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

Professor Ülo Mander, PhD
Institute of Ecology and Earth Sciences
University of Tartu
Estonia

Opponent: Research Fellow Marcela Hernández, PhD
School of Environmental Sciences
University of East Anglia
United Kingdom

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II **Kazmi, F.A**., Espenberg, M., Pärn, J., Masta, M., Ranniku, R., Thayamkottu, S., Mander, Ü. 2025. Meltwater of freeze-thaw cycles drives N_2O-governing microbial communities in a drained peatland forest soil. *Biology and Fertility of Soils* **61**, 667–680. https://doi.org/10.1007/s00374-023-01790-w

III Ranniku, R., **Kazmi, F.A**., Espenberg, M., Truupõld, J., Escuer-Gatius, J., Mander, Ü., Soosaar, K. 2025. Springtime soil and tree stem greenhouse gas fluxes and the related soil microbiome pattern in a drained peatland forest. *Biogeochemistry* **168**, 48. https://doi.org/10.1007/s10533-025-01238-3

IV **Kazmi, F.A**., Mander, Ü., Ranniku, R., Öpik, M., Püssa, K., Soosaar, K., Kasak, K., Masta, M., Ah-Peng, C., Espenberg, M. 2025. Nitrogen cycling genes abundance in soil and aboveground compartments of tropical peatland cloud forests and a wetland on Réunion Island. *Scientific Reports.* **15**, 27155. https://doi.org/10.1038/s41598-025-12367-y

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The author's contribution to the articles is denoted by: '*' a minor contribution, '**' a moderate contribution, '***' a major contribution.

	Articles				
	I	**II**	**III**	**IV**	**V**
Original idea	*	**	*	**	***
Study design	*	**	*	**	***
Data collection	**	***	***	***	***
Analyses and interpretation	**	***	***	***	***
Manuscript writing	**	***	**	***	***

ABBREVIATIONS

C	carbon
N	nitrogen
GHG	greenhouse gas
CO_2	carbon dioxide
CH_4	methane
N_2O	nitrous oxide
NO_3^-	nitrate
NH_4^+	ammonium
SWC	soil water content
WTD	water table depth
mcrA	methyl coenzyme M reductase subunit A
pmoA	particulate methane monooxygenase subunit A
n-DAMO	nitrite/nitrate-dependent anaerobic methane oxidation
MOB	methane-oxidizing bacteria
nifH	nitrogenase reductase component of the nitrogenase complex
amoA	ammonia monooxygenase subunit A
AOA	ammonia-oxidizing archaea
AOB	ammonia-oxidizing bacteria
COMAMMOX	complete ammonium oxidation
ANAMMOX	anaerobic ammonium oxidation
DNRA	dissimilatory nitrate reduction to ammonium
nirS	cytochrome cd1 nitrite reductase
nirK	copper-containing nitrite reductase
nosZ	nitrous oxide reductase
qPCR	quantitative polymerase chain reaction
DNA	deoxyribonucleic acid
16S rRNA	small subunit ribosomal ribonucleic acid
PCA	principal component analysis
ANOVA	analysis of variance
SEM	structural equation model

ABSTRACT

Peatlands in their pristine state act as net carbon sinks. However, when drained, they can become sources of greenhouse gases (GHG), mainly nitrous oxide (N_2O) and carbon dioxide (CO_2), in addition to methane (CH_4) during periods of inundation. High temporal variability and seasonal fluctuations in groundwater levels, substrate availability, and soil moisture contribute to shifts in the microbial processes occurring in these ecosystems. Temporal studies of N_2O-related microbiomes in the hemiboreal peatland forests are rare. Moreover, microbial research on freeze-thaw events, which are important N_2O hot moments in northern ecosystems, is also limited to mineral soils. There is also a significant research gap regarding the GHG-related microbial analyses of tropical forests, especially the rare cloud forests growing on peat soils. This research focused on the dynamics of greenhouse gas fluxes and aimed to identify microbial processes by analyzing substrate quantities and related microbial gene abundances in the peat soils of the forests located in temperate (Estonia) and tropical regions (Réunion Island). In Estonia, a hemiboreal drained peatland forest (featuring *Picea abies* and *Betula pubescens*) was chosen for the studies, while two tropical cloud forests (one featuring *Erica reunionensis,* and the other a mix of *E. reunionensis* and *Alsophila glaucifolia*) growing on peat soils were selected on Réunion Island. The gas fluxes were determined using gas chromatography and portable trace gas analyzers from soils and tree stems, respectively. The genes involved in N-transforming processes and those related to the emission and consumption of N_2O and CH_4 were quantified using qPCR. Metagenomic analyses were performed additionally for the tropical cloud forests to observe the microbiome structure related to CH_4.

The long-term study on N_2O fluxes in the temperate drained peatland forest showed that the forest was a net source of N_2O (annual mean: 33.1 ± 4.70 μg N m^{-2} h^{-1}). Most of the N_2O emissions occurred during the freeze-thaw period. The *in-situ* emissions during the artificial thawing of topsoil in this forest increased significantly and peaked (128.5 ± 17.1 μg N m^{-2} h^{-1}) within a narrow range of soil moisture (0.7−0.8 m^3 m^{-3}). The temporal N_2O fluxes were correlated with the abundance of the bacterial and archaeal *amoA* genes, in addition to fungal and prokaryotic *nirK* genes, indicating that both nitrification and denitrification influenced the N_2O fluxes. The correlation between N_2O fluxes and *nirK* gene abundance was strongest during the autumn months. However, during the artificial freeze-thaw cycles, a significant correlation was only observed between N_2O and the *nirK* gene. Meanwhile, *nir*:*amoA* and *nir*:*nosZ* ratios remained high in the thawing phase. During the springtime N_2O fluxes reduced to 13.2 ± 1.4 μg N m^{-2} h^{-1} which was due to the high *nosZ*:*nir* ratio, and the relationship between springtime N_2O fluxes and *nosZ*-II abundance was significantly negative (−0.78, $p < 0.05$). Meanwhile, springtime CH_4 fluxes from the drained peatland forest were also minimal (5.08 ± 1.38 μg CH_4-C m^{-2} h^{-1}). During the spring period, the methanogenic potential of the peat soil (*mcrA*:(*pmoA* +

n-DAMO 16S rRNA) ratio) decreased significantly, changing topsoil from a potential source to a sink. However, the CH_4 from tree stems increased, suggesting deep soil methane production and uptake by tree roots. The dissolved CH_4 fluxes in groundwater and sap of birch trees also increased simultaneously.

In the tropical cloud forests, peat soil exhibited minimal soil N_2O emissions (1.06 μg N m^{-2} h^{-1} in the mixed forest, 0.37 μg N m^{-2} h^{-1} in the Erica forest). The nitrogen-fixing *nifH* gene abundance was positively correlated with soil NH_4^+-N levels in the forest soils, especially the sites dominated by endemic shrub species *E. reunionensis*. Meanwhile, the nitrification process was mainly driven by archaea. Despite having high NO_3^--N levels, the N_2O fluxes were minimal, which was attributed to the low pH in these peat soils, as low pH potentially slowed down the *nir*-type denitrification. High abundance and proportions of both clades of the *nosZ* gene indicated a significant potential for N_2O reduction in these soils. Meanwhile, the peat soils in these forests acted as CH_4 sink (-23.8 ± 4.84 μg C m^{-2} h^{-1}) and CO_2 source (55.5 ± 5.51 μg C m^{-2} h^{-1}), with higher CH_4 uptake in sites dominated by *E. reunionensis*. Aerobic methanotroph's *pmoA* gene was found abundant in the mixed forest only, while the anaerobic methanotroph's n-DAMO 16S rRNA gene was found abundant in both cloud forest peat soils. However, this gene was found significantly higher in the Erica forest. Metagenomics also showed the highest relative abundance of NC-10 bacteria in the peat soils of the Erica forest. Overall, the high abundance of n-DAMO 16S rRNA gene was correlated with soil NO_3^--N levels, CH_4 uptake, and CO_2 emissions indicating a high anaerobic methane oxidation potential in these forests. Meanwhile, different N-transforming and CH_4-related functional genes were also detected in the above-ground components of these forests (canopy soils and leaves). In addition to *nirS*, *nirK*, and *nosZ*-I, the canopy soils also exhibited a significant abundance of n-DAMO 16S rRNA gene, suggesting a decent potential for the above-ground denitrification and methane oxidation processes. Similarly, the *nosZ*-I and *mcrA* genes were also detected in the leaves of these forests, suggesting the potential of N_2O reduction and CH_4 production, respectively. Metagenomics also revealed the presence of methylotrophs in the leaf samples. This also shows that the above-ground parts of cloud forests, especially the phyllosphere, can be an important player in the forest biogeochemical process and GHG exchange, independent of soils. The findings of this dissertation emphasize the necessity for temporal studies on microbiomes associated with GHG fluxes across various global forest ecosystems. Additionally, these results highlight the significance of above-ground forest components for future research on global GHG modeling and the analysis of related microbiomes in forest ecosystems.

