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Department of Geography, Institute of Ecology and Earth Sciences, Faculty of Science and Technology, University of Tartu, Estonia.

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Supervisors: Prof. Ülo Mander
Institute of Ecology and Earth Sciences
University of Tartu, Estonia

Prof. Kalle Kirsimäe
Institute of Ecology and Earth Sciences
University of Tartu, Estonia

Associate Prof. Jaan Pärn
Institute of Ecology and Earth Sciences
University of Tartu, Estonia

Associate Prof. Mikk Espenberg
Institute of Ecology and Earth Sciences
University of Tartu, Estonia

Opponent: Prof. Dr. Gerhard Gebauer
University of Bayreuth
Germany

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CONTENTS

ORIGINAL PUBLICATIONS

This thesis is based on the following publications which are referred to in the text by Roman numerals. Published papers are reproduced in print with the permission of the publisher.

I. **Masta, M.,** Sepp, H., Pärn, J., Kirsimäe, K., Mander, Ü. 2020. Natural nitrogen isotope ratios as a potential indicator of N_2O production pathways in a floodplain fen. *Water* 12, 2, 409. https://doi.org/10.3390/w12020409

II. **Masta, M.,** Espenberg, M., Gadegaonkar, S.S., Pärn, J., Sepp, H., Kirsimäe, K., Sgouridis, F., Müller, C., Mander, Ü. 2022. Integrated isotope and microbiome analysis indicates dominance of denitrification in N_2O production after rewetting of drained fen peat *Biogeochemistry*. https://doi.org/10.1007/s10533-022-00971-3

III. **Masta, M.,** Espenberg, M., Pärn, J., Kuusemets, L., Thayamkottu, S., Sepp, H., Kirsimäe, K., Sgouridis, F., Kasak, K., Soosaar, K., Mander, Ü. 2022. ^{15}N tracers and microbial analyses reveal in situ N_2O sources in contrasting water regimes on drained peatland forest. *Soil Biology & Biochemistry*. Under review.

Author's contribution to the articles denotes: '*' minor contribution, '**' moderate contribution, '***' major contribution.

Categories			
	I	II	III
Original idea	**	**	**
Study design	**	***	**
Data processing and analysis	**	**	**
Interpretation of the results	**	***	***
Writing the manuscript	***	***	***

ABBREVIATIONS AND ACRONYMES

ANOVA	analysis of variance
C	carbon
CH_4	methane
CO_2	carbon dioxide
comammox	complete ammonia oxidation
CRDS	cavity ring-down spectroscopy
DNRA	dissimilatory nitrate reduction to ammonium
ECD	electron capture detector
FID	flame ionization detector
FTIR	Fourier-transform infrared spectroscopy
GHG	greenhouse gas
GPP	gross primary production
GC	gas chromatograph
GWP	global warming potential
IPCC	The Intergovernmental Panel on Climate Change
N	nitrogen
NEE	net ecosystem exchange
N_2O	nitrous oxide
ORP	oxidation-reduction potential
PCA	principal component analysis
PVC	polyvinyl chloride
QCLS	quantum cascade laser absorption spectrometer
qPCR	quantitative Polymerase Chain Reaction
SWC	soil water content

1. INTRODUCTION

Peatlands are one of the important regulators of greenhouse gases even though they cover a small land area. Riparian ecosystems and floodplains are important buffers which control water quality and other ecosystem services but also are sources of nitrous oxide, a powerful ozone-depleting gas (Mander et al., 2017; Liu et al., 2020). With increasing population and food demand, fertilization application has also increased. This has increased the extent of nitrogen-rich soils, which leads to an increase in N_2O emissions (Butterbach-Bahl et al., 2013). Consequently, nitrogen load on riparian ecosystems and floodplains has been increasing and resulted in potentially high N_2O emissions. N_2O production in soil is affected by many factors, such as electron availability, soil oxygen concentration and availability of microbes (Masta et al., 2020). It has been observed that N_2O emissions follow a parabolic relation with changing soil moisture (Pärn et al., 2018). Peaks in N_2O emissions followed by a sudden drop and lower soil nitrate levels have been observed as aftereffects of flooding. These sudden and short-lived peaks are major contributors to global N_2O emissions (Inubushi et al., 2003; Toyoda et al., 2011; Yano et al., 2014; Pärn et al., 2018, Schindler et al., 2020).

Many microbial processes, such as nitrification, denitrification and dissimilatory nitrate reduction to ammonium (DNRA), are responsible for the production of N_2O (Hu et al., 2015; Denk et al., 2017). Nitrogen (N) pathways are controlled by many key genes such as *nirS* and *nirK* genes for denitrification, bacterial *amoA*, archaeal *amoA*, and complete ammonia oxidation (comammox) *amoA* genes for nitrification and *nrfA* genes for DNRA (Hu et al., 2015). Nitrification is the oxidation of ammonia under aerobic conditions and is a two-step process, where the first step is catalyzed by *amoA* (Freitag et al., 1987). On the other hand, heterotrophic denitrification reduces oxidized mineral forms of nitrogen and leads to gaseous products such as N_2O or N_2 (Philippot et al., 2007). Due to heterogenic nature of soil, anaerobic and aerobic zones can coexist, which leads to a variety of microbial activity at the same time. This makes it difficult to calculate the individual contributions of each process (Mathieu et al., 2006). Complete denitrification (reduction of N_2O) is the only process which consumes N_2O and reduces it to inert dinitrogen (N_2) (Butterbach-Bahl et al., 2013). The key microbes responsible for this reduction step are *nosZ* clade I and II genes (Jones et al., 2014).

Currently, the methods available to attribute N_2O fluxes to its production or consumption process are limited. Nitrification inhibitors have been used for over two decades to differentiate nitrogen pathway processes. A small amount of acetylene has been found to eliminate the production of N_2O from ammonia oxidation and nitrifier denitrification (Zhu et al., 2013). In this method, the concentration of acetylene is very critical as high levels of acetylene can also inhibit the last step (reduction of N_2O) of denitrification and lead to N_2O accumulation (Klemedtsson et al., 1988; Abed et al., 2013). One of the common methods used for this has been ^{15}N tracer gas flux measurement (Sgouridis et al., 2015; Kulkarni

et al., 2014). The process involves the addition of a ^{15}N tracer in the form of ammonium and nitrate. The applied tracer can be detected via the IRMS system, and we can partition the sources between nitrate and ammonium. This method can also be used under *in situ* conditions as it offers minimal disturbance to soil. The N_2O derived from $^{15}N\text{-}NO_3^-$ is generally quantified to denitrification and nitrification derived N_2O is associated with $^{15}N\text{-}NH_4^+$ (Baggs, 2008). In some studies, the application of $^{14}NH_4\ ^{15}NO_3$ and $^{15}NH_4\ ^{14}NO_3$ to calculate the relative contribution of nitrification and denitrification for N_2O emissions (Baggs and Blum, 2004; Müller et al., 2014). The limitation of the ^{15}N tracer method is that it cannot be used to partition individual processes as bacterial or archaeal ammonia oxidation (Wrange et al., 2005). Isotopologues of N_2O, which differ by terminal (β – beta) or central (α – alpha) position of nitrogen, are termed as isotopomers. These have been studied to connect N_2O fluxes to respective production processes over the last two decades (Yu et al., 2020). The difference in the enrichment of the central and terminal position of ^{15}N is called its site preference ($SP = \alpha - \beta$), which has been considered as a potential indicator of nitrous oxide production or consumption processes (Toyoda et al., 2002). Based on isotopic fractionation, we can also gain additional information on N_2 fluxes, which are otherwise hard to measure (Decock et al., 2013). One common interpretation method that is used to understand site preference values is isotopic mapping. In this method, a dual isotope plot is made, which presents the information and relations between different isotopic parameters. With this, we get insight into different N_2O production and consumption processes (Ostrom et al., 2007; Toyoda et al., 2011; Zou et al., 2014; Lewicka-Szczebak et al., 2017; Buchen et al., 2018; Yu et al., 2020). N_2O production via different processes can lead to enrichment of ^{15}N on both positions, and a wide variety of site preference values have been reported for different N_2O production and consumption processes (Sutka et al., 2003, 2004, 2006, 2008; Well et al., 2009; Hu et al., 2015). The enrichment of ^{15}N on the central (α) position is generally attributed to nitrification, and denitrification has been associated with lower site preference with enrichment at both positions (Santaro et al., 2011). This method with natural abundances can be used to partition individual processes (Mathieu et al., 2006; Well et al., 2008; Kato et al., 2013).

To solve the problem of overlapping site preference values, microbial analysis of soil can provide strong support and help us distinguish between simultaneously active N-pathway processes. Relative shares of key genes which are responsible for different microbial processes can be measured using qPCR (quantitative Polymerase Chain Reaction), which is a precise and sensitive method (Levy-Booth et al., 2014; Smith and Osborn, 2009; Smith et al., 2006). qPCR can show the potential for N cycle processes as they are dependent on many key controlling genes. When gene abundances and N_2O emissions are compared, then qPCR can also suggest gene activity. Due to possible bias in PCR amplification and poor primers coverage, qPCR can also offer some disadvantages (Kuypers et al., 2018). Hence, with combination of microbial and isotopic analyses, it is possible to make a more precise estimate of N_2O production and consumption processes.

In a limited number of previous studies, the integration of ^{15}N tracer and nitrogen cycle control gene studies have been used to identify sources of N_2O fluxes. Ma et al. (2008) used it for analysing the relationship between nitrifier and denitrifier community composition and abundance in predicting N_2O emissions from pothole peat soils. Suenaga et al. (2021) used the combination of ^{15}N tracer and microbial analyses to disclose the N_2O sink potential of the anammox community. In their review papers, Hu et al. (2015) and Yu et al. (2020) also mentioned the importance of combined approach. Nevertheless, the full range of nitrogen cycle control genes has not been used in previous studies.

The conceptual diagram of combined ^{15}N tracer and microbiome methods to study N_2O fluxes from peatland soils in this thesis is shown on Figure 1.

