Elzen et al. (2020) reported N_2 fixation to vary from 30 to 600 μg N m^{-2} d^{-1} in their study whereas Larmola et al. (2014) found the activity to reach up to almost 36 000 μg N m^{-2} d^{-1} at some plots. Other studies have found intermediate rates like 250–1250 μg N m^{-2} d^{-1} (Grannhal & Selander, 1973) and 1692 μg N m^{-2} d^{-1} (Rousk & Michelsen, 2017). My N_2 fixation results varied between 0.02 – 0.72 μg N m^{-2} d^{-1}. These low values might be partly due to leaking, that was observed when analyzing samples, together with several other factors that influence N_2 fixation. Therefore, these results are probably not representative for the total N_2 fixed. In spite of that, differences between plots should reflect the actual differences since the setup was same during all our measurements. My results about the connection of N_2 fixation activity and light and GPP support earlier findings. However, I did not find connection between N_2 fixation and many of the other factors presented in literature. This indicates that there might be some parameter that affects the rate of N_2 fixation even more than the parameters we measured and covers the effects of them. Therefore, further studies are needed to understand these results. Finally, as there are no other studies of the connection of N_2 fixation activity and vascular plant LAI, and LAI has an effect on ecosystem productivity and global C cycle, this interaction should be studied more to confirm my findings.

4.5. Feasibility and risk assessment

In Finland accessing nature and collecting plants is allowed unless it is not specifically prohibited (protected areas or species). The study area was not protected and did not have endangered or protected species and therefore, this study did not raise any ethical issues. The methods applied in this study are commonly used and generally accepted in scientific field. However, many parts of the study include uncertainties because of possible human errors. For example, leaf area indexes presented here are estimates calculated based on our estimates of plant coverage in the plots. To minimize errors, we repeated these estimations throughout the summer and used their average in

final calculations. This kind of open-air studies always include a risk of inaccuracies. This was our first time to try acetylene reduction assay and thus, there were some difficulties with the chamber we used and measurement times. However, the results should be reliable after correction calculations.

5. Conclusions

Northern peatland ecosystems are nitrogen limited environments, that are characterized by mosses, especially from genus *Sphagnum*. Moss-associated N_2 fixation is the major input of N in these areas and thus, the whole ecosystem functioning is dependent on N_2 fixation. Future changes like climate change and increasing N deposition are likely to affect N_2 fixation process and this way affect the productivity and carbon sequestration of these wide-spread ecosystems. Therefore, it is extremely important to understand the dynamics of N_2 fixation.

The purpose of this study was to figure out what factors affect N_2 fixation and whether N_2 fixation explains the variation in different processes or not. I investigated the effect of environmental variables (soil moisture, soil temperature, air temperature, relative humidity and radiation), methane (CH_4) flux and potential production (GPP1200) and found out, that radiation (PAR) and GPP1200 explained the rate of N_2 fixation during the first ARA measurement. However, none of the factors explained variation during the second ARA measurement. I also studied if N_2 fixation could explain vascular plant leaf area (LAI), *Sphagnum* growth, CH_4 flux and GPP1200 and found out that variation in LAI was explained by N_2 fixation activity. These results prove the strong connection between *Sphagnum* associated N_2 fixation and ecosystem-level processes like photosynthesis and plant growth. Conflicting results in literature about the factors affecting N_2 fixation reveal the demand for further studies, especially in the light of future changes that these vulnerable ecosystems will meet.

6. Acknowledgement

I want to thank my supervisor Juha Mikola for the opportunity to work with this topic and learn so much new. I am grateful for all the guidance, comments and support through the year. Thank you

Milja Männikkö for your huge work input for my thesis, patience with my endless questions and most importantly, your great company. I want to thank Kathrin Rousk and Yinliu Wang for your significant help in analyzing samples and for the opportunity to get to work with you. Thank you also Mika Aurela and Tarmo Virtanen for guidance and help with the field work. I express my gratitude towards Heidi Andersson who donated the grant for me. Last, I am extremely thankful for all my close ones for their support!

7. References

Aerts, R., Wallen, B. O., & Malmer, N. (1992). Growth-limiting nutrients in *Sphagnum*-dominated bogs subject to low and high atmospheric nitrogen supply. *Journal of Ecology*, 131-140.