1. INTRODUCTION

1.1 Biogeochemical processes and greenhouse gas emissions in peatland forests

Peatlands are ecosystems with soils that contain a high content of partially decayed plant-based organic matter. This accumulation happens under oxygen-deprived conditions, which are sustained by a consistently high water table that prevents the respiration of the organic matter (Page & Baird, 2016). In their pristine state, these ecosystems are significant reservoirs of carbon (C) and nitrogen (N), globally storing ~644 Gt of C (21% of the global terrestrial C) and 5.9–25.9 Gt N (Page et al., 2011; Ribeiro et al., 2021; Watmough et al., 2022; Yin et al., 2022). The C storage is continuously increasing at a rate of 0.14 Pg C yr^{-1}, making peatlands a major long-term carbon sink (Gallego-Sala et al., 2018). In water-logged conditions, as a result of microbial processes, peatlands release methane (CH_4), a highly radiative greenhouse gas (GHG); however, their long-term ability to absorb CO_2 from the atmosphere and store organic C means that peatlands can remain a net C sink (Mitsch et al., 2013). The storage of C in peatlands is only maintained until the conditions are waterlogged; as soon as the water table drops, the oxygen penetrates the peat, and microbial degradation of the peat enhances, resulting in substantial carbon loss in the form of carbon dioxide (CO_2) to the atmosphere (Page & Baird, 2016). Similarly, the drainage of peatlands also results in the emission of nitrous oxide (N_2O), which is another GHG (298 times stronger than CO_2 in terms of its global warming potential) and ozone depletion agent (Butterbach-Bahl et al., 2013; Ravishankara et al., 2009). Since 1700, 0.51 Mkm^2 (11%) of global peatlands have been lost due to human activities, mainly land-use change (Fluet-Chouinard et al., 2023). Northern peatlands, located in boreal and hemiboreal regions, have primarily been drained for forestry (Westman & Laiho, 2003) and a small fraction for mining purposes (Rooney et al., 2012). Similarly, tropical peatlands or peat swamp forests are often drained and converted into agricultural lands, including oil palm plantations (Miettinen et al., 2012, 2016; Wan Mohd Jaafar et al., 2020). These conversions affect the normal biogeochemical processes occurring in peat soil, primarily due to fluctuating soil moisture levels and shifts in the soil's microbiome structure (Espenberg et al., 2018; Mander et al., 2025).

Compared to undisturbed peatlands, peatland forests represent more complex ecosystems regarding GHG emissions and related biogeochemical processes. In boreal drained peatland forests, the C balance depends on different factors like water table, temperature, and, most importantly, soil fertility (availability of nutrients). This implies that a nutrient-poor peatland forest can be a net C sink, but C loss would happen in nutrient-rich forest soils (Ojanen et al., 2013). In forested peatland ecosystems, CH_4 fluxes are directly related to groundwater levels (GWL) and soil water content (SWC), while CO_2 fluxes are negatively correlated with these factors (Mander et al., 2025). A long-term study on a nutrient-poor, drained

peatland forest in Finland indicated that it functioned as a small CH_4 sink and a minor source of N_2O, with carbon accumulation rates reportedly higher than those of natural peatlands (Lohila et al., 2011). Other studies on nutrient-rich temperate peatland forests have revealed hot moments of soil N_2O release, especially during winter and spring freeze-thaw events, wet summers, (Rautakoski et al., 2024) and spring freeze-thaw (Mander et al., 2021). These events, especially freeze-thaw cycles, contribute to the majority of N_2O fluxes at an annual scale in temperate peatland forests, even if they are nutrient-poor (Pihlatie et al., 2010). In the case of tropical peatland forests, even the primary pristine forests differ from the secondary forests regarding the GHG fluxes (Prananto et al., 2020). A long-term study focusing on Malaysian peat swamp forests indicated that while the CO_2 fluxes were the same, CH_4 fluxes varied significantly among different forest types and were highly dependent on various soil physicochemical properties (Busman et al., 2023). They found that soil organic matter decomposability and the availability of Fe and SO_4^{2-} were the important factors determining the CH_4 fluxes.

The complexity is not limited to the below-ground part of the peatland forests, but the above-ground compartments like tree stems, cryptogamic covers, and the phyllosphere can influence the forests' GHG exchange. During inundated conditions, the tree stems have been reported to release enormous amounts of CH_4 in peatland forests in Estonia (Ranniku et al., 2024) as well as in tropical wetland forests of the Amazon River basin (Pangala et al., 2017; Soosaar et al., 2022). Meanwhile, a long-term study in the Estonian drained peatland forest suggested that the forest canopy absorbs the soil N_2O through some unknown process (Mander et al., 2021). Additionally, the microbial processes happening in the phyllosphere and other parts of the plants can also affect the GHG fluxes (Putkinen et al., 2021).

1.1.1 Carbon cycle

Peatlands are significant carbon (C) reservoirs, functioning as active sinks for atmospheric CO_2 when they are in their pristine, water-saturated conditions. However, under these anoxic/hypoxic conditions, during the microbial degradation of the organic matter, CH_4 is generated (methanogenesis) by the activity of methanogenic archaea, which are subdivided into classes I and II based on the different metabolic pathways for CH_4 production. While class I methanogens mainly reduce methanol to produce CH_4, class II methanogens use a broader range of substrates and can reduce CO_2 to CH_4 (Borrel et al., 2019). The metabolic process involved is governed through the methyl-coenzyme M reductase (MCR) enzyme complex activity (Thauer, 1998) which is encoded by the *mcrA* gene. The CH_4 produced in the anoxic environments can also be oxidized by some other microbes within the same environment, and the process is known as anaerobic oxidation of methane (AOM) and is governed by a group of anaerobic methanotrophic Euryarchaeota (ANME) (Timmers et al., 2017). A type of ANME; ANME-2d can perform independent oxidation of CH_4 through reverse methanogenesis, utilizing nitrate as an electron acceptor, hence linking the carbon and nitrogen

cycles (Haroon et al., 2013). The process in which methanotrophs use nitrate or nitrite as an electron acceptor is also known as nitrate/nitrite-dependent anaerobic methane oxidation (n-DAMO). Some bacteria, especially those belonging to the NC-10 phylum, also perform the n-DAMO process by actively taking part in denitrification using the *narG* and *napA* genes (Ettwig et al., 2009). The n-DAMO process (Fig. 1) can also be governed by the consortium of ANME-2d archaea (*Methanopredens nitroreducens*), the ANAMMOX (*Kuenenia*), and NC-10 type bacteria (*Methylomirabilis oxyfera*) (Haroon et al., 2013; Raghoebarsing et al., 2006).

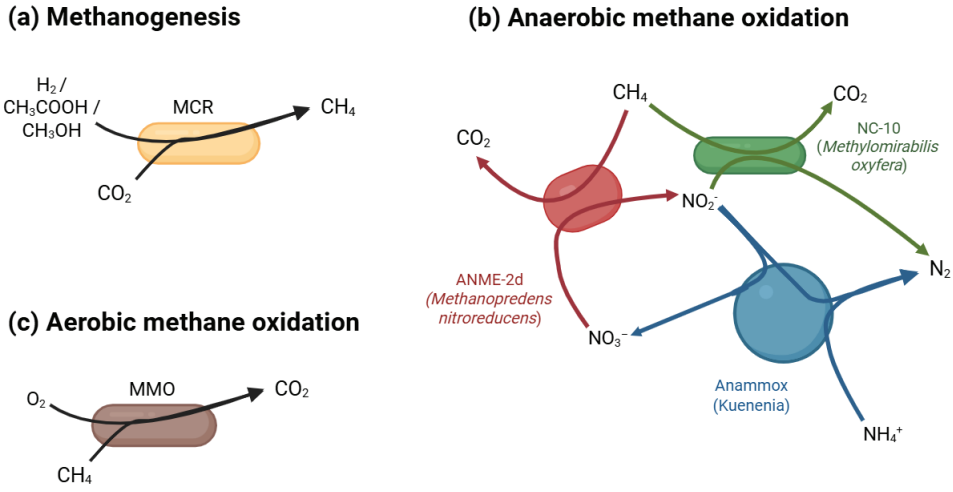

Figure 1. The schematic pathways of **(a)** methanogenesis, **(b)** n-DAMO interactions between ANME-2d archaea (*Methanopredens nitroreducens*), ANAMMOX (*Kuenenia*), and NC-10 type bacteria (*Methylomirabilis oxyfera*), if the anaerobic soil environment has all three substrates, viz. CH_4, NO_3^-, and NH_4^+, adopted from Haroon et al. (2013) and reedited, and **(c)** aerobic methane oxidation.

The aerobic methanotrophs oxidize CH_4, facilitated by specific enzymes known as methane monooxygenases (MMOs). There are two types of MMOs: particulate MMO (pMMO), which is encoded by the *pmoA* gene, and soluble MMO (sMMO). These enzymes first convert CH_4 into methanol, which is then further oxidized to formaldehyde and eventually to CO_2 through a chain of reactions (Dedysh & Knief, 2018). There are different microbial groups undertaking aerobic methanotrophy, such as microbes belonging to *Gammaproteobacteria*, *Alphaproteobacteria*, and *Verrucomicrobia* phyla (extremophiles). Most of the aerobic methanotrophs thrive in a wide range of environments, including extreme conditions (Guerrero-Cruz et al., 2021).

1.1.2 Nitrogen cycle

Nitrogen (N) is a crucial component of many biomolecules, including nucleic acids and proteins, which means that the nitrogen supply in an ecosystem plays a vital role in regulating its primary productivity. The N cycle is typically divided into several major processes: ammonification, nitrification, and denitrification (Fig. 2). Different groups of microorganisms carry out each of these processes under varying oxic conditions. Ammonification occurs through two primary mechanisms: nitrogen fixation (Dixon & Kahn, 2004) and dissimilatory nitrite reduction to ammonium (DNRA) (Stein & Klotz, 2016). Biological nitrogen fixation (BNF) in peatlands can be performed by free-living cyanobacteria, quasi-symbiotic cyanobacteria linked with *sphagnum* moss, and other heterotrophic bacteria regulated by the functional gene *nifH* (which encodes nitrogenase reductase component of the nitrogenase complex) (Kox et al., 2020; Schmidt et al., 2024; Yin et al., 2022). The enzyme nitrogenase catalyzes the BNF and converts atmospheric N_2 to bioavailable NH_3 under ambient conditions (Chalkley et al., 2020).