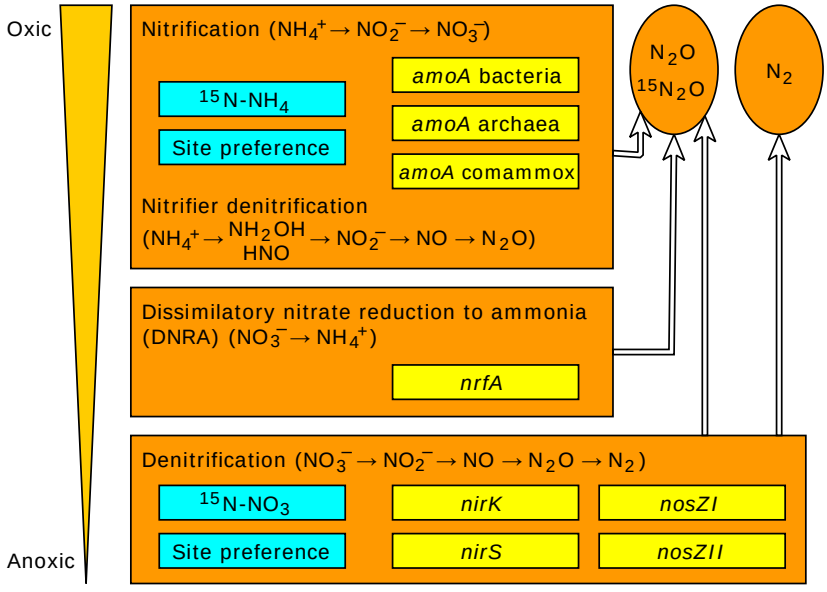

Figure 1: Conceptual diagram of combined ^{15}N tracer and microbiome methods to study N_2O fluxes from peatland soils. Blue boxes indicate isotopic methods, and yellow ones indicate key marker genes of nitrogen cycle. Site preference is based on natural abundance isotopic signatures.

The main objectives of this study were:

(1) to estimate the impact of water table and oxygen content on N_2O pathways using natural isotope composition and flooding in drained peatlands.

(2) to analyse the dependence of N_2O fluxes on soil moisture regime (dry, intermediate and flooded) and identify key source processes of N_2O fluxes, supported by isotopic signatures and gene abundances in a floodplain fen.

(3) to understand and identify key processes responsible for N_2O fluxes in peat forest using gene abundances and isotopic signatures.

We hypothesised the following: (1) N_2O emissions are higher from drained peat than in flooded (Articles I & III), (2) based on the labelled ^{15}N, NH_4 is the dominant source of N_2O in the drained peat whereas NO_3 dominates N_2O production under the flooded conditions (Articles II & III), (3) bacterial, archaeal and comammox nitrification, and nitrifier denitrification are the dominant processes of N_2O emissions in drained peat, whereas incomplete and complete denitrification are responsible for N_2O fluxes in flooded peat (Articles II & III).

2. MATERIALS AND METHODS (Articles I–III)

2.1 Site description (Articles I & III)

For the study in **Article I**, we sampled gas in at Kardla (Kärevere) drained fen located in the floodplain of Emajõgi river near Tartu, Estonia (58°25'N; 26°30' E; Fig. 2). The temporarily flooded fen has been managed more than 60 years as hayfield, last two decades for the nature conservation purpose. Intact soil columns for the laboratory experiment in **Article II** were taken from the same study area.

The study site for **Article III**, was a drained peatland near Agali (58°17'N; 27°19' E, Fig. 2) village in Kastre municipality of southeast Estonia. The experimental site is an *Oxalis*-type drained peatland forest mainly dominated by birch and spruce trees. The depth of the nitrogen-rich peat is more than 50 cm. The site is located close to the Apna Canal, from which water was taken to conduct flooding during the experiment.

Figure 2: Location of study sites in Estonia. 1 – Kardla floodplain fen, 2 – Agali drained peatland forest.

2.2 Experimental Setup

For **Article I,** the samples were taken from three different positions between 05.08.2018 and 12.09.2018. The positions differed in terms of their distance from the river, which affected the soil moisture and oxygen concentration. The three positions were labelled as A, B and C depending on their distance from the river (58°25′41.0″ N 26°30′30.3″ E; 58°25′38.2″ N 26°30′45.6″ E and 58°25′35.5″ N 26°31′02.8″ E; Fig. 3). Position A was farthest from the river, and position C was closest. The chambers for gas collection were installed in a shape of equilateral triangle with side length of 1.6 meters. On position C, wooden planks were installed to create walls for the triangle. The height of the sidewall for this triangle was 20 cm above soil surface. This position (C) was also flooded with water from the nearby ditch. The flooding lasted for 2 hours before each sampling session, and a garden pump was used to pump the water to achieve anoxic conditions. We also installed oxygen sensors at depths of 5 cm, 25 cm, and 50 cm to get a vertical profile for soil oxygen concentration. Gas samples were collected in 50 ml vials using white 65-liter chambers, which were used on top of collars at all three positions.

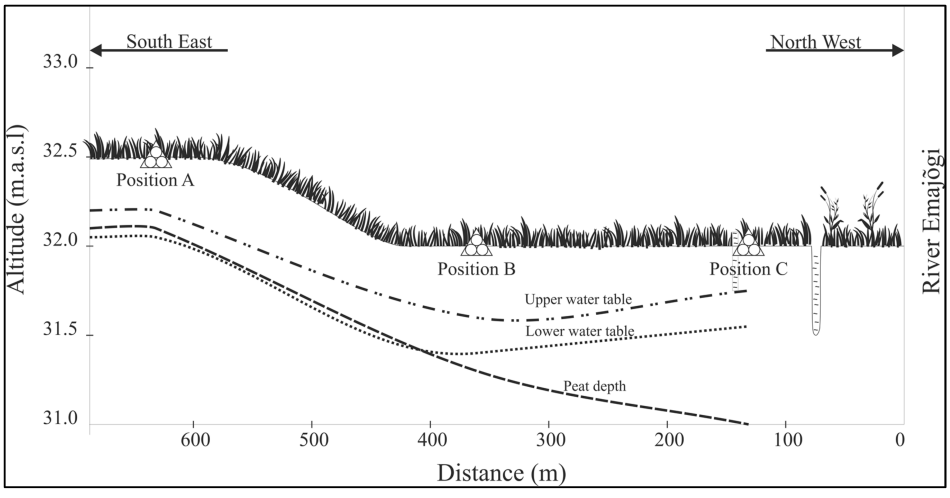

Figure 3: Diagram for the study site showing peat depth and variation of ground water level at Kardla near Tartu, Estonia.

For **Article II**, the experimental setup was designed in laboratory environment at the University of Tartu in Estonia. We used thirty-six 7-liter plastic microcosms for two arrays. These microcosms were filled with topsoil peat monoliths taken from a floodplain sedge fen in Kardla, a nature reserve close to Tartu in Estonia. The water content at this site varies from season to season with a range of 0.9 during spring to 0.3 in dry summer. The microcosms were filled with peat up to the height of 10 cm, and we left a headspace of 12 cm, which also facilitated plant growth during the experiment (Fig. 4). We also installed an oxygen sensor

(4-spot Presens Fibox) and water table indicator on each microcosm. After initial air drying, the bulk density of the peat was from 0.44 g cm^3 to 0.56 g cm^3 and then we further air-dried the microcosms on a laboratory table-top and the final moisture value was 0.12 ± 0.008 m^3/m^3. Then we added to achieve intermediate (0.53 ± 0.03 m^3/m^3) and flooded (0.85 ± 0.06 m^3/m^3) conditions. The flooding lasted for one hour before the beginning of each sampling session.

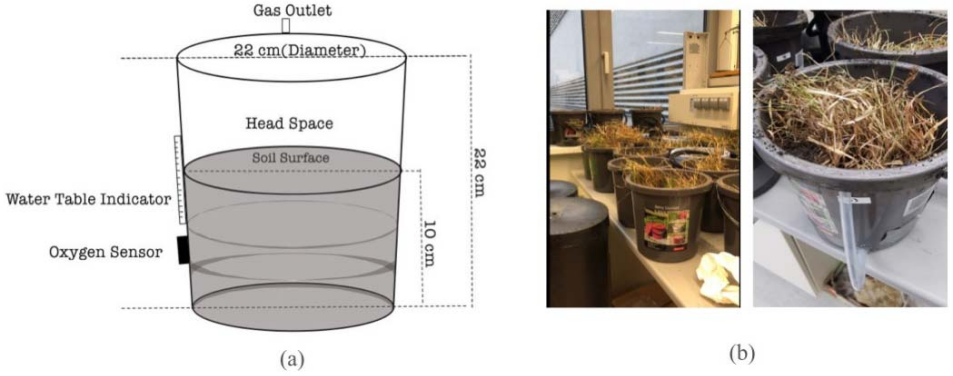

Figure 4: Laboratory experimental setup. (a) Schematic for the microcosm design at the lab with dimensions. (b) Top and side view of the microcosm in the laboratory.

The experiment in the drained peatland forest site (**Article III**) began in July 2020 and six equilateral triangles with each of side length of 1.6 m were constructed to create two different soil moisture regimes (flooded and drained). The experiment site was a mixed forest dominated by birch and spruce trees. The soil moisture for drained triangles was in the range of 0.50–0.65 m^3/m^3 and for flooded triangles the range was 0.71–0.92 m^3/m^3. We used wooden plans to make sides for these triangles and each of these sides had a depth of 40 cm in inside soil and 20 cm above soil surface. Water table observation wells were installed in all triangles along with draining/flooding wells. For gas measurements, three circular collars of 65 cm diameter were installed per triangle (Fig. 5). Flooded and drained regime triangles were 10 meters apart, and the flooding experiment began in October 2020. The water for flooding was taken from the Apna Canal. In the drained as well as the flooded triangles, pumps were used to drain and flood the water. The flooding started pre-treatment and continued for the whole experiment. The soil on the site had high organic matter, which indicated rich variability of material for the formation of peat via decomposition. The overall soil properties for drained and flooded triangles were similar.