Alm, J., Schulman, L., Walden, J., Nykänen, H., Martikainen, P. J., & Silvola, J. (1999). Carbon balance of a boreal bog during a year with an exceptionally dry summer. *Ecology*, *80*(1), 161-174.

Bengtsson, F., Granath, G., & Rydin, H. (2016). Photosynthesis, growth, and decay traits in *Sphagnum*–a multispecies comparison. *Ecology and Evolution*, *6*(10), 3325-3341.

Berg, A., Danielsson, Å., & Svensson, B. H. (2013). Transfer of fixed-N from N_2-fixing cyanobacteria associated with the moss *Sphagnum riparium* results in enhanced growth of the moss. *Plant and soil*, *362*(1), 271-278.

Bragina, A., Berg, C., Müller, H., Moser, D., & Berg, G. (2013). Insights into functional bacterial diversity and its effects on Alpine bog ecosystem functioning. *Scientific reports*, *3*(1), 1-8.

Carrell, A. A., Kolton, M., Glass, J. B., Pelletier, D. A., Warren, M. J., Kostka, J. E., ... & Weston, D. J. (2019). Experimental warming alters the community composition, diversity, and N_2 fixation activity of peat moss (*Sphagnum fallax*) microbiomes. *Global Change Biology*, *25*(9), 2993-3004.

Cleveland, C. C., Townsend, A. R., Schimel, D. S., Fisher, H., Howarth, R. W., Hedin, L. O., ... & Wasson, M. F. (1999). Global patterns of terrestrial biological nitrogen (N_2) fixation in natural ecosystems. *Global biogeochemical cycles*, *13*(2), 623-645.

Clymo, R. S. (1970). The growth of *Sphagnum*: methods of measurement. *The Journal of Ecology*, 13-49.

Dilworth, M. J. (1966). Acetylene reduction by nitrogen-fixing preparations from *Clostridium pasteurianum*. *Biochimica et Biophysica Acta (BBA)-General Subjects*, *127*(2), 285-294.

Granhall, U., & Selander, H. (1973). Nitrogen fixation in a subarctic mire. *Oikos*, 8-15.

Gundale, M. J., Nilsson, M., Bansal, S., & Jäderlund, A. (2012). The interactive effects of temperature and light on biological nitrogen fixation in boreal forests. *New Phytologist*, *194*(2), 453-463.

Hardy, R., Burns, R. C., & Holsten, R. D. (1973). Applications of the acetylene-ethylene assay for measurement of nitrogen fixation. *Soil Biology and Biochemistry*, *5*(1), 47-81.

Hedwall, P. O., Brunet, J., & Rydin, H. (2017). Peatland plant communities under global change: negative feedback loops counteract shifts in species composition. *Ecology*, *98*(1), 150-161.

Houlton, B. Z., Wang, Y. P., Vitousek, P. M., & Field, C. B. (2008). A unifying framework for dinitrogen fixation in the terrestrial biosphere. *Nature*, *454*(7202), 327-330.

Hsu, S. F., & Buckley, D. H. (2009). Evidence for the functional significance of diazotroph community structure in soil. *The ISME journal*, *3*(1), 124-136.

Igarashi, R. Y., & Seefeldt, L. C. (2003). Nitrogen fixation: the mechanism of the Mo-dependent nitrogenase. *Critical reviews in biochemistry and molecular biology*, *38*(4), 351-384.

IPCC (2022). *Climate Change 2022: Impacts, Adaptation, and Vulnerability.* Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Pörtner, H.-O., Roberts D.C., Tignor M., Poloczanska E.S., Mintenbeck K., Alegría A., Craig M., Langsdorf S., Löschke S., Möller V., Okem A., Rama B. (eds.)]. Cambridge University Press, *in press*.

Kraiser, T., Gras, D. E., Gutiérrez, A. G., González, B., & Gutiérrez, R. A. (2011). A holistic view of nitrogen acquisition in plants. *Journal of experimental botany*, *62*(4), 1455-1466.

Larmola, T., Leppänen, S. M., Tuittila, E. S., Aarva, M., Merilä, P., Fritze, H., & Tiirola, M. (2014). Methanotrophy induces nitrogen fixation during peatland development. *Proceedings of the National Academy of Sciences*, *111*(2), 734-739.