The NH_3 or ammonium (NH_4^+) in soil can either be taken up by plants (especially in the case of peatland forests) or further transformed by ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) into nitrite ($NH_3 \rightarrow NO_2^-$) using enzyme ammonia monooxygenase (AMO) whose subunit A is encoded by *amoA* genes (Rotthauwe et al., 1997). But since peat soils are slightly acidic, the role of AOA would be more prominent (Lehtovirta-Morley et al., 2011; Zhang et al., 2012). The AOA are usually the dominant nitrifiers in the most extreme environments (Hernández et al., 2014; Zhou et al., 2016). The dominance of AOA in nitrification can also result in the aerobic N_2O production (Stieglmeier et al., 2014). The second step of nitrification involves nitrite oxidation ($NO_2^- \rightarrow NO_3^-$) and is performed by nitrite-oxidizing bacteria (NOB) (Costa et al., 2006). However, recently discovered *Nitrospira* bacteria, or complete ammonia oxidizers (comammox), can convert ammonia all the way to nitrate (NO_3^-) independently (van Kessel et al., 2015). These organisms use the enzymes AMO, hydroxylamine oxidoreductase (HAO – encoded by the *hao* gene), and nitrite oxidoreductase (NXR) for this purpose (Koch et al., 2015). Under oxygen-stressed conditions, the nitrifying bacteria can also reduce the NO_2^- to nitric oxide (NO) and then to N_2O, and the process is called nitrifier denitrification (Wrage et al., 2001). Overall, there are two different pathways through which nitrifiers can contribute to the emission of N_2O: one governed under aerobic conditions and the other under anaerobic conditions (Fig. 2).

Like nitrification, denitrification is also performed by multiple microbial groups in multiple steps. The process is mainly anaerobic and involves the conversion of NO_3^- to NO, and N_2O to N_2. The first step ($NO_3^- \rightarrow NO_2^-$) is catalyzed by the membrane-bound nitrate reductase (NarGHI) and periplasmic nitrate reductase (NapAB). The second step ($NO_2^- \rightarrow NO$) is then performed by copper-containing nitrite reductase (encoded by the gene *nirK*) and cytochrome cd1 nitrite reductase (encoded by the gene *nirS*) (Kraft et al., 2011; Zumft, 1997). In addition to

bacteria, fungi can also perform denitrification, regulated by fungal *nirK* and *p450nor* (Aldossari & Ishii, 2021). The NO produced in the second step is then converted to N_2O by various bacteria using the enzyme nitric oxide reductase (NOR). In the final step, the N_2O is reduced to N_2 by microbes using the copper-containing enzyme nitrous oxide reductase (NOS), which is encoded by the gene *nosZ* (Kraft et al., 2011). The release of N_2O can occur when the N_2O reducers are unable to convert all of the N_2O produced by the denitrifiers, meaning that the net N_2O flux will result from the balance between the N_2O production and consumption within the anoxic environment.

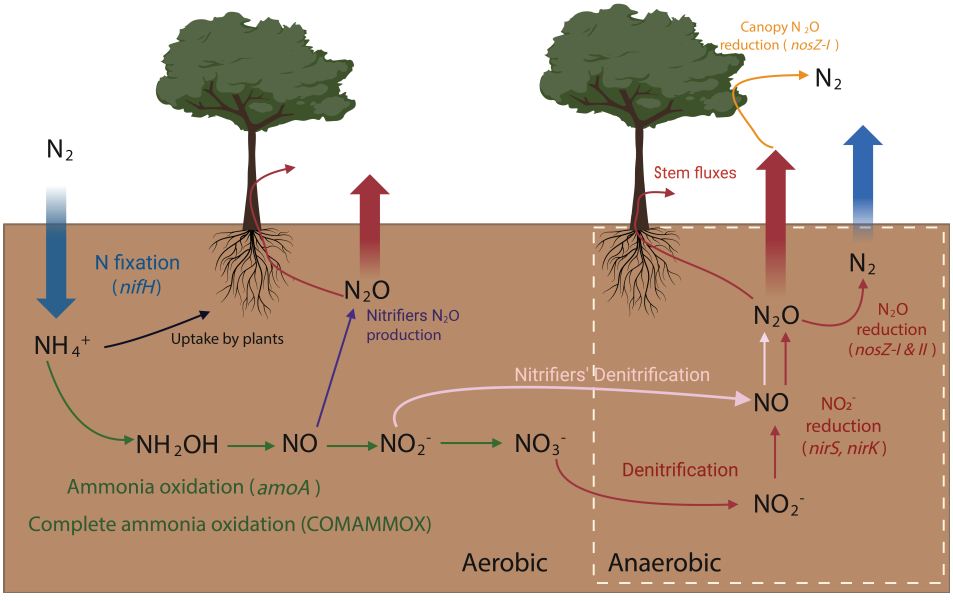

Figure 2. Schematic illustration of the microbial nitrogen cycle in forest soils. The arrows indicate the direction of the reactions, while those with faded tails represent the processes occurring in gaseous form. The red arrows represent the soil N_2O fluxes, while the blue arrows represent the soil N_2 fluxes.

1.2 Role of above-ground forest compartments in biogeochemical cycles

In addition to soil, the above-ground compartments of forests also play a crucial role in biogeochemical cycles. Leaves absorb atmospheric CO_2 and convert it into complex organic compounds via the process called photosynthesis. Terrestrial ecosystems are a major C sink due to the photosynthesis and storage of CO_2 in living and dead organic matter. For example, forest ecosystems store the C as lignin and other relatively resistant polymeric carbon compounds. This process of transferring atmospheric CO_2 into biotic and soil carbon pools is known as terrestrial carbon sequestration (Lal, 2007). Plant tissues, especially leaves, are

home to a wide range of microorganisms. This microbial habitat, referred to as the phyllosphere, is known to support a variety of microorganisms that interact with plants, affecting their fitness. Phyllosphere microorganisms adhere to the cuticle, a waxy layer, on both the upper (adaxial) and lower (abaxial) surfaces of the leaf. In addition to their role in plant functions, these microorganisms can influence different ecological processes, such as the water and nutrient cycles (Vacher et al., 2016). The microbial communities of the phyllosphere are reported to be distinct from those inhabiting the soils (Siegenthaler et al., 2024); however, their connection with the biogeochemical cycles cannot be ignored completely. Previous research has detected the methanotrophs (Iguchi et al., 2012) as well as nitrogen fixers, nitrifiers, and denitrifiers (X. Shi et al., 2023) in the phyllosphere. This shows that the microbial communities of the phyllosphere can influence both C and N cycles, either independently or alongside soil processes within an ecosystem. The detection of methanogenic archaea and methanotrophic bacteria has already been reported in the foliage of different boreal forests (Putkinen et al., 2021), in addition to the aerobic methane production in the Scots pine shoots (Tenhovirta et al., 2024). Another recent study estimated that canopy nitrification is responsible for 80% of the nitrate that reaches the soil via throughfall (Guerrieri et al., 2024). This study also found various microbial groups of nitrifiers on the foliar surfaces in addition to the detection of bacterial and archaeal *amoA* genes. Given the presence of nitrifiers and denitrifiers in the phyllosphere, along with the availability of substrates, the phyllosphere microbiome may also contribute to the production or consumption of N_2O under varying aerobic conditions. This can only be tested if the shoot flux for the GHG is measured.

In different European forests, the stems and shoots have also been found to be sinks of N_2O (Machacova et al., 2024) and sources of N_2O and CH_4 (Machacova et al., 2016). Tree stems can also act as conduits for the CH_4 produced in the saturated soils in forests (Barba et al., 2019; Pangala et al., 2013), or can act as an independent source or sink of CH_4, highly depending on the microbial processes going on in their bark (Gauci et al., 2024). Studies have also shown that the woody surfaces of trees can uptake CH_4, attributed to the methanotrophic activity within the wood cervices (Gauci et al., 2024; Jeffrey et al., 2021). The GHG flux from stems or other vegetative parts of the forests can be highly dependent on the plants' age, taxonomic status, and other physiological traits, in addition to the soil properties and seasonal variations (Moisan et al., 2024; Vainio et al., 2022). However, the microbial analyses of the above-ground forest compartments can enhance our understanding of the potential of these components in playing a role in forest biogeochemical cycles.

The main aim of this dissertation was to explore the dynamics of different microbial processes in the temperate and tropical peatland forests, which are responsible for the greenhouse gas fluxes. Under this theme, the objectives included:

1. The determination of the abundance and structure of different functional genes and microbiomes involved in carbon and nitrogen cycling in peat soils (Articles I–IV) as well as in the above-ground forest compartments (Articles IV, V), including the relationship with GHG fluxes (Articles I–IV).
2. Quantifying the microbial genes involved in the temporal dynamics of nitrogen cycling in a drained peatland forest to observe the connections between the gene abundance and hot moments of N_2O release (Article I, II).

The following hypotheses were drawn based on the aims:

I. Nitrification is the main process behind the overall N_2O fluxes from the drained peatland forest, because of the oxic conditions in the topsoil.

II. Due to fluctuating soil moisture levels, freeze-thaw N_2O fluxes result mainly from the incomplete denitrification process.

III. In temperate drained peatland forest, tree stem CH_4 and N_2O fluxes are related to the shifts in soil microbiome during springtime.

IV. The above-ground components of tropical cloud forests exhibit different CH_4 and N_2O-related microbiomes compared to the peat soils.