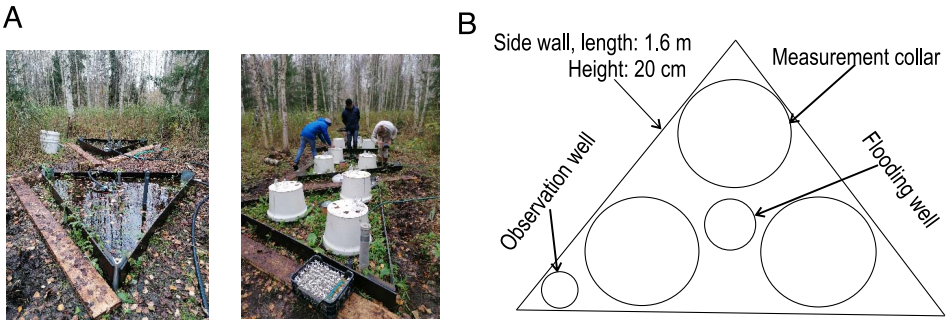

Figure 5: Photo for geographical location of study site (A) and the schematic diagram of study site design (B).

2.3 Gas flux analyses

Gas samples were collected at the beginning of each session which was followed by sampling after every twenty minutes for an hour. We used 50 ml glass vials for the collection of gas samples for N_2O flux measurement and 100 ml glass vials for the gas samples for measurement of N_2O isotopes. The sample for isotope analysis was taken at the end of each session with two duplicate samples. This was done as we measured N_2O and NO^- in two sequences and used one vial for N_2O and two vials for NO^-. This was done to ensure enough concentration in the IRMS. We used an electron detector equipped gas chromatograph for the measurement of N_2O emissions. The targeted gas concentration was determined using Shimadzu GC-2014 which as equipped with an electron capture detector and a flame ionisation detector and they were coupled with a Loftfield auto-sampler (Loftfield et al., 1997).

For determining the isotopic composition of N_2O, we used a Delta V Advantage mass spectrometer (Thermo Fisher Scientific, Germany) which was coupled with a modified Precon and Gas Bench II (Masta et al., 2020). The amplifier resistor values for the Faraday cups were 3×10^8, 3×10^{10} and 1×10^{11} for masses 44, 45, 46, respectively. The IRMS was also equipped with a Universal 3 collector. The amplifier resistor for mass 30 was 3×10^8 and for mass 31 was 3×10^{11}. Due to the three-cup calibration of our IRMS, it was not possible for us to measure all the masses (44, 45, 46, 30 and 31) together. Therefore, we measured N_2O and NO^- with two sequences. All the calculations for gas isotopes were based on Toyoda et al., 1999. Our lab standards were calibrated with Johann Heinrich von Thünen Institute's standards (Well et al., 2009), which are calibrated in accordance with the Tokyo Institute of Technology's (Toyoda et al., 1999) standards. ^{15}N was calibrated against air and ^{18}O was calibrated against V-SMOW.

2.4 Isotopologue studies

We calculated the ^{15}N site preference (SP) and isotopomers ratios (bulk $\delta^{15}N_{gas}$ from the untreated control microcosms) according to Toyoda et al. (1999) and performed a ^{15}N calibration with Thünen Institute (Braunschweig, Germany). VSMOW was used to calibrate ^{18}O and isotopomer ratios were defined as follow:

$$\delta^{15}Ni = {}^{15}R_{sample}i/{}^{15}R_{standard} - 1 \text{ (i = } \alpha, \beta, \text{ or bulk)}$$

$^{15}N^{\alpha}$ and $^{15}N^{\beta}$ were defined as the central (α) and terminal (β) positions of the linear N_2O molecule. The site preference values were measured according to $SP = \delta^{15}N^{\alpha} - \delta^{15}N^{\beta}$ and $^{15}R_{bulk}$ was used to denote the average of $^{15}N/^{14}N$ ratios. The site preference and δ values were expressed in per mil (‰). For conversion of measured ratios to isotopomer ratios, we used the following equations as in Toyoda et al. (1999):

$$^{45}R = {}^{15}R^{\alpha} + {}^{15}R^{\beta} + {}^{17}R$$

$$^{46}R = {}^{18}R + ({}^{15}R^{\alpha} + {}^{15}R^{\beta})\ {}^{17}R + {}^{15}R^{\alpha}\ {}^{15}R^{\beta}$$

$$^{31}R = {}^{15}R^{\alpha} + {}^{17}R$$

$$^{32}R = {}^{18}R + {}^{15}R^{\alpha}\ {}^{17}R$$

Here mass 45 and 46 are denoted by ^{45}R and ^{46}R respectively and ^{17}R denotes the heavy oxygen with ^{32}R and ^{31}R isotopomers.

2.4.1 Natural abundances of ^{15}N isotopologues

We collected the soil samples from the top 5 cm and analysed them for total N isotopes. The soil samples were first dried for 48 hours at 60 °C for homogenization and removal of moisture. For N and carbon (C) isotope analysis, we used 1 milligram of the sample and packed it into tin capsules. We used a Delta V Plus mass spectrometer which was coupled with a Flash HT element analyser for the calculation of ^{15}N. The isotope calibration was done with IAEA-N1 and IAEA-N2 international standards. Delta notations were used to express the isotopic ratios as (Schmidt et al., 2004):

$$\delta X = (\ R_{Sample} / R_{Standard} - 1) \times 100$$

Here X = ^{15}N and R = $^{15}N/^{14}N$ and AIR is the international standard for ^{15}N.

2.4.2 Tracer studies

For the addition of ^{15}N-labelled tracer, we used ^{15}N-labelled ammonium-chloride (>98 atom%, Sigma Aldrich) and potassium nitrate (>98 atom %, Sigma Aldrich). The tracer was added on the basis of already present nitrate and ammonia concentration in the soil. A 5% enrichment to the ambient nitrate and ammonia values was made and the tracer was mixed with water to make a solution which was then injected to the top 5 cm of the soil (Kulkarni et al., 2014). In case of **Article II**, we used tap water to make a 50ml solution and in **Article III**, we used the water from the Apna Canal and made a 250 ml solution. After the tracer application, we waited for 15 minutes for the tracer to take effect, which was followed by gas sampling for one hour.

2.5 Microbiome studies

We isolated the DNA from the soil samples using a DNeasy Powersoil Pro Kit (Qiagen, USA) and followed the protocol provided by the manufacturer. We isolated the DNA from 0.25 g of wet soil and used Precellys 24 (Bertin Technologies, France) to homogenize the samples for 20 seconds at 5000 rpm. To assess DNA quality and concentration, an Infinite 200 M spectrophotometer (Tecan AG, Austria) was used, and the isolated DNA was stored at −20 °C in the freezer (**Articles II & III**).

We used quantitative polymerase chain reaction (qPCR) to quantify the 16S rRNA genes in archaea and bacteria. We also quantified the abundances of nitrification (bacterial and archaeal *amoA*) and denitrification (*nirK*, *nirS*, *nosZI* and *nosZII*) genes. qPCR reactions were performed in a Rotor-Gene Q thermocycler (QIAGEN, Germany). The reaction mixer volume was 10 µl and it contained forward and reverse primers. Triplicates were used to amplify every sample, and all qPCR measurements received three DNA-free negative control samples. Rotor-Gene® Q software v.2.0.2 (QIAGEN) and LinRegPCR v.2020.2 (Netherlands) were used to calculate qPCR results. The number of gene copies was calculated using standard curve ranges, and the gene copies were expressed in the number of gene copies per gram of dry matter (copies/g dw). The detailed qPCR methodology can be found in Espenberg et al. (2018).

2.6 Ancillary soil and water analysis

The soil samples for physiochemical analysis were collected from the top 10 cm of the soil surface. The chemical analyses were done at Plant Biochemistry Laboratory at the Estonian University of Life Sciences according to standard methods (APHA,1989). We calculated ammonium (NH_4^+-N), nitrate (NO_3^-), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and pH values along with total nitrogen (N), percentage of dry and organic matter.

2.7 Statistical analysis

We used t-tests to determine differences in nitrate and ammonia gene copy numbers and concentrations using STATISTICA v.7.1 (Tibo, USA). For comparing enrichment of $R^{45/44}$ vs $R^{46/44}$ under different amendments, we used linear regression. Soil physiochemical parameters and gene copy number correlations were determined using spearman's correlation. R package "akima" v.0.6-2.1 (Akima et al., 2020) was used to make interpolated contour plots. The dot size represented N_2O fluxes and colours were used to specify each treatment. Gene abundances were put to Z variable. X and Y axis represented SP and $\delta^{18}O$, respectively.

3. RESULTS AND DISCUSSION

3.1 N_2O source attribution in a floodplain fen

3.1.1 ^{15}N natural isotope ratios (Articles I & II)

In our study for **Article I**, we observed negative site preference values for all three positions of our experiment. Under sub-oxic conditions (Position B), we found a positive correlation of site preference with $\delta^{18}O$. Similar increasing trend has also been observed in previous studies (Köster et al., 2013; Mothet et al., 2013; Mander et al., 2014; Lewicka-Szczebak et al., 2017). Hence in our study, the low site preference values and the positive correlation of site preference and $\delta^{18}O$ indicate nitrifier denitrification as the major contributor of N_2O fluxes in a floodplain fen (Fig. 6; Butterbach-Bahl et al., 2013; Hu et al., 2015; Wrange-Mönnig et al., 2018).

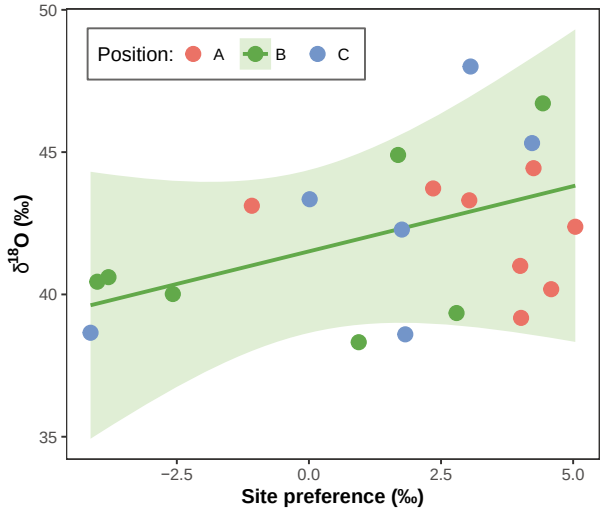

Figure 6: N_2O SP (site preference) vs $\delta^{18}O$ relationship for all measurements in the Kardla site (Article I). Position A (drained with water table of 54 cm), B (drained with water table of 45 cm) and C (flooded) show the site location of sampling, which was based on the distance from the Emajõgi River, position C (150 m) being closest to the river and position A (650 m) as farthest.