Lawrence, D. B., Schoenike, R. E., Quispel, A., & Bond, G. (1967). The role of *Dryas drummondii* in vegetation development following ice recession at Glacier Bay, Alaska, with special reference to its nitrogen fixation by root nodules. *The Journal of Ecology*, 793-813.

Leppänen, S. M., Rissanen, A. J., & Tiirola, M. (2015). Nitrogen fixation in *Sphagnum* mosses is affected by moss species and water table level. *Plant and Soil*, *389*(1), 185-196.

Lett, S., & Michelsen, A. (2014). Seasonal variation in nitrogen fixation and effects of climate change in a subarctic heath. *Plant and Soil*, *379*(1), 193-204.

Liengen, T. (1999). Conversion factor between acetylene reduction and nitrogen fixation in free-living cyanobacteria from high arctic habitats. *Canadian Journal of Microbiology*, *45*(3), 223-229.

Malmer, N., Albinsson, C., Svensson, B. M., & Wallén, B. (2003). Interferences between *Sphagnum* and vascular plants: effects on plant community structure and peat formation. *Oikos*, *100*(3), 469-482.

Mikola, J., Juutinen, S., Räsänen, A., Virtanen, T., Penttilä, T., Heiskanen, L., & Aurela, M. (2022) Weather variation, microtopography and soil fertility as drivers of CO_2, CH_4 and N_2O fluxes on a northern peatland. *Unpublished manuscript*.

Näsholm, T., Ekblad, A., Nordin, A., Giesler, R., Högberg, M., & Högberg, P. (1998). Boreal forest plants take up organic nitrogen. *Nature*, *392*(6679), 914-916.

Opelt, K., Chobot, V., Hadacek, F., Schönmann, S., Eberl, L., & Berg, G. (2007). Investigations of the structure and function of bacterial communities associated with *Sphagnum* mosses. *Environmental microbiology*, *9*(11), 2795-2809.

Pirinen P., Simola H., Aalto J., Kaukoranta J-.P., Karlsson P. & Ruuhela R. (2012). *Climatological statistics of Finland 1981–2010.* Finnish Meteorological Institute.

Rinnan, R., Michelsen, A., & Jonasson, S. (2008). Effects of litter addition and warming on soil carbon, nutrient pools and microbial communities in a subarctic heath ecosystem. *Applied Soil Ecology*, *39*(3), 271-281.

Rousk, K., Sorensen, P. L., & Michelsen, A. (2016). Nitrogen transfer from four nitrogen-fixer associations to plants and soils. *Ecosystems*, *19*(8), 1491-1504.

Rousk, K., & Michelsen, A. (2017). Ecosystem nitrogen fixation throughout the snow-free period in subarctic tundra: effects of willow and birch litter addition and warming. *Global Change Biology*, *23*(4), 1552-1563.

Rousk, K., Sorensen, P. L., & Michelsen, A. (2018). What drives biological nitrogen fixation in high arctic tundra: moisture or temperature?. *Ecosphere*, *9*(2), e02117.

Räsänen, A., Juutinen, S., Kalacska, M., Aurela, M., Heikkinen, P., Mäenpää, K., ... & Virtanen, T. (2020). Peatland leaf-area index and biomass estimation with ultra-high resolution remote sensing. *GIScience & Remote Sensing*, *57*(7), 943-964.

Sorensen, P. L., & Michelsen, A. (2011). Long-term warming and litter addition affects nitrogen fixation in a subarctic heath. *Global Change Biology*, *17*(1), 528-537.

Stewart, W. D., Fitzgerald, G. P., & Burris, R. (1967). In situ studies on N_2 fixation using the acetylene reduction technique. *Proceedings of the National Academy of Sciences of the United States of America*, *58*(5), 2071.

Stewart, K. J., Grogan, P., Coxson, D. S., & Siciliano, S. D. (2014). Topography as a key factor driving atmospheric nitrogen exchanges in arctic terrestrial ecosystems. *Soil Biology and Biochemistry*, *70*, 96-112.