2. MATERIALS AND METHODS

This dissertation consists of a temporal study (article I), two seasonal experiment studies (articles II & III) in a hemiboreal drained peatland forest in Estonia, and two case studies covering tropical cloud forest with peat soils on Réunion Island (articles IV & V).

2.1 Descriptions of the study sites

2.1.1 Agali drained peatland forest (Articles I, II, & III)

For studying the temperate peatland forest ecosystem, a drained peatland mixed forest in Estonia was selected. The forest is situated near the Agali village of Kastre Municipality, in Southeast Estonia (58.290184° N, 27.31725° E). This forest site is classified in the hemiboreal vegetation zone (Ahti et al., 1968) and was drained during the 1920s by an open-ditch network drainage system (Mathiesen, 1927). The forest is dominated by *Picea abies* (Norway spruce) and *Betula pubescens* (Downy birch).

The sampling for a year-long study (article I) was conducted between October 2020 and November 2021. The freeze-thaw experiment (article II) was performed in March 2022, while the birch sap experiment (article III) took place in April 2023 in the Agali forest.

Figure 3. The map of the drained peatland forest site near the Agali village of Kastre Municipality, in Southeast Estonia. The forest is a drained peatland with open ditches. The red marked area on the main picture represents the sampling area. The satellite imagery depicts conditions of the forest during the summer, **(a)** during the freeze-thaw experiment (Article II), and **(b)** during the spring-time experiment (Article III). Photo credits: Fahad Ali Kazmi and Reti Ranniku.

2.1.2 Réunion Island (Articles IV & V)

Two tropical cloud forests with peat soils (article V) in addition to a wetland site (article IV) on the Réunion Island, an overseas French territory located in the Indian Ocean (Fig. 4), were selected for these studies. The two cloud forests were in Plaine des Cafres in Le Tampon municipality (21.145343° S, 55.569692° E) and Plateau de Thym, in Forêt de Bébour region of Saint-Benoît commune (21.097139° S, 55.548028° E). These forests are situated between the elevations of 1500–1600 meters above sea level, which is classified as the montane cloud forest vegetation band. The wetland was situated close to the city of Saint Paul (20.991416° S, 55.294264° E).

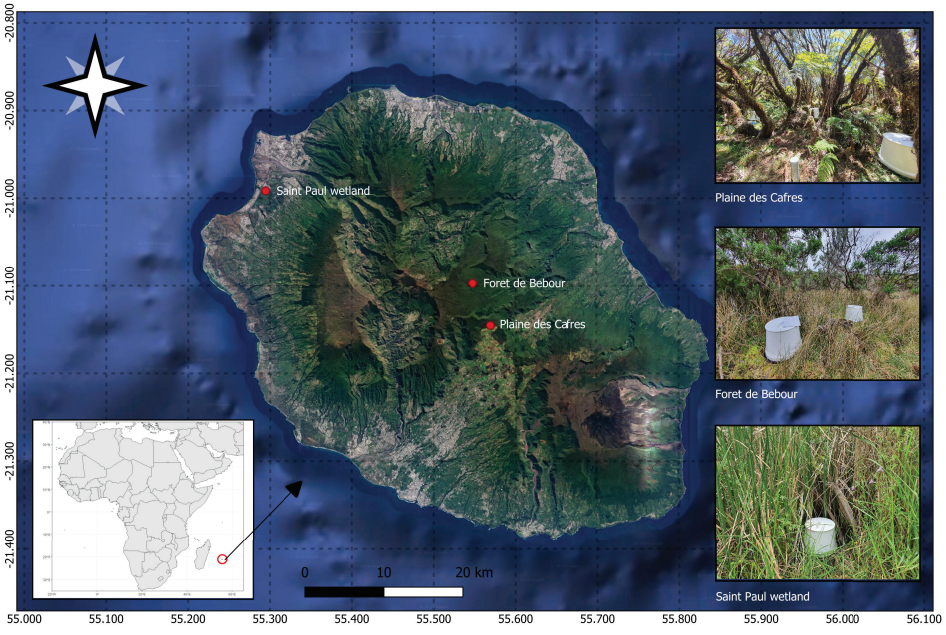

Figure 4. The site map and locations of the tropical peatland cloud forests (Plaine des Cafres and Forêt de Bébour) and the wetland (Saint Paul) on the Réunion Island. The embedded pictures show the actual environmental and vegetative conditions on the site at the time of sampling (November 2022). All three sites were studied in Article IV, while only the cloud forest sites were studied in Article V. Photo credits: Fahad Ali Kazmi.

The peatland forest of Plaine des Cafres featured a mix of endemic shrub species, including *Erica reunionensis*, as well as the endemic tree fern species *Alsophila glaucifolia*. The epiphytic vegetation was primarily dominated by *Cordyline mauritiana* and various fern species, such as *Hymenophyllum inaequale* and *Blechnum attenuatum*. In the understory, the most common species included *Embelia angustifolia*, *Anthoxanthum odoratum*, and *Cynorkis ridleyi*. The bryophyte layer was patchy and mainly consisted of *Sphagnum* species. This forest has been characterized as a mixed forest with two sub-sites, distinguished by the dominance of either *E. reunionensis* or *A. glaucifolia* in articles IV and V.

The peatland forest of Plateau de Thym was mainly dominated by the endemic shrub *E. reunionensis,* followed by the *Hubertia ambavilla*. In the understory, we found *Erica galioides*, *Juncus effusus*, with a patchy dominating *Sphagnum* species. The peatland in this forest is 25,000 years old (Roux et al., 2024), and the site has been described as an Erica forest in articles IV and V.

The wetland was only included in article IV, in addition to the cloud forests. The dominant vegetation in the wetland was *Typha domingensis*. Also, in the riparian zone of the wetland main species were *Setaria geminata*, aquatic *Lemna spp*, along with exotic *Schinus terebinthifolius* and *Ipomoea cairica* along the edges.

The field campaigns to the Réunion Island sites were made in November 2022, which is during the early spring dry season on the island.

2.2 GHG flux sampling and measurement

In all studies except for article II, soil flux sampling was performed under absolute natural conditions. However, in article II, the topsoil was cleared of snow and then heated using cables to create artificial thawing conditions required for the experiment (Fig. 5a,b). To measure the gas fluxes of CO_2, CH_4, and N_2O from the soil surface, the static chamber method was employed (Soosaar et al., 2011) in articles II, IV, and V. In this method, a 65-liter polyvinyl chloride (PVC) chamber (surface area = 0.0196 m^2, volume = 0.065 m^3, diameter = 0.5 m, height = 0.4 m) was fixed to a pre-installed collar in the peat soils, making sure the connection is airtight. Gas samples were obtained in pre-vacuumed 50 ml glass bottles from the top of chambers every 20 minutes for a period of one hour. The concentrations of CO_2 and N_2O in these samples were measured using a gas chromatograph (GC-2014, Shimadzu, Kyoto, Japan) with an electron capture detector (GC-ECD), while for the CH_4, the gas chromatograph was used coupled with a flame ionization detector (GC-FID). The GC was equipped with a Loftfield-type autosampler (Loftfield et al., 1997). In the case of articles I and III, the soil fluxes were measured using the automated dynamic chambers (surface area = 0.16 m^2, volume = 0.032 m^2), connected with a multiplexer for facilitation. The automated chambers closed for nine minutes for each measurement. A gas analyzer (G2508, Picarro Inc., Santa Clara, California, United States) measured the concentrations of CO_2, CH_4, and N_2O by employing cavity ring-down spectroscopy. Every day, these chambers made 12 flux measurements (one measurement per two hours).

To measure the fluxes of CO_2, CH_4, and N_2O from the surfaces of tree stems in articles III, IV, and V, static chambers (total area = 0.0108 m^2 stem surface, total volume = 0.00119 m^3) were installed on the stem surfaces in all studied forests. The static chambers were made of transparent plastic (Lock & Lock, Seoul, South Korea) with their bottoms removed manually. A black neoprene band was glued to the bottom edges, allowing for attachment to the tree stem surfaces and ensuring an airtight seal. Each chamber was closed with a lid, which was further

connected to the trace gas analyzers via nylon tubes (Fig. 5c). An automated chamber is shown in Fig. 5c. To quantify the concentrations of CO_2 and CH_4, a trace gas analyzer (LI-COR, LI-7810 $CH_4/CO_2/H_2O$, Li-Cor Biosciences, Lincoln, NE, USA) was used, and for N_2O, another analyzer (LI-7820 N_2O/H_2O, Li-Cor Biosciences, Lincoln, NE, USA) was used. The fluxes were measured by circulating the air in the chamber's headspace and the gas analyzers in a closed loop over a ten-minute interval.

The quality of each gas sample was assessed by assessing the adjusted R^2 values from the linear regression of the CO_2 concentrations. This analysis verified the seals or closure quality of the chambers. Flux measurements were considered acceptable only when the R^2 values for the CO_2 slope were greater than 0.9. Any measurement not following this rule was visually inspected to see the meaningful patterns; for example, the low-flux measurements demonstrating acceptable changes in concentration during visual inspection were included in the analysis to prevent the systematic exclusion of valid low-flux data.

In addition to the *in situ* fluxes, the potential fluxes were also determined from the intact soil cores. For this purpose, the metallic cylinders (diameter = 6.8 cm) were used to extract intact peat soil cores. The soil cores were incubated in the laboratory by flushing them with a gas mixture (21.0% O_2, 358 ppm CO_2, 0.313 ppm N_2O, 1.67 ppm CH_4, 5.97 ppm N_2, and the rest Helium) and providing them with actual site conditions in terms of temperature. The incubated conditions were achieved by continuously flushing the headspace of the vessel with the mixture gas at 20 mL/minute after 12–24 hours (Butterbach-Bahl et al., 2002; Mander et al., 2014). Meanwhile, the flushing time depended on the soil moisture levels. The N_2, N_2O, and CO_2 concentrations were determined after two hours of closure (with a 40-minute interval) using the gas chromatograph (GC-2014, Shimadzu, Kyoto, Japan) equipped with a thermal conductivity detector (GC-TCD).