We recorded site preference values ranging from −22‰ to +42‰ during our laboratory experiment for **Article II**. This range indicates that multiple overlapping processes were in operation. The site preference values indicate dominance of bacterial denitrification during this experiment. Ammonia oxidation and fungal denitrification were also observed as secondary processes which contributed to N_2O fluxes (Hu et al., 2015; Yu et al., 2020) (Fig. 7).

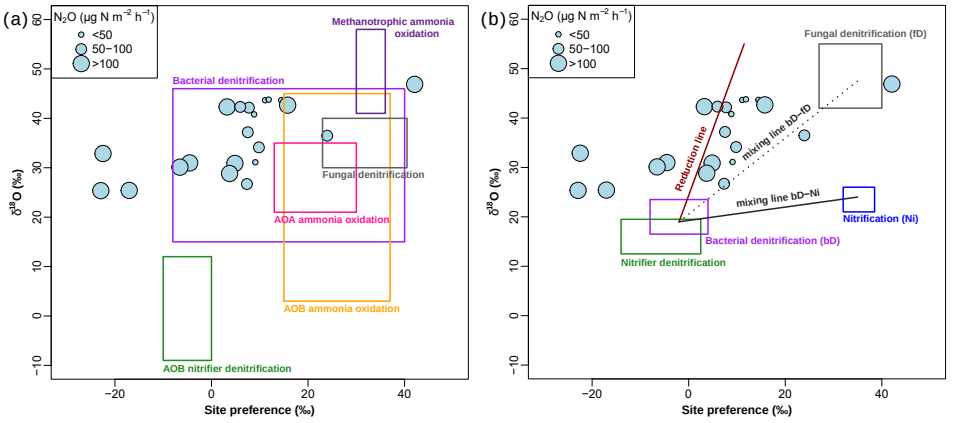

Figure 7: $\delta^{18}O$, site preference and N_2O emission values for all water treatments in control microcosms (which did not receive tracer). The expected ranges for different microbial processes are shown by the coloured boxes based on (a) Hu et al. (2015) and (b) Yu et al. (2020). The size of the bubble shows the magnitude of N_2O emission.

Here it is clear that site preference values offer the problem of overlapping of different microbial processes. Therefore, for a more precise estimate of N_2O sources, we used a combined approach of tracer, physio-chemical, microbial and isotopomer analyses. On the combination of different methods, we observed that *nir*, *nos* and archaeal *amoA* share similar abundance zones when compared with site preference and $\delta^{18}O$ (Fig. 8). We observed that *nir* and *nosZ* genes were major sources of N_2O emissions under flooded conditions. Denitrification was found to be the major source of N_2O emissions on combination of microbial and isotopic analyses. During the ^{15}N tracer study, Mathieu et al. (2006) also reported that most N_2O emissions were contributed by denitrification under saturated conditions. In organic soils, the dominance of denitrification has also been reported under flooded conditions, and different flooding rates affect N_2O production (Tomasek et al., 2019). Anoxic conditions are favoured by the denitrification mechanism, which could be affected by flooding, and hence we observed that our results support previous studies from isotopic and microbial analysis (Yu et al., 2020).

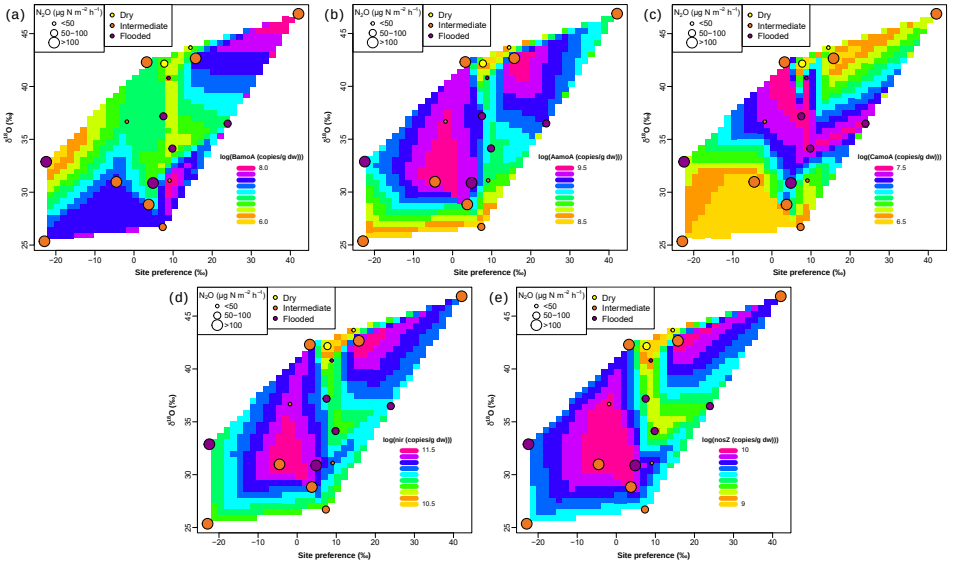

Figure 8: Relationships of abundances or gene copy numbers (nitrification genes: bacterial *amoA* (BamoA) with site preference and $\delta^{18}O$, (a), archaeal *amoA* (AamoA) (b), comammox *amoA* (CamoA) (c); denitrification genes: *nir* (d), *nosZ* (e)) and N_2O fluxes between the control mesocosms under the treatments (dry, intermediate and flooded). The gene abundances were plotted as the z dimension, with SP and $\delta^{18}O$ on the x-axis and y-axis, respectively. Size of dots showed N_2O fluxes, and water treatments were colored differently.

For intermediate treatment, we observed dominance of nitrification genes (bacterial *amoA* and archaeal *amoA*). In SP ranges of +8‰ to +11‰ and +21‰ to +40‰, bacterial *amo*A showed dominance. Archaeal *amo*A was more dominant in the SP range of −10‰ to +8‰ and +14‰ to +27‰. Hence, our results indicate denitrification via *nir* genes as the second major source of N_2O emissions under this treatment. It has also been shown that almost 88% of N_2O emissions are attributed to nitrification under laboratory conditions under intermediate moisture conditions (Well et al., 2008; Huang et al., 2014; Hu et al., 2015). Nitrification has also been observed as a dominant process in agricultural soils under intermediate conditions (Liu et al., 2016).

3.1.2 ^{15}N tracer and microbial analysis (Article II)

^{15}N tracer application showed that labelled nitrate under flooded conditions was the largest source of N_2O emissions (130 µg N m^{-2} h^{-1}) (Fig. 9). The second highest contribution was observed by labelled nitrate pool under intermediate treatment. Labelled ammonia showed low consumption and low N_2O emissions under all treatments. Similar denitrification rates have been reported under flooded conditions in agricultural soil (Mathieu et al., 2006). Khalil and Baggs

(2005) also attributed the highest N_2O emissions to denitrification under high water content. Loick et al. (2021) have reported dominance of denitrification under high moisture content during their ^{15}N tracer study.

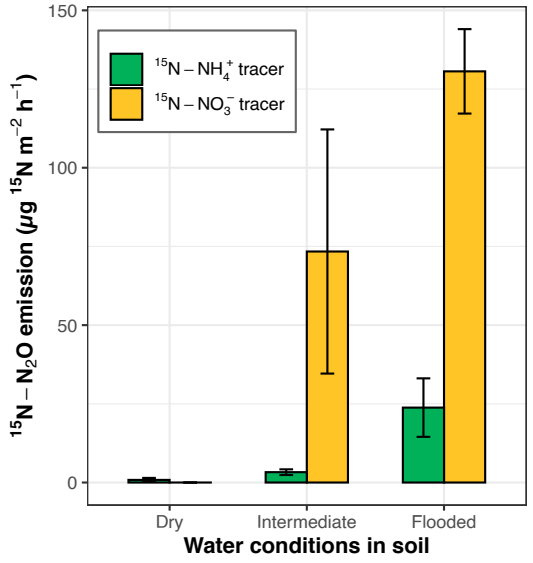

Figure 9: ^{15}N-N_2O emissions proportions from ^{15}N-NO_3 and ^{15}N-NH_4 tracer amendment for all three moisture manipulation treatments (n=8). Standard deviations and averages are also shown.

The abundances of bacterial 16S rRNA and archaeal 16S rRNA genes differed significantly between all the treatments ($p<0.05$ and $p<0.02$, respectively). Similar was observed for *nirS, nirK, nosZI, nosZII*, comammox *amoA* and *nrfA* gene abundances ($p<0.01$, $p<0.01$, $p<0.02$, $p<0.05$, $p<0.05$, $p<0.02$, respectively). The decreasing order of the functional genes in total abundances was as follow: *nirK*, *nirS*, *nosZI*, archaeal *amoA*, *nosZII*, bacterial *amoA*, comammox *amoA*, *nrfA*. Flooded treatment recorded the highest surge in gene copy numbers as compared to other treatments. comammox *amoA* showed significant increase with ammonium tracer as compared to nitrate (Fig. 10). Archaeal *amoA* genes showed a spike in comparison to bacterial *amoA*. *nirS* and *nirK* genes showed an increase only in microcosms with nitrate tracers. Under anoxic conditions, *nosZI* and *nosZII* gene numbers showed a rise (Fig. 11).