The Finnish Meteorological Institute. *Temperature and precipitation statistics from 1961 onwards*. https://www.ilmatieteenlaitos.fi/vuodenaikojen-tilastot

Turetsky, M. R., Bond-Lamberty, B., Euskirchen, E., Talbot, J., Frolking, S., McGuire, A. D., & Tuittila, E. S. (2012). The resilience and functional role of moss in boreal and arctic ecosystems. *New Phytologist*, *196*(1), 49-67.

Van Cleve, K., & Alexander, V. (1981). Nitrogen cycling in tundra and boreal ecosystems. *Ecological Bulletins*, 375-404.

Van Den Elzen, E., Kox, M. A., Harpenslager, S. F., Hensgens, G., Fritz, C., Jetten, M. S., ... & Lamers, L. P. (2017). Symbiosis revisited: phosphorus and acid buffering stimulate N_2 fixation but not *Sphagnum* growth. *Biogeosciences*, *14*(5), 1111-1122.

Van Den Elzen, E., Bengtsson, F., Fritz, C., Rydin, H., & Lamers, L. P. (2020). Variation in symbiotic N_2 fixation rates among *Sphagnum* mosses. *PloS one*, *15*(2), e0228383.

Virtanen, T. & Räsänen, A. (2022).*Unpublished manuscript*.

Verhoeven, J. T. A., & Liefveld, W. M. (1997). The ecological significance of organochemical compounds in *Sphagnum*. *Acta Botanica Neerlandica*, *46*(2), 117-130.

Vile, M. A., Kelman Wieder, R., Živković, T., Scott, K. D., Vitt, D. H., Hartsock, J. A., ... & Wykoff, D. D. (2014). N_2-fixation by methanotrophs sustains carbon and nitrogen accumulation in pristine peatlands. *Biogeochemistry*, *121*(2), 317-328.

Vitousek, P. M., & Howarth, R. W. (1991). Nitrogen limitation on land and in the sea: how can it occur?. *Biogeochemistry*, *13*(2), 87-115.

Witkamp, M. (1969). Cycles of temperature and carbon dioxide evolution from litter and soil. *Ecology*, *50*(5), 922-924.

Zechmeister-Boltenstern, S. (1995). Nitrogenase activity by $^{15}N_2$-fixation. *Methods in soil biology. Springer, Berlin*, 130-134.

Appendices

Appendix 1. Variation in N_2 fixation, gas fluxes and vegetation properties among study plots