In article III, the dissolved CO_2 and CH_4 concentrations from the birch sap and soil water were also determined. This involved the collection of birch sap with syringes connected with tubes, which were inserted into the tree stems (Fig. 5d). Birch sap (30 ml) was collected in the syringe, and 30 ml of ambient air was added to the headspace to achieve equilibrium between the liquid and atmosphere. To determine dCH_4 and dCO_2, water samples were collected into syringes from the water table surface in groundwater wells at each sampling point. The syringes were shaken for a minute, and the headspace was then injected into the pre-vacuumed glass vials (Fig. 5e). The gas concentrations were determined using gas chromatography (GC-2014, Shimadzu, Kyoto, Japan).

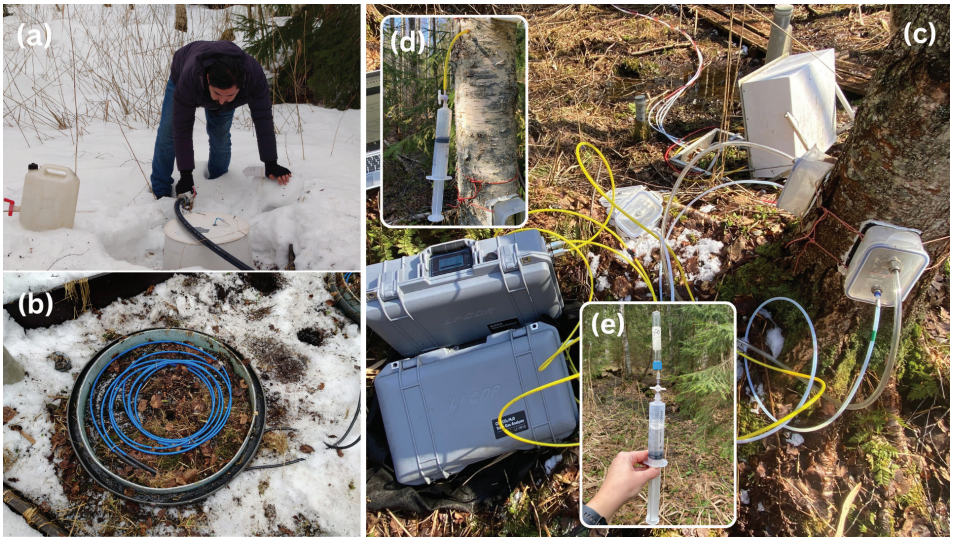

Figure 5. Experimental conditions during the **(a)** freeze-thaw experiment, **(b)** heating cables used to thaw the frozen topsoil. **(c)** Tree stem flux measurement system. An automated chamber is also visible in a non-measuring state behind the tree. **(d)** Birch sap collection system and **(e)** dissolved flux collection from birch sap. Photo credits: Mohit Masta and Reti Ranniku.

2.3 Physical and chemical analyses of peat samples

The temperature at the surface and at depths of 10 cm and 20 cm in all peat soil was measured during each gas sampling session by using the probes (model CS 107, Campbell Scientific Inc., Logan, UT, USA). The soil water content was also determined at the same time by using a ProCheck moisture sensor (Decagon Devices, USA). The water table depth (WTD, cm) dynamics were measured using perforated polypropylene pipes (Ø 7.5 cm) as monitoring wells. Soil chemical properties, including the measurements of total nitrogen, total carbon, and pH, were determined in the Estonian Environmental Research Center in Tartu, Estonia. Soil NH_4^+-N and NO_3^--N levels were determined by extracting with 2M KCl (1:10 ratio) using flow-injection analyses as per standard methods (APHA-AWWA-WEF, 2005).

2.4 Microbiological analyses

The microbiological analyses involved careful sampling of various materials of interest within the ecosystems studied. In temperate peatland forests, the primary focus was on the peat soil. In contrast, in tropical cloud forests, both the peat soil and above-ground compartments were sampled to investigate the microbiome and its potential contribution to GHG fluxes. All articles involved qPCR analyses, while in article V, metagenomics was additionally employed to understand the microbial communities. The analyses are discussed in detail in the following subsections.

2.4.1 Peat soil and above-ground sampling

For microbiological analyses, 10–50 g of soil samples from the topsoil (10 cm depth) were obtained using disinfected equipment (spade and spoon) and packed into sealed grip bags in all studies. It was ensured that the sample was representative of the whole sampling point by taking the soil from at least three different locations within a sampling point (composite sampling). The samples were then transferred to the −20 °C until DNA extraction. In case of tropical cloud forests, the above-ground samples were also included for microbiological analyses. For this purpose, the canopy soil (defined as the decomposing epiphytic material from the tree stems) was scratched using a disinfected spoon/spade and put into the tea bags, which were then packed with the active silica gel in the sealed grip bags. Tree stem cores were drilled using a disinfected Teflon-coated three-cutting threaded incremental borer (length = 400 mm, diameter = 5.15 mm), and samples were obtained with an extraction needle (Haglöf Sweden AB, Langsle, Sweden). And leaves were sampled from different branches into the tea bags and packed with silica gel. The silica gel was replaced continuously until all the moisture was removed from these samples. The drying was performed to preserve the samples for a long time, and the method is suitable for DNA-based microbial analyses (Smenderovac et al., 2024). All the sampling equipment (spoons, spade, and stem corer) was disinfected between sampling points using 80% ethanol for locations outside of Estonia, while 80–90% methanol was used during the sampling from the Estonian sites.

2.4.2 DNA extraction and quantitative PCR

Before the extraction of DNA, the frozen soil samples were partially thawed, and the above-ground samples were crushed using a coffee grinder. From the soil samples, an amount of 0.25 g was taken from different parts of the sample for DNA extraction, while in the case of the above-ground samples, 0.12 g was obtained for extraction. The DNeasy PowerSoil Pro kit (Qiagen, Hilden, Germany) was used to extract DNA from all samples, following mainly the manufacturer's instruction manual. The lysis step was aided by homogenizing the sample with CD1 (the lysis buffer) using a Precellys 24 Homogenizer (Berlin Technologies,

Montigny-le-Bretonneux, France) at 5000 rpm for 20 seconds. In case of above-ground samples (canopy soils, leaves, and stem cores), the amount of CD1 increased to 70% to enhance the lysate quantity. The DNA concentrations were measured using a Tecan infinite M200 spectrophotometer (Tecan AG, Grodig, Austria) and then stored at −20 ºC until the quantitative polymerase chain reaction (qPCR). A RotorGene ® Q machine (Qiagen, Hilden, Germany) was used to perform qPCR on all genes of interest. The details of primers and the profiles are provided in Table 1. Each qPCR run was performed with positive and negative controls in addition to different samples. Positive control samples were the pre-tested DNA samples that had consistently yielded the same amplification and melting values in the qPCR for specific genes over time. Only replicates and samples that exhibited amplification patterns similar to the positive controls were selected for further analysis. The qPCR data were later processed in the software LinRegPCR (version 2020.0), where all samples were grouped in certain amplicon groups and the N_0 value was obtained, which is the estimated starting amount of targeted nucleic acid by fitting fluorescence data into the PCR efficiency model. The noisy samples (those outside the 15% of the amplicon median, and without a plateau phase) were disqualified for further analyses. Gene copy numbers were calculated from the N_0 values of each replicate and normalized based on the sample's dry weight percentage and indicated as gene copies per gram of sample's dry weight (copies g^{-1} dw).

Table 1. Primers and qPCR profile details for the functional genes studied in all articles

Target gene	Primer	Primer reference	Amplicon size (bp)	Primer conc. (µM)	qPCR program
Bacterial 16S rRNA	Bact517F	(Z. Liu et al., 2007)	530	0.6	95°C 10 min; 35 cycles: 95°C 30 s; 60°C 45 s; 72°C 45 s
	Bact1028R	(Dethlefsen et al., 2008)			
Archaeal 16S rRNA	Arc519F	(Espenberg et al., 2016)	393	0.6	95°C 10 min; 45 cycles: 95°C 15 s; 56°C 30 s; 72°C 30 s
	Arch910R				
nirS	nirSCd3af	(Kandeler et al., 2006)	431	0.8	95°C 10 min; 45 cycles: 95°C 15 s; 55°C 30 s; 72°C 30s, 80°C 30 s
	nirSR3cd				
nirK	nirK876	(Henry et al., 2006)	165	0.8	95°C 10 min; 45 cycles: 95°C 15 s; 58°C 30 s; 72°C 30s, 80°C 30 s
	nirK1040				
nosZI	nosZ2F	(Henry et al., 2006)	267	0.6	95°C 10 min; 45 cycles: 95°C 15 s, 60°C 30 s, 72°C 30 s, 80°C 30 s
	nosZ2R				