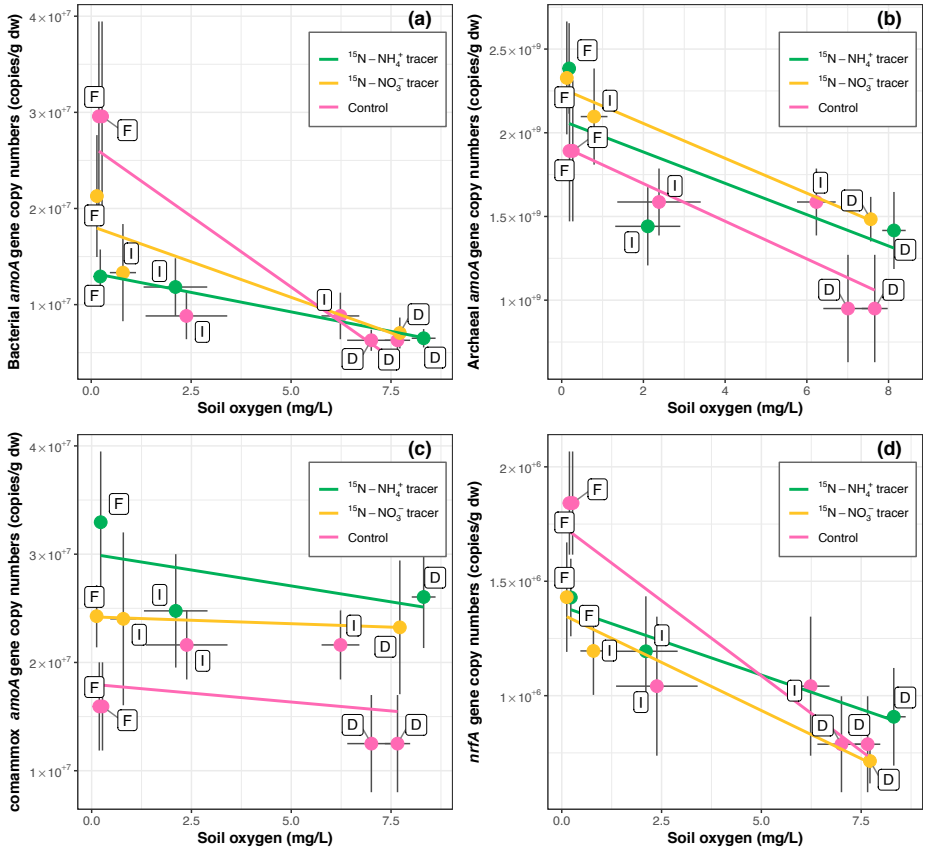

Figure 10: Soil oxygen and nitrification trends (i.e. bacterial *amoA* (a), archaeal *amoA* (b) and comammox *amoA* (c)) and DNRA (i.e. *nrfA* (d)) genes after different treatments (dry (D), intermediate (I), flooded (F)) and amendments (added tracers and control).

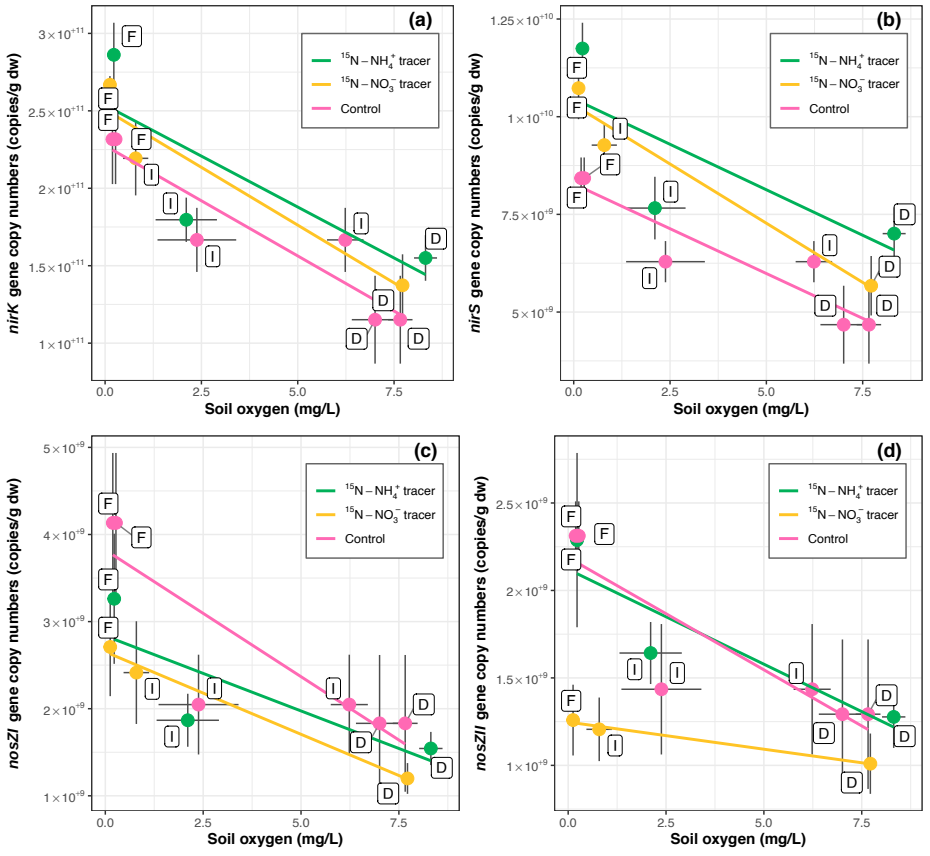

Figure 11: Soil oxygen and denitrification genes (i.e. *nirK* (a), *nirS* (b), *nosZI* (c) and *nosZII* (d)) trends after different treatments (dry (D), intermediate (I), flooded (F)) and amendments (added tracers and control).

3.2 N_2O source attribution in a drained peatland forest (Article III)

During our study at Agali forest, we observed differences in labelled ^{15}N-N_2O fluxes between flooded and drained treatments. Almost negligible N_2O emissions were recorded from flooded treatment. The N_2O emissions from the ammonia tracer (which indicates nitrification) peaked at 147 µg ^{15}N m^{-2} h^{-1} (Fig. 12). This indicated that nitrification was the dominant process producing ^{15}N-N_2O fluxes in drained peat forest.

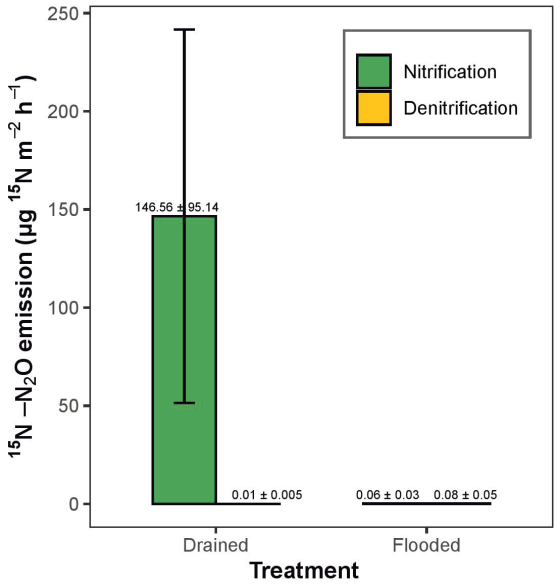

Figure 12: ^{15}N-N_2O emissions from drained and flooded treatments.

Although the contribution of denitrification was low for ^{15}N-N_2O, we recorded ^{15}N enrichment under both tracer amendments. Under nitrate amendment, the N_2O was enriched at both positions (α and β) relative to mass 44 with both mass 46 and 45, while under ammonia amendment, the enrichment was selective to one position with preference to mass 45 (Fig. 13). As nitrification was the major source under ammonia amendment, our results suggest enrichment at α position (Fig. 13). This difference in selectivity of mass 45 and 46 enrichment also suggests the presence of two different processes (nitrification and denitrification).

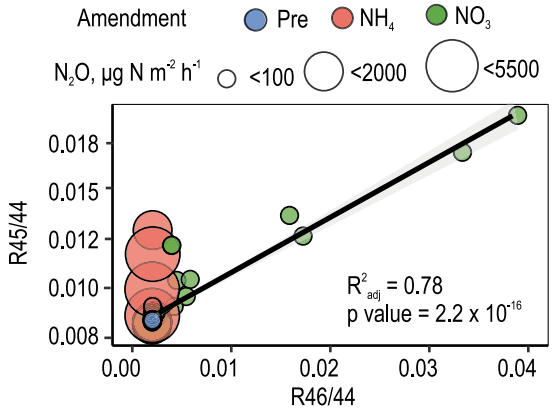

Figure 13: $R^{46/44}$ vs $R^{45/44}$ with total N_2O flux under different treatments.

During our study in the forest of Agali, we did not observe differences between bacterial gene copy numbers under different treatments. Gene copy numbers for archaea were found higher under drained treatment and ammonia amendment. Bacterial genes showed higher abundances under flooded treatment (Fig. 14).

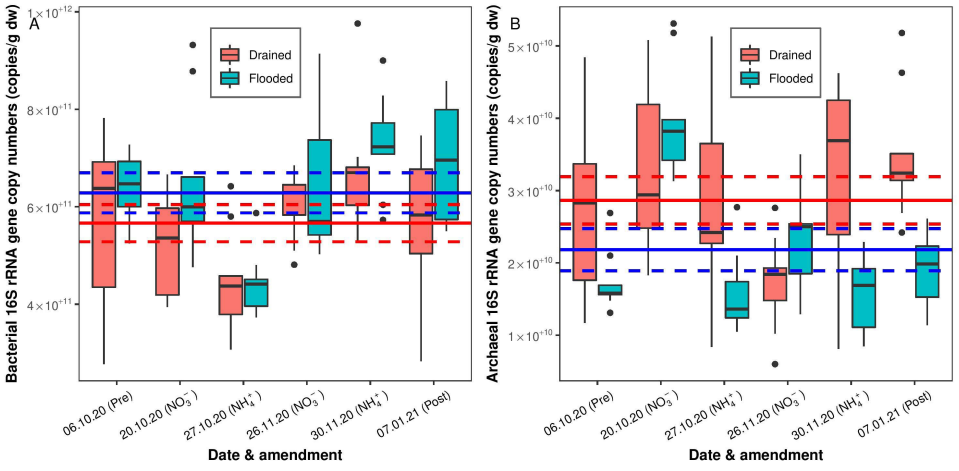

Figure 14: Abundances of bacterial (A) and archaeal (B) 16S rRNA gene copies under different treatments during the experiment. The central lines indicate median values, box edges indicate the 25th and 75th percentiles, whiskers represent the 95% confidence interval, and dots indicate outliers. The overall mean and 95% confidence intervals are shown by the solid and dashed lines, respectively, for drained (red) and flooded (blue) treatments.