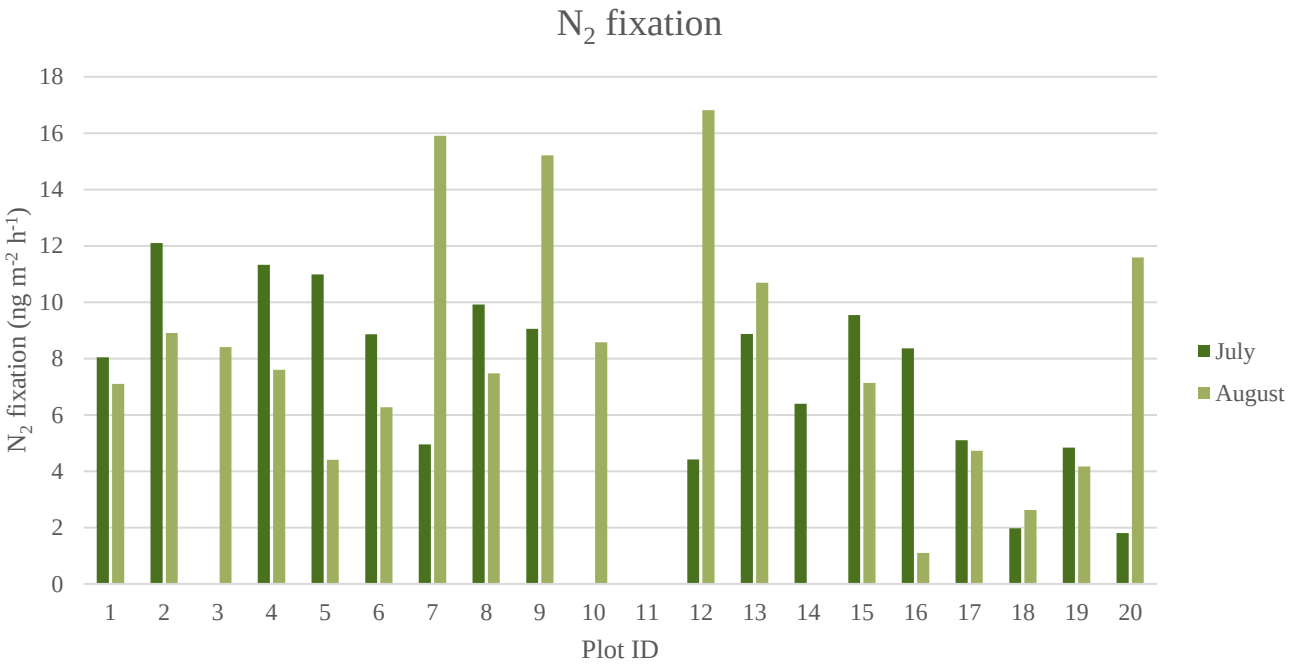


Figure A. The total N_2 fixation rate in July and in August.

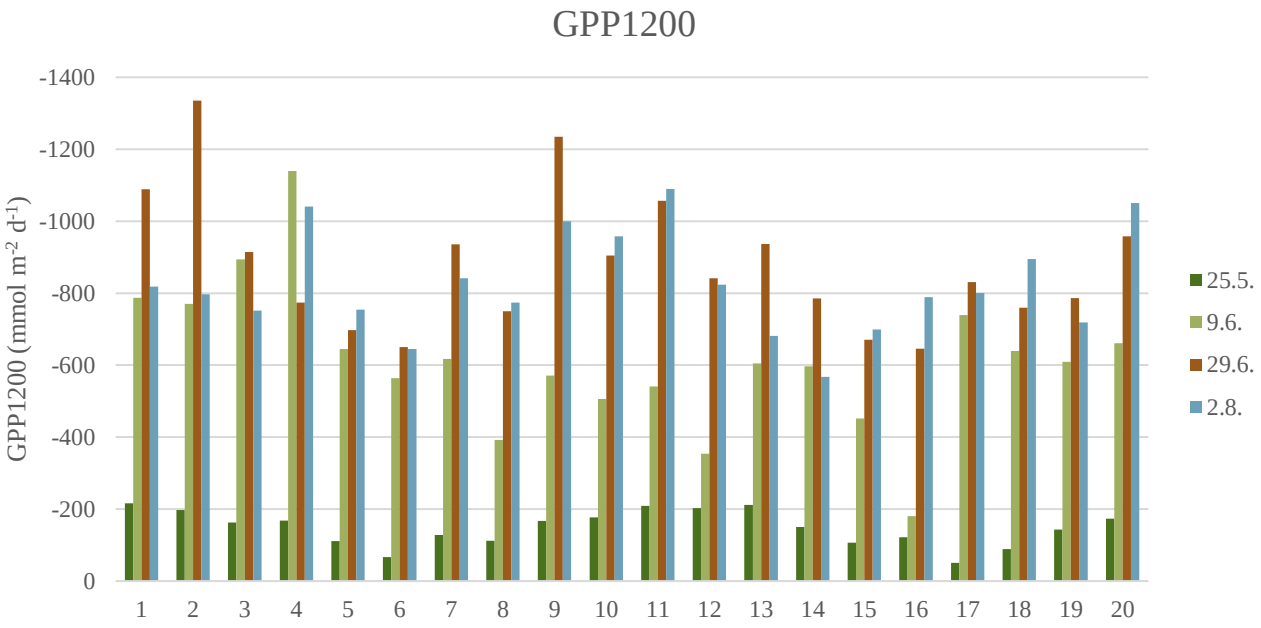


Figure B. Potential gross primary production in plots in different measurement days when PAR is 1200 µmol m^{-2} s^{-1}.