Target gene	Primer	Primer reference	Amplicon size (bp)	Primer conc. (µM)	qPCR program
nosZII	nosZIIF nosZIIR	(Jones et al., 2013)	~700	0.6	95°C 10 min; 45 cycles: 95°C 30 s, 54°C 45 s, 72°C 45 s, 80°C 45 s
Bacterial *amoA*	amoA-1F amoA-2R	(Rotthauwe et al., 1997)	491	0.8	95°C 10 min; 45 cycles: 95°C 30 s; 57°C 45 s; 72°C 45 s
Archaeal *amoA*	CrenamoA 23F CrenamoA 616R	(Tourna et al., 2008)	~600	0.8	95°C 10 min; 45 cycles: 95°C 30 s; 55°C 45 s; 72°C 45 s
COMAMMOX *amoA*	comamoA AF comamoA SR	(Wang et al., 2018)	436	0.8	95°C 10 min; 40 cycles: 95°C 15 s, 55°C 30 s, 72°C 30 s
nifH	Ueda19F Ueda407R	(Ueda et al., 1995)	390	0.8	95°C 10 min; 45 cycles: 95°C 30 s, 53°C 45 s, 72°C 45 s
nrfA	6F 6R	(Takeuchi, 2006)	390	0.8	95°C 10 min; 45 cycles: 95°C 15 s, 55°C 30 s, 72°C 30 s
Fungal *nirK*	FnirK-F3 FnirK-R2	(H. Chen et al., 2016)	233	0.8	95°C 10 min; 45 cycles: 95°C 15 s, 56°C 30 s, 72°C 30 s
n-DAMO 16S rRNA	pq2F pq2R	(Ettwig et al., 2009)	292	0.8	95 °C 10 min, 40 cycles: 95 °C 15 s, 60°C 30 s, 72 °C 30 s, 80 °C 30 s
mcrA	METH-F mcrAqR	(Espenberg et al., 2016)	135	0.8	95 °C 10 min, 55 cycles: 95 °C 15 s, 51 °C 30 s, 72 °C 30 s
pmoA	A189F Mb661R	(Costello & Lidstrom, 1999)	510	0.6	95 °C 10 min, 35 cycles: 95 °C 15 s, 57 °C 30 s, 72 °C 30 s

2.4.3 Preparation of DNA libraries for metagenomics analysis, sequencing, and data processing

For article V, the metagenomics analyses were also performed. In these analyses, the raw metagenomic reads were quality filtered and adapters were trimmed using *fastp* v0.23.2 (S. Chen et al., 2018) using the following options: -q -y -g -Y 10 -l 100 -b 150 -B 150. The clean reads from each sample were *de novo* assembled individually using MEGAHIT v1.2.9 (Li et al., 2015) with the following options: --min-contig-len 200 --k-list 21,41,61,81,101,121. Assembled contigs were used to predict protein-coding sequences (CDSs) with Prodigal v2.6.3 (Hyatt et al., 2010) in metagenomic mode. To reduce sequence redundancy, redundant genes were clustered using CD-HIT v4.8.1(Fu et al., 2012; W. Li & Godzik, 2006) with parameters: -c 0.95 -aS 0.9. CDSs were annotated using DIAMOND v2.1.10.164 in blastp mode (-max-target-seqs 1 --evalue 1e-5 -d uniprot_db) against the UniProtKB/SwissProt reference database (release 2024_05). Assembled contigs of each sample were indexed using bowtie2-build, and clean reads were aligned to the assemblies using bowtie2 v2.5.4 (Langmead & Salzberg, 2012). The obtained SAM files were converted to BAM format and sorted using SAMtools v1.21 (Danecek et al., 2021). Gene abundance was quantified with *featureCounts* v2.0.8 (Liao et al., 2014) to obtain raw read counts. The annotations were then mapped to the corresponding gene IDs based on the matching of gene identifiers from the GFF file used during the *featureCounts* step. Genes associated with aerobic CH_4 oxidation, methanol oxidation, and methanogenesis were identified using Python (v3.12.3) libraries such as pandas (v2.2.3), glob, and os. Specifically, gene annotations were filtered by searching for gene name identifiers (GN) corresponding to functional genes involved in these pathways. The targeted keywords included: 'GN=pmo', 'GN=mmo', 'GN=xox', 'GN=mdh', 'GN=mox', 'GN=mcr', 'GN=mtr', 'GN=mtb', 'GN=mtm', 'GN=mtt'. To normalize for sequencing depth and compositional biases, we used the R package DESeq2 (V1.44.0) (Love et al., 2014) with the 'poscounts' size factor estimation method. Normalized counts were extracted using the DESeq2 function “counts (dds, normalized = TRUE)”, ensuring comparability across samples.

Microbial community composition, including all prokaryotes (bacteria and archaea), was analyzed based on small subunit (SSU) rRNA sequences. These sequences were extracted from clean reads of metagenome sequencing data using SortMeRNA v4.3.6 (Kopylova et al., 2012) then the data was used to estimate the relative abundances of microbial taxa. Nucleotide sequence alignment was performed using Nucleotide-Nucleotide BLAST 2.5.0+ (Altschul et al., 1990) against the SILVA SSU r138.2 database with the following parameters: -evalue 1e-50 -max_target_seqs 1. Library preparation and sequencing were performed by Biomarker Technologies (BMK) GmbH (Münster, Germany). Briefly, the constructed library was sequenced on the Illumina NovaSeq X platform using a paired-end (PE) 150 bp strategy.

2.5 Statistical analyses

Gene copy numbers were checked for normality using Shapiro-Wilks tests and then log10 transformed before using them in multivariate tests like principal component analysis (PCA). In addition to the normality tests, Q-Q plots and histograms were also used to observe the normality in the data. The factors that were considered important in determining any variation in all kinds of data variables (chemical properties, fluxes, and gene abundances) were used in comparing the means of variables. To accomplish this, one-way analysis of variance (ANOVA) and t-tests were performed. In article III, Kruskal–Wallis one-way ANOVA was performed, followed by Dunn's multiple comparison test with Bonferroni adjustment for post-hoc analysis. In other articles, Welch ANOVA and t-tests were employed to account for any violation of the homogeneity of the variances. Similarly, Games-Howell pairwise tests were used after Welch ANOVA as post hoc tests. For correlations, Spearman's rank correlations were employed in the correlation matrix as well as the regression plots. ANOVA tests were performed using the *ggstatsplot* package (Patil, 2021) in RStudio (Posit team, 2024). Normality of the data was assessed using *Jamovi* (The jamovi project, 2023) and RStudio. All figures presented in the articles (II, III, IV, & V) were prepared using the *ggplot2* package in RStudio. Python was additionally used to perform analyses and produce figures in the case of article I. For spatial analysis (article I), QGIS was used to map changes in environmental factors and soil microbial communities. The Inverse Distance Weighting (IDW) method was used for interpolation. The structural equation model (SEM) was performed using the *lavaan* and *semplot* packages in RStudio (Rosseel, 2012).

3. RESULTS AND DISCUSSION

3.1 Nitrous oxide cycle dynamics

Due to the complex nature of N_2O emissions via different processes and the fluctuating dominance of these processes in the soil, it is very difficult to predict and model N_2O emissions. So far, the temporal studies on N_2O fluxes have identified the short-lived spikes in the emissions, often termed as hot moments, in different ecosystems. Such fluctuations have been reported to contribute a significant portion to the annual N_2O emissions from these ecosystems. In this dissertation, the temporal study of flux patterns and microbial gene abundances (article I) allowed us to identify the major events or hot moments in N_2O emissions.

3.1.1 Temperate peatland forests

The temporal analyses of N_2O in the temperate peatland forest showed that the forest was a net source of N_2O (mean: 33.1 ± 4.70 μg N m^{-2} h^{-1}). A spike in N_2O emissions was observed from mid-March to mid-April (Fig. 6a). This period coincides with the thawing of frozen topsoil during the day and refreezing at night, as temperatures fluctuate between –5 °C and 5 °C in Estonia during this time of year.

Usually, the N_2O flux results from both nitrification as well as denitrification, as discussed in detail earlier. In the temporal study, the N_2O flux was found to be positively correlated with the *amoA* gene abundances (article I). There were instances where correlations between N_2O and other genes were statistically significant for more than half of the sampling locations (for example, fungal *nirK*) but were insignificant for the overall area. This suggested that the relationship was significant locally but may not be meaningful when considering a larger area. Moreover, at a specific sampling location, where the N_2O emissions were the highest throughout the year, N_2O showed positive correlations with archaeal *amoA*, fungal *nirK*, and *nirK* genes. The peat from this forest is slightly acidic, which favors ammonia-oxidizing archaea (AOA) over the ammonia-oxidizing bacteria (AOB), and the nitrification is thus mainly driven by the archaea (Stempfhuber et al., 2015; Zhalnina et al., 2012). The fungal *nirK* gene was also correlated with the N_2O emissions. Recent studies suggest that fungal denitrifiers may play a greater role in forest soils (Bösch et al., 2023). Since fungi lack the final step of converting N_2O to N_2, they function solely as emitters rather than consumers of N_2O.