Bacterial *amoA* gene copy abundances were found slightly higher under drained treatment with ammonia amendment. Overall, the archaea *amoA* abundances were also found higher under drained treatment (Fig. 15).

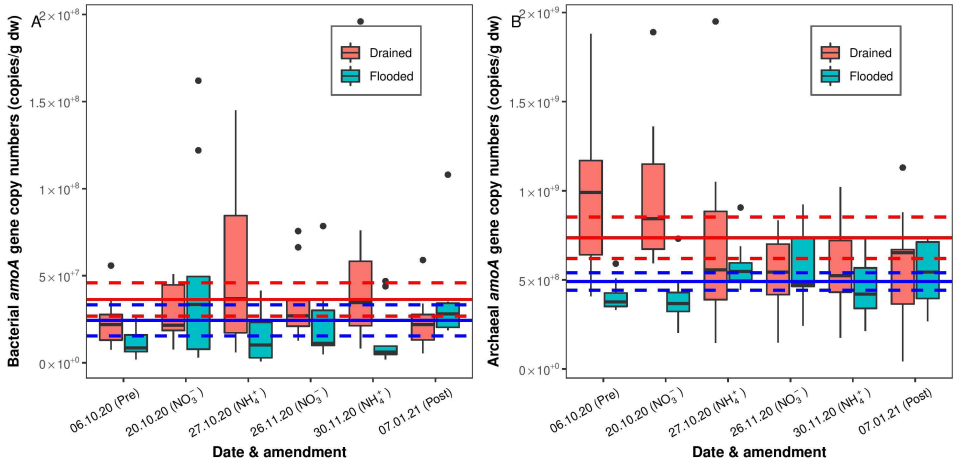

Figure 15: Abundances of bacterial (A) and archaeal (B) *amoA* gene copies in flooded and drained treatments during the experiment. The central lines indicate median values, box edges indicate the 25th and 75th percentiles, whiskers represent the 95% confidence interval, and dots indicate outliers. The overall mean and 95% confidence intervals are shown by the solid and dashed lines, respectively, for drained (red) and flooded (blue) treatments.

Under nitrate amendment, *nirS* gene copy abundances were found higher under drained treatment, although overall abundances were similar. A temporal trend for *nirS* was observed as the gene copy numbers decreased with lower temperatures. *nirK* gene copies were found lower in drained treatment under both tracer amendments. It was also observed that overall *nosZI* gene copy numbers were higher under flooded treatment (Fig. 16). Here, it is worth to mention that part of nitrate could be formed via the oxidation of organic nitrogen to NO_3^-, a recently considered pathway, accounted in lab experiments up to 83% of total NO_3^- production being higher in lower P concentration in soil (O'Neill et al., 2021). High organic matter and relatively low P content in soil, important preferences for this pathway, are typical for our study area.

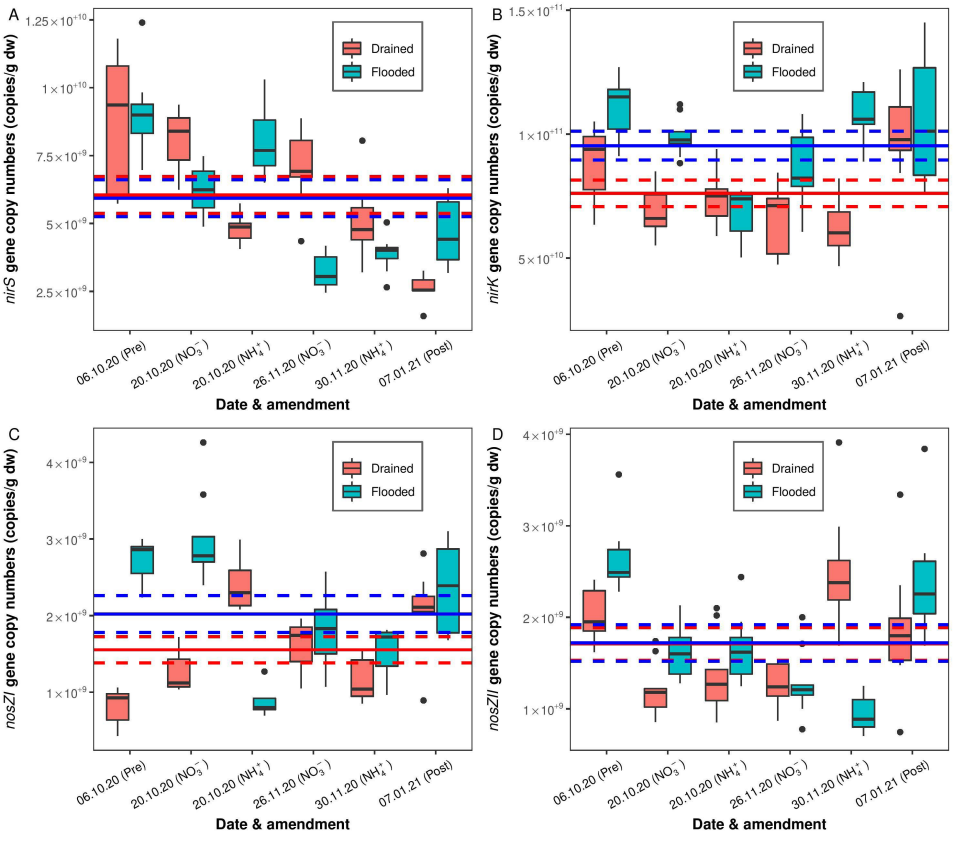

Figure 16: Abundances of *nirK* (A), *nirS* (B), *nosZI* (C), and *nosZII* (D) gene copies from both treatments during the experiment. The central lines indicate median values, box edges indicate the 25th and 75th percentiles, whiskers represent the 95% confidence interval, and dots indicate outliers. The overall mean and 95% confidence intervals are shown by the solid and dashed lines, respectively, for drained (red) and flooded (blue) treatments.

The presence of higher archaea genes under drained conditions could be the specificity of these genes towards harsh environments in peatlands, as observed by Espenberg et al. (2018). Similar was observed when we compared different amendments, as archaea genes preferred ammonia amendment, which indicates nitrification. This also suggest a low potential for archaeal nitrification under flooded conditions. Also, the amount of nitrate under drained treatment was higher, indicating nitrate consumption via denitrification under flooded treatment. The same was also observed for denitrification genes (*nirK* and *nosZI)* as well, as the process favours anoxic conditions (McKenney et al., 2001). A negative correlation as found between *nosZII* in drained treatment and *nosZI* in flooded treatment when compared against N_2O emissions. Espenberg et al. (2018) have reported such relations in their study of the tropical peat bog of French Guiana. This indicated ecological niches of *nosZI* and *nosZII* genes (Jones et al., 2014).

The site preference and $\delta^{18}O$ from our natural sites showed the problem of overlapping when compared with two previous studies (Hu et al., 2015; Yu et al., 2020). Therefore, it was difficult to separate N_2O production-consumption processes only on the basis of site preference. We coupled the microbial analysis with site preference values and found that it was not easy to distinguish nitrification and denitrification only from the isotopic analysis. Nitrification genes (*amoA*) were abundant in comparison to denitrification (*nir*) genes and showed major relations with N_2O fluxes from drained sites. On comparison of N_2O consumers (*nosZ*) and producers (*nir* and *amoA*), we found that the lowest emissions came from flooded treatment where *nosZ* was high in abundance (Fig. 17).

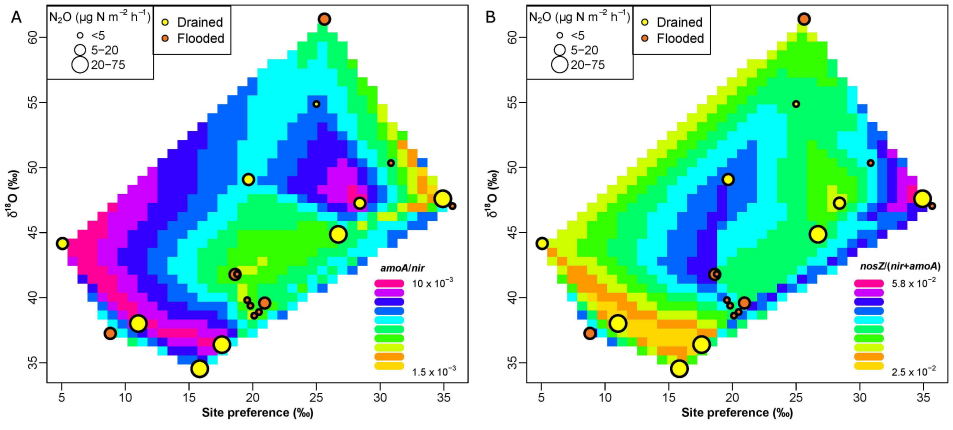

Figure 17: Site preference and $\delta^{18}O$ values relations with genetic parameter ratios and N_2O fluxes between all treatments (drained and flooded) and control. *amoA* to *nir* ratio (A) and N_2O consumers to producers genes ratio (B). The gene abundances were plotted as the z dimension, with SP and $\delta^{18}O$ on the x-axis and y-axis, respectively. Size of dots showed N_2O fluxes, and water treatments were colored differently.

Under both treatments (drained and flooded), site preference values increased with $\delta^{18}O$ and were positive. Positive site preference ranges have been reported for different processes such as fungal denitrification (+22‰ to +40‰), ammonia oxidation (+13‰ to +34‰) and bacterial denitrification (−7‰ to +24‰) (Well et al., 2009; Hu et al., 2015; Yu et al., 2020). These overlapping values clearly show the problem of the partitioning of N_2O fluxes on the basis of isotopic analysis alone.

Combining microbial and isotopic methods, we recorded higher abundances of *amoA*, *nosZII* and archaeal 16S rRNA under drained treatment and as the overall proportion of *nos*Z genes is lower than *amo*A in forest soil, which indicates the dominance of nitrification. Site preference range from (+5‰ to +17‰) coupled with gene abundances, strongly indicating the dominance of nitrification under drained conditions. High ^{15}N-N_2O flux, which was recorded under drained treatment, also supports our findings.