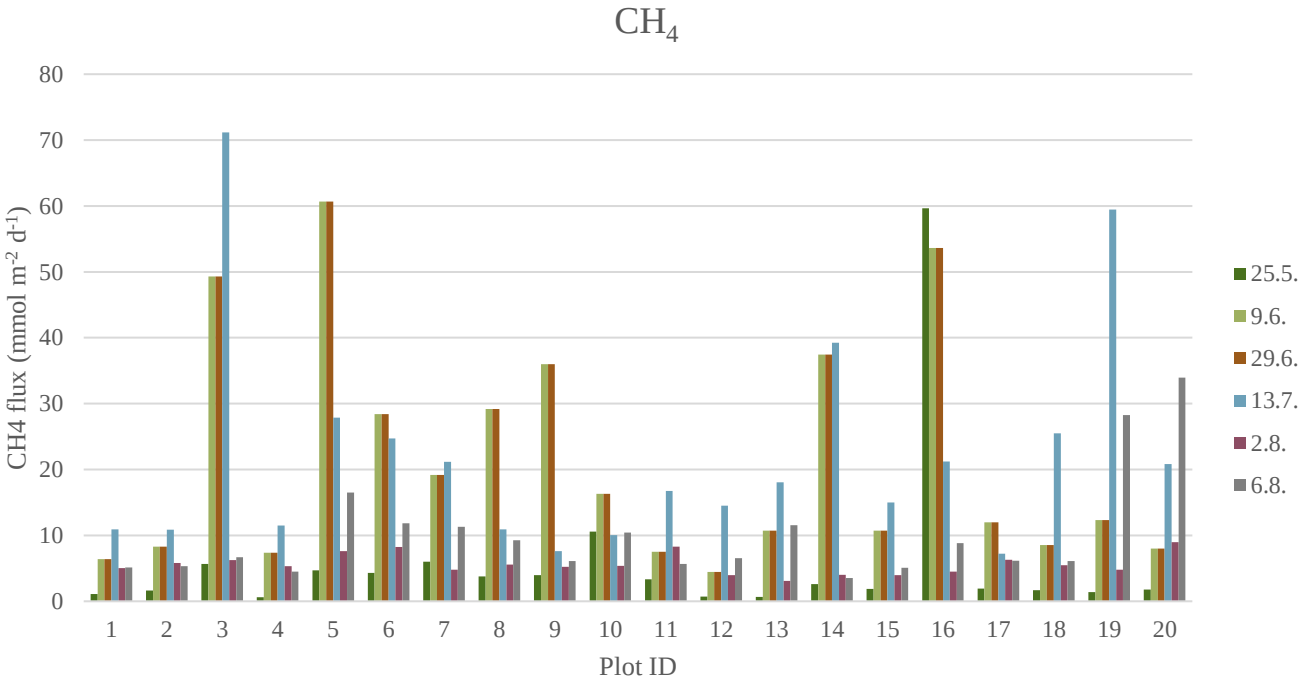


Figure C. Methane emissions varied significantly between plots and measurement days.