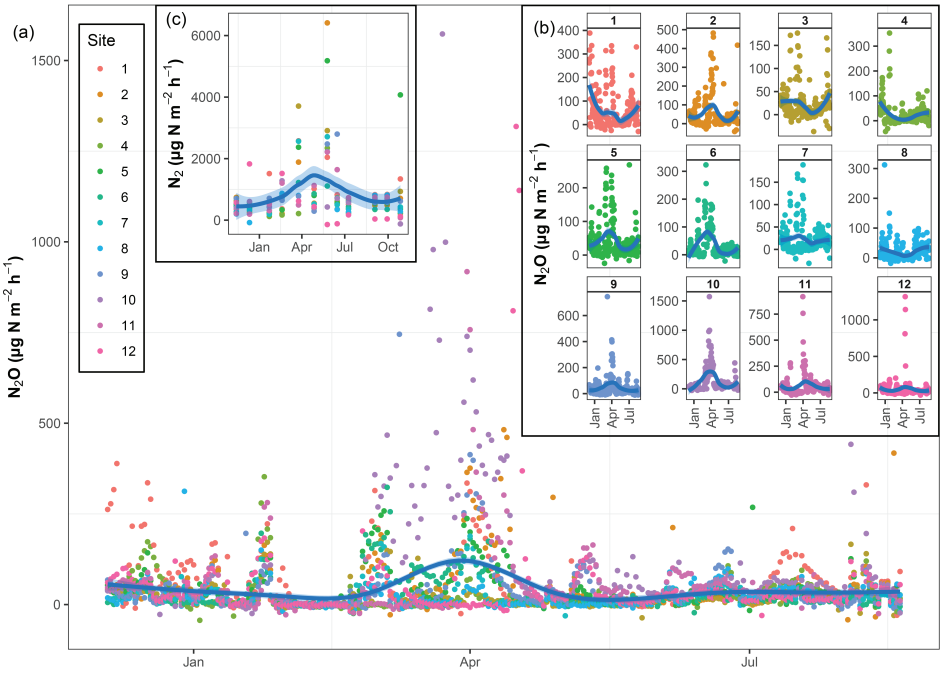

Figure 6. (a) The N_2O flux temporal variability in the year-long study (article I), **(b)** at different sampling points within the peatland forest, **(c)** along with the potential N_2 flux measured in the laboratory after incubating the intact soil cores. Different colors represent the different sampling locations in the peatland forest (article I).

The spikes of N_2O could also be seen during the late winter when temperatures were fluctuating around 0 °C, which can cause the freeze-thaw effect on the soil fluxes. Hence, the identified hot moments were mainly the winter and spring freezing and thawing of the topsoil in article I. However, due to the limited number of samples for microbial analyses (once per month), the thawing was not coupled exactly with the microbial sampling. Hence, generalizing the fluxes with archaeal nitrification or fungal denitrification was considered insufficient. For this purpose, it was needed that repeated sampling of soil be performed before, during, and after the thawing of frozen topsoil in a time when natural spring thaw had not occurred (ideally just before that). This could give us control over the conditions, and an exact snapshot of freeze-thaw could be captured in sampling. Hence, article II covered these aspects of the freeze-thaw events, which happen over a staggering terrestrial area in the northern hemisphere and contribute to more than 70% of the annual N_2O emissions in most of the ecosystems (Mander et al., 2021).

During the thawing of topsoil artificially with the heating cables, an increase in soil water content was found to correlate with soil temperature as well as the N_2O emissions from the soil. The emissions were significantly higher in the thawing phase as compared to the frozen soil. This was also reflected in the

abundances of different functional genes, such as the archaeal *amoA* gene, which peaked in session 3 (after 4 hours of heating). Meanwhile, the bacterial *amoA* abundance decreased in that session following a peak in session 2 (2 hours of heating ~ partial thawing). The microbial response to thawing was interesting as nitrifiers behaved differently to thawing, showing that AOA are more resilient to enhanced SWC during the freeze-thaw as compared to the AOB. However, the *nirK* gene grew significantly throughout the heating session and peaked in session 3 (Fig. 7a). Proportion-wise, *nirK* constituted 5% of the total prokaryotic abundance in S1 and increased to 10% in S2, while it dominated in S3 with 17%. In addition to the *nirK,* a continuous increase in the abundance of the *nirS* gene was observed during the experiment (Fig. 7b). Based on the co-occurrence patterns of *nirS* and *nosZ* genes in denitrifiers, it is evident that the *nirS*-containing microbes are more capable of complete denitrification. In contrast, the ecosystems dominated by *nirK*-type denitrifiers would emit more N_2O (Graf et al., 2014). While the potential N_2O producers increased their abundance during thawing, the N_2O-reducing gene *nosZ* did not increase in a similar pattern at the same time. Especially, there was no significant increase in the abundance of *nosZ*-II (Fig. 7c; 7d). During thawing, the oxic phase is followed by anoxia in the topsoil, during which only the microbes containing the *nosZ*-II gene can respond quickly, as *nosZ*-I containing microbes take a longer time to be active (Sennett et al., 2024).

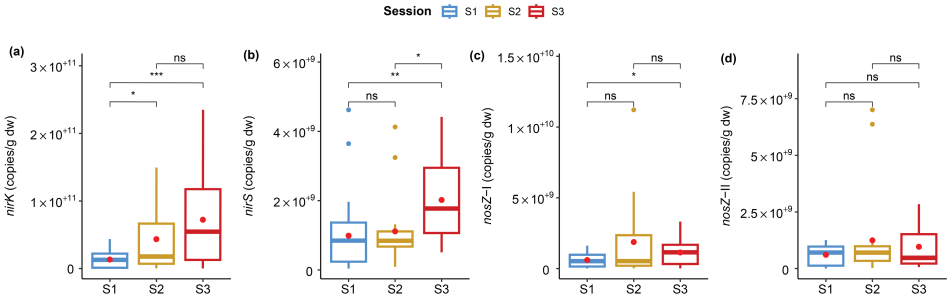

Figure 7. The abundance of denitrification genes **(a)** *nirK*, **(b)** *nirS*, and N_2O-reducing gene *nosZ* **(c)** clade I, and **(d)** clade II (derived from article II). The box boundaries indicate data points between the 25th and 75th percentiles, whiskers show the range of all data points excluding the outliers, the intersected line in the box represents the median, and red dots represent mean values. The significance of the pairwise relationships is indicated by ns (not significant), * ($p \leq 0.05$), ** ($p \leq 0.01$), and *** ($p \leq 0.001$).

In article I, only the N_2O fluxes during the autumn (September – November) were correlated with *nirK* abundance, while this correlation was not significant for spring or any other season. However, in the freeze-thaw experiment, this correlation was finally captured. This correlation indicated that during the freeze-thaw events, the main producers of the N_2O are the *nirK*-type denitrifiers. The abundances of *nir* genes (*nirK* and *nirS*) dominated over the abundances of ammonia oxidation genes (*amoA* genes), as well as the *nosZ* gene (both clades I & II), and

positively correlated with the N_2O fluxes (Fig. 8a and b). The multiple regression models (Fig. 8c) showed that when N_2O fluxes were correlated with the abundance and proportions of all the potential N_2O producer genes, only the abundance and proportion of the *nirK* gene were found to be significantly correlated.

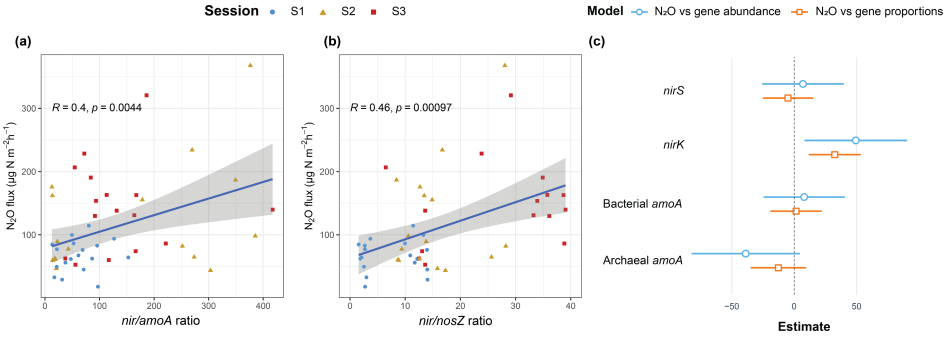

Figure 8. The relationship between the **(a)** ratios of nir (*nirK* and *nirS*) and *amoA* (bacterial, archaeal, and comammox), and **(b)** ratios of *nir* (*nirK* and *nirS*) and *nosZ* (I & II). **(c)** The multiple regression between N_2O fluxes and the abundances and proportions of all genes responsible for N_2O production (article II).

There was no significant change in *nosZ* gene abundances (both clades) in response to thawing during the experiment because thawing did not create inundated conditions. The soil surface temperature also did not exceed 4 °C during the experiment. Although the abundance of N_2O-producing denitrifiers is unaffected by such low temperatures, the N_2O-reducers, on the other hand, have shown a decrease in their abundance and breakdown in their activity at temperatures below 5 °C, resulting in an increase in N_2O fluxes (Holtan-Hartwig et al., 2002). The N_2 emissions in the thawed soil were higher than in the frozen soil, meaning that the N_2O-reduction process was active during the thawing, but not enough to reduce all the produced N_2O, hence the N_2O: (N_2 + N_2O) ratio also increased in the thawed soil.

During freeze-thaw events, the diminishing of the frozen layer from the topsoil may also cause a physical release of the underlying trapped N_2O (Teepe et al., 2001). But this release would only be significant when the topsoil is thawed for the first time after winter. Some studies emphasize the *de novo* production of N_2O during the freeze-thaw events, making them the hot moments of N_2O production (Risk et al., 2013; Wagner-Riddle et al., 2010). Nitrification and denitrification depend contrastingly on soil oxygen levels, with nitrification preferring oxic conditions and denitrification occurring under anoxic conditions. During thawing, the consumption of NH_4^+ and an increase in NO_3^- levels were observed. The increase in soil NO_3^- indicated that thawing initiated nitrification. The increase in NO_3^- was consistent throughout the measurement in S2 because this session provided aerobic conditions for the topsoil microbiome, which only dropped during S3 (complete thawing).