Under flooded treatment, we recorded site preference values ranging from (+8‰ to +20‰) and dominance of denitrifier genes. This indicates denitrification as a major source process under flooded treatment. The fluxes recorded under flooded treatment were very low and did not contribute much to the overall fluxes during the experiment.

The difference in mass enrichment for mass 45 and 46 also indicate the presence of two separate processes. The N_2O formed during nitrification is more depleted in ^{15}N and ^{18}O as compared to denitrification. The differences between $\delta^{15}N$ have been observed to occur while the reduction of N_2O (Baggs., 2008). This indicates that during our study, ^{15}N-NH_4 was precursor under drained treatment and ^{15}N-NO_3 was the precursor of N under flooded treatment.

4. SYNTHESIS

Even though the nitrogen cycle has been studied over a century, it has been challenging to associate N_2O emissions with its production and consumption processes. Extensive research has been done in two last decades to develop methods to partition the N_2O fluxes between the sources and processes. Source partitioning based on ^{15}N tracer method allows to distinguish only a relatively small fraction of the total N_2O flux (compare Figs. 9, 11 and 13). Therefore, other methods are needed to get more adequate results. In this thesis combination of ^{15}N tracer-based source partitioning method with microbiome analysis and isotopologue mapping was used to apportion N_2O fluxes. Results gained in these studies were promising and give a positive perspective for future research.

Figure 18 illustrates general outcomes of the experiments conducted in this thesis. The fen and the peatland forest showed different N_2O source processes related to water regime and availability of different mineral nitrogen forms. Accordingly, denitrification and nitrification were important in the flooded nitrate-rich fen peat depending on the soil oxygen level. The isotopologue mapping showed dominance of nitrifier denitrification in drained in-situ condition in the fen. Dry conditions achieved in the lab experiment suppressed practically all microbial activity. Drained forest peat showed the highest N_2O emissions from nitrification which was supported by isotopologue mapping and higher abundance of nitrifier genes (archaeal, bacterial and comammox *amoA*). In contrast, flooded forest peat showed signs of complete denitrification, which was supported by higher abundance of denitrifier genes (*nirK* and *nosZI*), and resulted in low N_2O emissions. In addition, n*rfA* genes (performing less-known DNRA process) showed significant importance in N_2O flux formation in lab-flooded treatments.

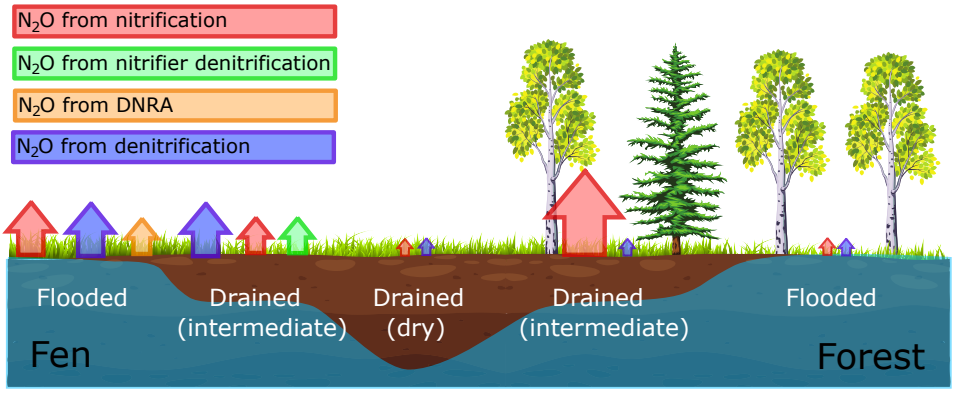

Figure 18: Generalized scheme of thesis outcomes in both lab and *in-situ* measurements: N_2O emissions depend on ecosystem type, groundwater depth and processes responsible for N_2O production. Mean values of N_2O emissions: high emissions (wide arrow) – >100 μg N_2O-N m^{-2} h^{-1}; medium emissions (medium-size arrow) – 20–100 μg N_2O-N m^{-2} h^{-1}, low emissions (narrow arrow) – 1–20 μg N_2O-N m^{-2} h^{-1}, and close to zero emissions (the smallest arrows) – 0.0–1.0 μg N_2O-N m^{-2} h^{-1}. Please note that figure uses source partitioned N_2O flux values (see Figs. 9 and 11) and not total N_2O flux (see Fig. 13 for those).

Peatland drainage can result in dry or intermediate soil moisture conditions. In the case of dry regime, the thesis showed that the microbial activity reduced drastically and resulted in close to zero N_2O emissions. This experiment is simulating severe droughts, which are becoming more frequent with climate change, rather than artificial drainage alone. For intermediate soil moisture conditions, we found contribution from overlapping of multiple processes with dominance of nitrification as a major source.

Restoration of drained peatlands can result in hot spots of N_2O emissions, which could be caused by incomplete denitrification or nitrification. The results of this thesis could be valuable to avoid N_2O emissions via the optimisation of water regime during restoration and following maintenance.

5. CONCLUSIONS

In the anoxic conditions achieved in the *in-situ* water table manipulation of the floodplain fen, N_2O emission was low. Accumulation of the heavy nitrogen isotope in the soil, low values of site preference, and $\delta^{18}O$-N_2O values in the captured gas samples indicate nitrifier denitrification in the anoxic floodplain peat. In the suboxic peat, the isotopic signals were similar, but N_2O emissions were high, indicating that the denitrification was incomplete.

The laboratory study demonstrated that a combination of source partitioning, isotopomers and N-cycle functional gene analyses allows to adequately elucidate the microbial pathways of N_2O fluxes. The results show that the flooded and intermediate water regimes produced the highest N_2O fluxes, whereas the lowest flux was found in dry peat. Thus, the 1st hypothesis found only partial proof: we did not see the expected low N_2O emission in the flooded regime. Based on site preference values, we observed that bacterial denitrification was the dominating process responsible for N_2O emissions during our study. But, as isotopomer mapping reveals overlaps of multiple processes within the same ranges of SP, these estimates would not be precise. Therefore, for a more precise identification of processes, isotopic and microbial analyses were combined. Contrary to hypothesis 2, nitrate fuelled bacterial denitrification was the major source of N_2O emissions under the intermediate and flooded regimes followed by nitrification via comammox *amoA* as secondarily important. Evidence supporting the findings include high N_2O emissions under flooded (anoxic) and intermediate (sub-oxic) treatments, high ^{15}N-N_2O emissions from the flooded and intermediate peats, site preference and $\delta^{18}O$ range, and increase in *nirK*, *nirS* and *nosZ* after the flooding. Regarding hypothesis 3, nitrification via bacterial and archaeal *amoA* genes was a major source of N_2O emissions in intermediate moisture regime. This was supported by high archaeal and bacterial *amoA* gene copy numbers, and the site preference and $\delta^{18}O$ values within the ammonia oxidation range.

The *in-situ* peatland forest study indicated that nitrification was the major process of N_2O in the peat sites manipulated by artificial drainage. Denitrification dominated the low emission of N_2O under flooded conditions, possibly reduced by complete denitrification. *amoA* and *nirS* genes dominated the microbiome of the drained treatment, while *nosZ* and *nirK* were more abundant in the flooded treatment. As a novel finding, on the atomic level, it was observed that nitrification and denitrification enrich N_2O molecules with heavy isotopes differently, as nitrification exclusively creates mass 45 of the N_2O molecule while denitrification produces both masses 46 and 45.

The results highlight denitrification as a fundamental biogeochemical process that trumps other nitrogen-transformation mechanisms in nitrate-rich, ammonium-poor peatlands. Nitrification is the main source in ammonium-rich peatlands. This supports the use of nitrogen-form (ammonium or nitrate) based biogeochemical models for future research and nitrogen management.

A combination of isotopic and microbial techniques for partitioning N_2O fluxes yields additional useful information for N_2O source apportioning. Our results are important for better regulation of land use in nitrogen-rich peatlands to mitigate N_2O emissions.

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SUMMARY

Peatlands (natural or managed) can be a significant source for N_2O emissions, which is a strong greenhouse gas with a powerful global warming potential (265 times higher than that of carbon dioxide, CO_2), and the most dangerous stratospheric ozone layer depleting agent in the 21st century. N_2O can be produced by multiple processes in soil such as denitrification, nitrification, nitrifier and fungal denitrification, and dissimilatory nitrate reduction to ammonium (DNRA), which can be active simultaneously. To understand the individual contribution of the N_2O production and consumption processes the thesis combined isotopic and microbial analyses. The ultimate objective was to understand the relationship between key environmental factors (nitrogen (N) forms, soil moisture and oxygen content) and N_2O emissions. Knowledge acquired from the precise estimation of N_2O source processes should be applied in restoration and climate-smart management of peatlands with optimisation of water regimes.

The thesis is organised according to the following pathway: 1) natural abundances of N_2O isotopes (Articles I–III), 2) source (ammonium or nitrate) partitioning using ^{15}N tracers in combination with analysis of N cycle functional gene abundances to distinguish between N_2O production and consumption processes (Articles II and III). The effect of soil oxygen and water table manipulation on N_2O fluxes and natural abundances of nitrogen and oxygen isotopes in a floodplain fen was investigated (Article I). From suboxic peat (0.5–6 mg O_2/L), nitrous oxide emissions peaked at oxygen concentration of 6 mg/L and then decreased with decreasing oxygen. From anoxic peat and oxic peat (0 and >6 mg O_2/L, respectively), nitrous oxide emissions were low. Elevated $^{15}N/^{14}N$ ratios ($\delta^{15}N$ = 7–9‰ ambient N_2) indicated intensive microbial processing of nitrogen in the suboxic peat. With increasing depth, the $^{15}N/^{14}N$ ratios decreased, showing the most intensive microbial activity in topsoil. The low values of $\delta^{18}O$-N_2O and site preference (difference between the central and peripheral ^{15}N atoms in N_2O molecule) indicated dominance of nitrifier denitrification.