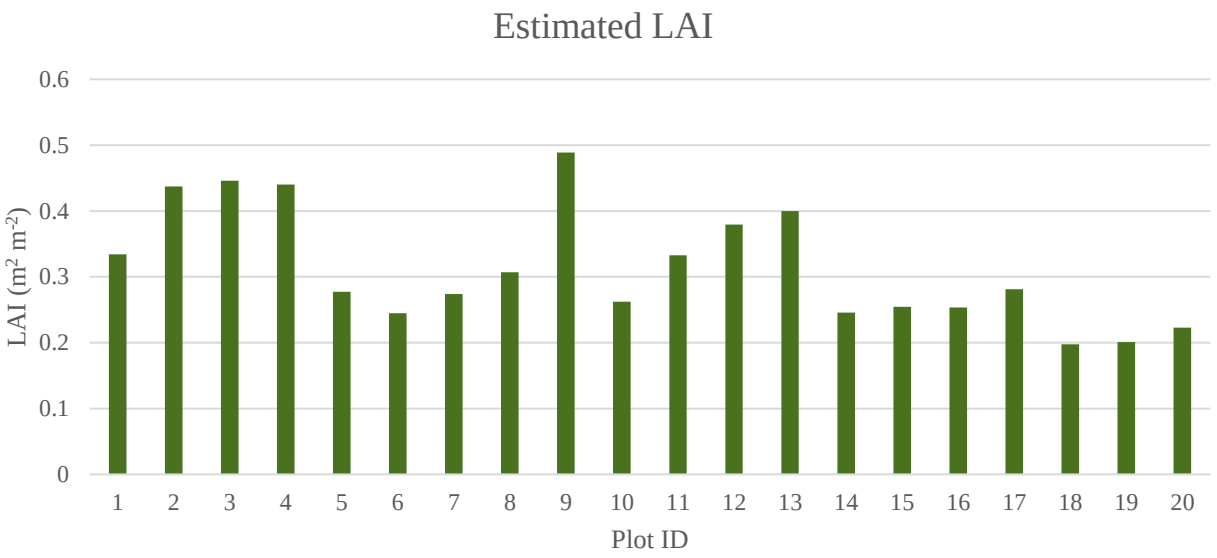


Figure D. Estimated leaf area index in plots.

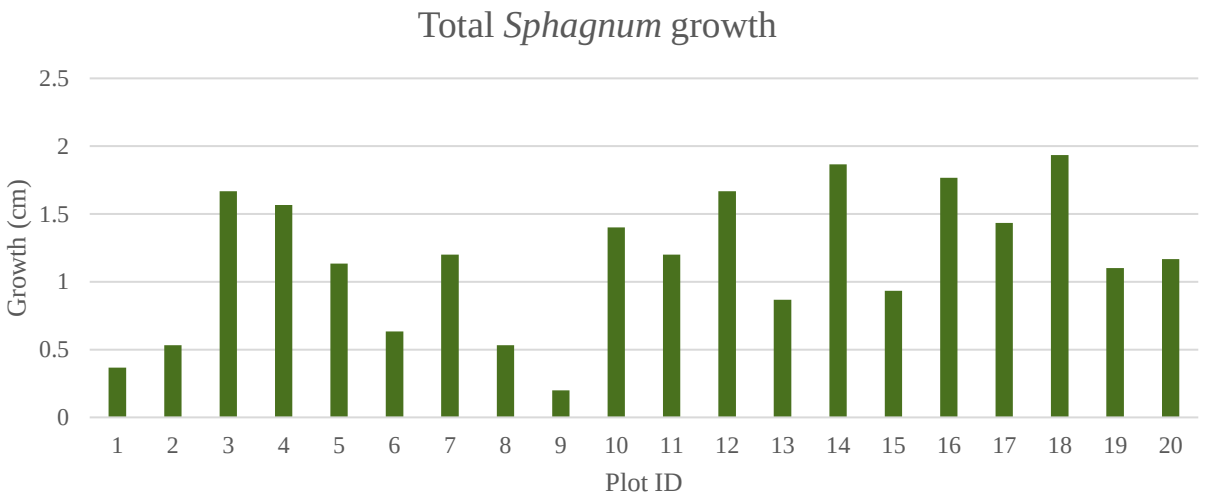


Figure E. *Sphagnum* growth varied notably between plots.

Appendix 2. Starting models of the linear models

Table A. Results of the linear model from July when all the explanatory variables were included.

July	Estimate	Std. Error	t value	Pr (>|t|)
Intercept	-4.956	7.108	-0.697	0.505
PAR	0.002	0.001	1.967	0.085
Relative humidity	0.067	0.044	1.521	0.167
Soil moisture	-0.884	0.81	-1.092	0.307
Air temperature	-0.039	0.095	-0.407	0.695
Soil temperature	0.199	0.274	0.726	0.489
GPP	0.003	0.001	2.105	0.068
$^{15}N_2$ fixation	0.048	0.109	0.444	0.669
CH_4 flux	-0.011	0.019	-0.576	0.581

Table B. Results of the linear model from August when all the explanatory variables were included.

August	Estimate	Std. Error	t value	Pr (>|t|)
Intercept	1.712	4.605	0.372	0.719
PAR	0.000	0.001	0.152	0.883
Relative humidity	0.003	0.010	0.325	0.752
Soil moisture	-0.026	0.067	-0.384	0.710
Air temperature	-0.139	1.036	-0.134	0.896
Soil temperature	-0.111	0.297	-0.373	0.718
GPP	-0.003	0.002	-1.207	0.258
$^{15}N_2$ fixation	0.062	0.065	0.950	0.367
CH_4 flux	-0.025	0.030	-0.828	0.429