After this, using ^{15}N tracing, the effects of different water regimes (dry, intermediate and flooded) on nitrous oxide emissions have been investigated in a laboratory experiment with peat taken from a floodplain fen (Article II). ^{15}N-labelled ammonium-chloride and potassium tracers were used assuming that NH_4 tracer is indicative for nitrification-based N_2O emissions whereas NO_3 tracer would be related to both nitrification- and denitrification-based N_2O emissions. For more adequate distinguishing between the source processes the isotopic composition was combined with qPCR analysis of abundant marker genes. It was found that bacterial denitrification was the predominant source of N_2O emissions followed by nitrification. This was confirmed by concomitant increase in *nirK*, *nirS* and *nosZ* after the flooding and by ^{15}N-N_2O fluxes under flooded and intermediate treatment. Site preference values offered overlapping of multiple processes, but combination of microbial and site preference values indicated bacterial denitrification as the major process responsible for N_2O emissions under both

treatments followed by nitrification (ammonia oxidation) as the secondary process. The high abundance of *amo*A genes and the second highest N_2O emissions support our findings. The increase in *nrfA* genes also showed that dissimilatory nitrate reduction to ammonium (DNRA) potentially contributed to N2O emission under flooded treatment.

Finally, N_2O sources were studied using combined microbial and ^{15}N tracer analysis in a drained peatland forest (Article III) under flooded and drained treatment in situ. As expected, higher abundances of nitrification genes were found in drained treatment and denitrification genes were higher under flooded treatment. This is because nitrification is an aerobic process and denitrification prefers anoxic conditions. It was observed that significant differences in labelled N_2O fluxes from flooded and drained treatment. N_2O emissions from flooded treatment were close to zero, and the highest (147 μg ^{15}N m^{-2} h^{-1}) labelled N_2O fluxes were recorded from drained treatment. This indicates nitrification as the major mechanism responsible for nitrous oxide fluxes in the drained peatland forest soil. It is also confirmed by the genetic results.

Conclusively, the previously found importance of soil water content (SWC) and N_2O fluxes in peat soils was confirmed. The integration of ^{15}N isotopologue and nitrogen cycle functional genes analyses allowed to distinguish more precisely the N_2O sources. In drained conditions the studied peatland soil emitted N_2O from nitrification process whereas denitrification dominated flooded peat (SWC > 0.80 m^3 m^{-3}). These results are potentially applicable for the restoration of drained peatlands forests: most likely, their flooding will decrease potential N_2O emissions.

SUMMARY IN ESTONIAN

Soomuldade naerugaasi-allikate määramine isotopoloogiliste ja mikrobioloogiliste meetoditega

Soomullad reguleerivad naerugaasi (N_2O) voogusid. N_2O on tugevatoimeline kasvuhoonegaas, mille kliimasoojendamise potentsiaal on 265 korda kõrgem kui süsihappegaasil. Samuti on N_2O ohtlik stratosfääri osoonikihi lõhkuja. N_2O toodavad mullas mitu protsessi, sh. denitrifikatsioon, nitrifikatsioon, nitrifitseerija-denitrifikatsioon, seente dentrifikatsioon ja dissimilatoorne nitraadi redutseerimine ammooniumiks (DNRA), mis võivad toimida samaaegselt. Analüüsimaks N_2O tootmist nende protsesside kaupa, kombineeris käesolev töö isotoopseid ja mikrobioloogilisi meetodeid. Lõppeesmärk oli selgitada peamiste keskkonnatingimuste (lämmastiku (N) vormide, mullaniiskuse ja -hapnikusisalduse) mõju N_2O voogudele. Sedavõrd täpse N_2O lähteprotsesside määramisega saadud teadmisi läheb tarvis soode taastamisel ja kuivendatud turbamaade majandamisel.

Väitekiri on üles ehitatud järgmise skeemi alusel: 1) N_2O isotoopide looduslikud arvukused (artiklid I–III), 2) N_2O allikate (ammoonium või nitraat) vaheline jaotus, kasutades ^{15}N märgistatud lämmastikku koos N-ringe funktsionaalsete geenide arvukuste analüüsiga N_2O tootmis- ja tarbimisprotsesside määramiseks (artiklid II ja III). Esmalt uuriti lammisoos mullahapniku ja veetaseme muutuse mõju N_2O voogudele ning N ja hapniku isotoopide looduslikele arvukustele (Artikkel I). N_2O vood tipnesid alaküllastunud hapnikutasemel (6 mg/L). Hapnikuga küllastunud ja hapnikuta mullast olid N_2O vood madalad. Alaküllastunud turba kõrgenenud $^{15}N/^{14}N$ suhe turbas ($\delta^{15}N$ = 7–9‰ atmosfääri N_2) osutas mullamikroobide intensiivsele N käitlusele. $^{15}N/^{14}N$ sügavusprofiil näitas kõrgeimat mikroobide tegevust maapinna lähedal. Madalad N_2O-$\delta^{18}O$ ja N_2O isotoopide asendieelistus (kesksete ^{15}N aatomite massi erinevus äärmistest) osutasid nitrifitseerija-denitrifikatsioonile olulise N_2O tootjana.

Järgnevalt, kasutades ^{15}N märgistatud lämmastikku, uurisime laborieksperimendi abil erinevate veerežiimide (kuiv, veega küllastunud ja vahepealne) mõju N_2O voogudele Emajõe lammiturbas (Artikkel II). Täpsemalt kasutasime ^{15}N märgistatud ammooniumi ja nitraati, eeldades, et ammooniumist pärinev N_2O on toodetud peamiselt nitrifikatsioonis ja nitraadist pärinev N_2O denitrifikatsioonis. Lähteprotsesside pädevamaks eristamiseks kombineerisime märgistatud lämmastiku meetodit funktsionaalsete geenide arvukuse qPCR analüüsiga. Saime teada, et peamine N_2O allikas oli denitrifikatsioon. Mõnel määral pärines N_2O ka nitrifikatsioonist. Seda kinnitasid isotopoloogide väärtused ning *nirK*, *nirS* ja *nosZ* arvukuste suurenemine veetaseme tõstmise järel veega küllastunud mullas. Kõrgenenud *nrfA* geeniarvukuse suurenemine vesises turbas viitas DNRAle.

Viimasena uurisime N_2O allikaid kuivendatud madalsoometsas ja selle eksperimentaalselt üleujutatud variandis. Ootuspäraselt tekitas nitrifikatsioon kuivendatud metsaturbas väga kõrgeid N_2O heitmeid (147 $\mu g\ ^{15}N\ m^{-2}\ h^{-1}$; Artikkel III). Seda toetas kõrge nitrifitseerija-geenide arvukus. Üleujutatud madalsoometsast N_2O õhku ei paiskunud. Denitrifitseerijageenide kõrge arvukus

ja N_2O isotoopkoostis aga viitasid sellele, et N_2O küll toodeti, aga tarbiti denitrifikatsiooni lõppfaasis ära.

Kokkuvõttes leidis kinnitust mulla veesisaldus N_2O võtmetegurina. Isotoopsete ja mikrobioloogiliste meetodite kombineerimine andis palju uut teavet N_2O allikate kohta. Katsetatud meetodeid ja nende põhjal saadud teadmisi saab rakendada põllumajanduse ja metsanduse kliimasõbralikul planeerimisel. Kõige suurem potentsiaal on veetaseme reguleerimisel, optimeerimaks kasvuhoonegaasiheitmete vähendamist majandusliku tulu ning väärtuslike elupaikade hoiu ja loomisega.

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PUBLICATIONS

CURRICULUM VITAE

Name: Mohit Masta
Date of birth: 14.05.1991
E-mail: mohitmasta@gmail.com

Education:

2018−2022	University of Tartu, PhD in Environment Technology
2015−2016	Newcastle University, MSc in Sustainable Chemical Engineering
2009−2013	DCR University of Science & Technology, B.Tech in Chemical Engineering
2005−2008	Rishikul Vidyapeeth (High School)

Work experience:

Feb 2021− present: Specialist in Environment Technology, Institute of Ecology and Earth Sciences, University of Tartu.

Research interests:

Environmental technology, Nitrous oxide source processes, Nitrogen isotopes & soil microbiology.

Publications:

Masta, M., Sepp, H., Pärn, J., Kirsimäe, K., Mander, Ü. 2020. Natural nitrogen isotope ratios as a potential indicator of N_2O production pathways in a floodplain fen. *Water* 12, 2, 409. https://doi.org/10.3390/w12020409

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Conference presentations:

Masta, M. (June 2022). Understanding N_2O production and consumption processes in peat soil with the help of isotopic and microbial analysis. Biogeomon, 10^{th} International Symposium on Ecosystem Behavior (2022). Tartu, Estonia.

Masta, M. (April 2021). Isotope and microbiome analysis indicates variety of N-cycle processes controlling N_2O fluxes in a drained peatland soil. On-line presentation at EGU General Assembly (2021). Vienna, Austria.

Masta, M. (June 2019). Fluctuating groundwater table enhances N_2O emissions from nitrification or denitrification in a floodplain fen. Oral presentation at the 8th International Symposium on Wetland Pollutant Dynamics and Control (WETPOL 2019). Aarhus, Denmark.

Masta, M. (April 2019). Fluctuating groundwater table enhances N_2O emissions from nitrification or denitrification in a floodplain fen. Poster presentation at EGU General Assembly (2019). Vienna, Austria.

ELULOOKIRJELDUS

Nimi: Mohit Masta
Sünniaeg: 14.05.1991
E-mail: mohitmasta@gmail.com

Haridus:

2018−2022	Tartu Ülikool, PhD keskkonnatehnoloogias
2015−2016	Newcastle'i Ülikool, MSc jätkusuutlikus keemiatehnoloogias
2009−2013	DCR Ülikool, B.Tech keemiatehnoloogias
2005−2008	Rishikul Vidyapeeth Keskkool

Töökogemus:

2021−	spetsialist keskonnatehnoloogias, Tartu Ülikool

Uurimisvaldkonnad:

Keskkonnatehnoloogia, mikrobioloogia

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Masta, M. (April 2019). Fluctuating groundwater table enhances N_2O emissions from nitrification or denitrification in a floodplain fen. Poster presentation at EGU General Assembly (2019). Viin, Austria.

DISSERTATIONES TECHNOLOGIAE CIRCUMIECTORUM UNIVERSITATIS TARTUENSIS

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