In the temporal study, the freeze-thaw N_2O spike was followed by the spring N_2 spike (Fig. 6c), which surprisingly did not correlate with the abundance of the *nosZ* gene. After thawing of all topsoil, the forest floor is mainly flooded with meltwater, and conditions become strictly anoxic in the peat soil. These conditions are conducive to complete denitrification (until they last); however, the nitrification can still be active after such an environment due to fluctuation in water table depth (WTD), potentially causing a release of N_2O. Due to the same problem as before (not being able to catch the dynamics of the conditions), another study was conducted (article III) to observe more closely the flux dynamics as well as the microbiome analysis after the inundated conditions are over, and when these conditions completely changed to normal.

There were minimal soil N_2O fluxes observed in the first two weeks (April 6 – April 22) of the study period (article III). N_2O fluxes increased after this continuously until the end of the study period (May 12). The mean flux for overall period of study was 13.2 ± 1.4 μg N m^{-2} h^{-1}. Tree stems (birch and spruce) also showed a similar pattern to the soil. Both soil water content and WTD lowered continuously throughout the study period. Soil and tree stem N_2O fluxes were negatively correlated with the WTD and SWC (Fig. 9). There was a negative correlation between NH_4^+ and NO_3^-, suggesting a potential nitrification throughout the study. However, in the initial weeks of spring, the N_2O was minimal (close to zero). The overall N_2O fluxes (soil and tree stems) correlated negatively with the abundance of *nosZ*-I and *nosZ*-II, while positively with the *nir*:*nosZ* ratio. The abundance of *nosZ*-I and *nosZ*-II dropped significantly after the first week, increasing the *nir*:*nosZ* ratio, which shows that the incomplete denitrification became the main cause of N_2O emissions in the late spring.

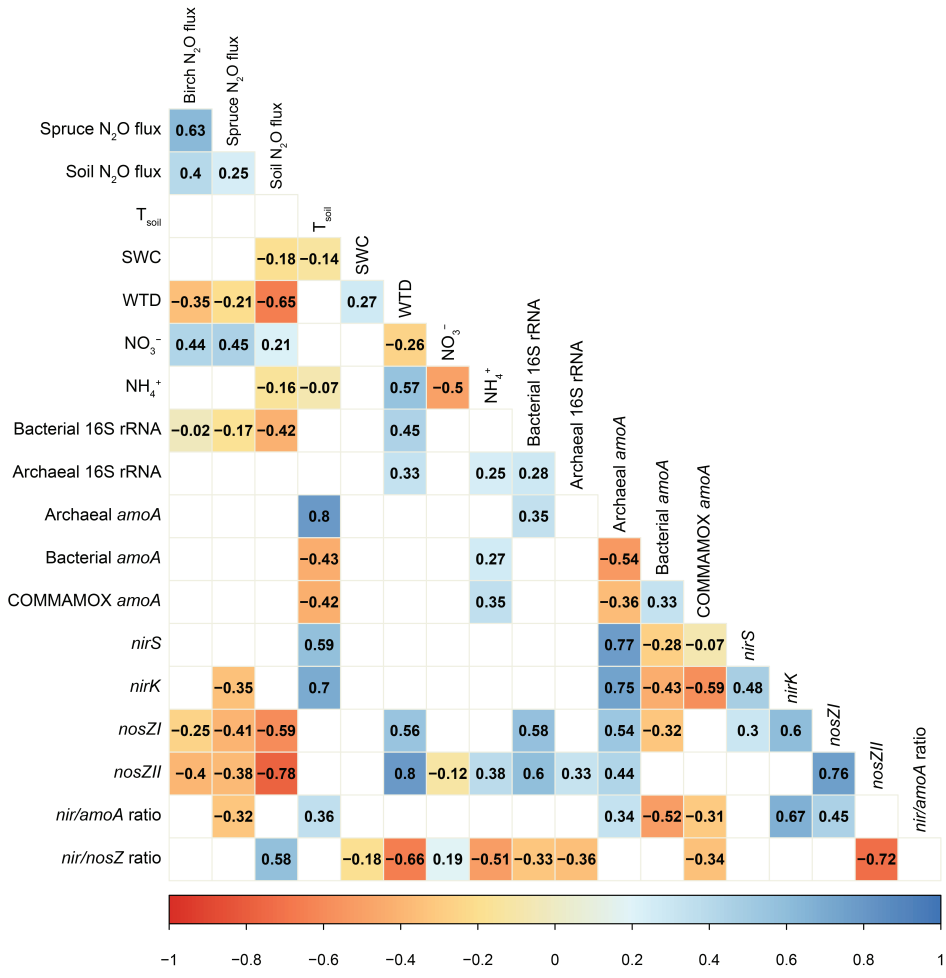

Figure 9. Correlation matrix (Spearman) between the N_2O fluxes (soil and tree stems), soil physical-chemical parameters, and the nitrogen cycling-related gene abundances and their ratios (article III).

3.1.2 Tropical peatland cloud forests

Nitrogen-rich organic soils under the dry and warm conditions have been reported to be the N_2O source globally (Pärn et al., 2018). Similarly, tropical wetland soils during drought periods have been reported as significant sources of N_2O (Liengaard et al., 2013). Such emissions are correlated with the functional diversity of different microbes, in addition to the N-related processes driven by ammonia-oxidizing archaea (Bahram et al., 2022). In case of cloud forests (article IV), it was found that during the warm and dry conditions, despite having high soil NH_4^+ and NO_3^- content, the N_2O emissions were minimal (1.06 µg N m^{-2} h^{-1} in the mixed forest, 0.37 µg N m^{-2} h^{-1} in the Erica forest). The relationship between soil temperature and N_2O fluxes was inconsistent and statistically

insignificant in the case of forest soil and the wetland. Although they correlated positively with the soil temperature in one of the forests (Fig. 10a), this inconsistency was probably due to very low N_2O values. Meanwhile, N_2O fluxes from the soil correlated with the increase in SWC in both cloud forests, and in the wetland this correlation seemed to be negative (Fig. 10b). The highest N_2O flux in cloud forest soils was observed between the SWC range of 0.6–0.7 m^3 m^{-3} while in case of wetland the highest seemed to be around 0.9. There was a visible gap in SWC between the cloud forest soils and wetland soils, where most of the previous global study reported their peak N_2O emissions at 0.6–0.8 m^3 m^{-3} (Pärn et al., 2018). Figure 10b shows that our study potentially missed the hot moment of N_2O if considering SWC only. Although the regression lines point out that any further increase in SWC might increase the amount of N_2O as well, the magnitude of fluxes observed can potentially claim a spike within a range of one unit of SWC. In such a situation, an investigation into microbial dynamics helps our understanding of the underlying mechanisms.

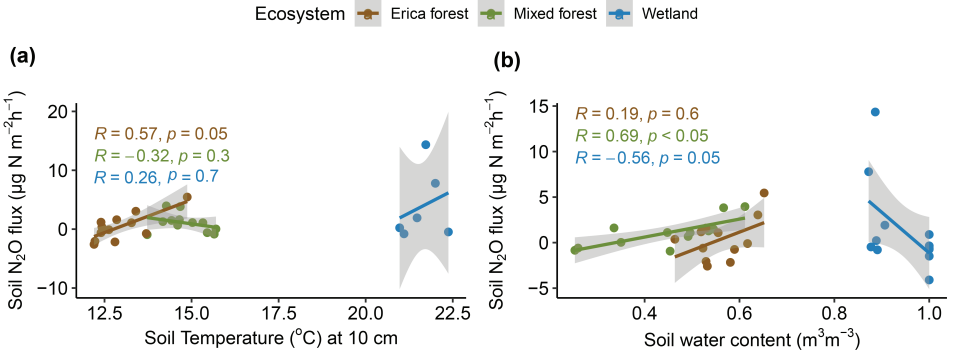

Figure 10. The correlation between soil N_2O fluxes and **(a)** soil temperature and **(b)** soil water content (article IV).

The abundance of *the nifH* gene positively correlated with high NH_4^+ levels in one of the cloud forests, as well as in the wetland, which was chosen for the comparison. This suggested a high potential of microbial nitrogen fixation in the peat soils of cloud forests. The ammonia oxidation was mainly driven by archaea (AOA), as the soil NO_3^- positively correlated with the abundance of archaeal *amoA* gene. The archaeal *amoA* gene abundance was significantly higher in the Erica forest (featuring *Erica reunionensis*) forest soil than in the mixed forest ($p = 0.005$) and the wetland ($p < 0.001$). Among tropical soils, cloud forest soils have previously been reported to have the highest abundance of archaeal *amoA* genes (Pett-Ridge et al., 2013). The high soil NO_3^--N also correlated with low soil pH values in both forests, which also suggests the active nitrification process, as the increased H^+ ions generated during the ammonia oxidation result in the acidification of the soil (Li et al., 2018; Tao et al., 2024). The peat soil in the studied forests was highly acidic. The mean soil pH was 4.5 and 4.3 in Erica and mixed

forests, respectively. These significantly differed from the wetland samples' mean pH (7.3, $p < 0.001$).

Under such acidic conditions, the *nir*-type prokaryotic denitrification is reported to be slowed down (Albina et al., 2019; Brenzinger et al., 2015; Nicol et al., 2008), and hence, the NO_3^- was being accumulated in these soils. Despite the high abundance of archaeal nitrifiers, very low N_2O emissions were observed in the cloud forest peat soils. This was inconsistent with a previous global study where soil N_2O emissions were positively correlated with archaeal *amoA* gene abundance (Bahram et al., 2022). In tropical soils, N_2O is often correlated with the *nirK* and fungal *nirK* abundances (Lourenço et al., 2022), but due to potentially slow denitrification, this process was not a prevalent factor anymore in our study sites. Although low pH also affects the activity of *nosZ*-carrying N_2O-reducers (Liu et al., 2014), in tropical soils, some acidophilic bacteria have been reported to reduce N_2O even at low pH levels (He et al., 2024).

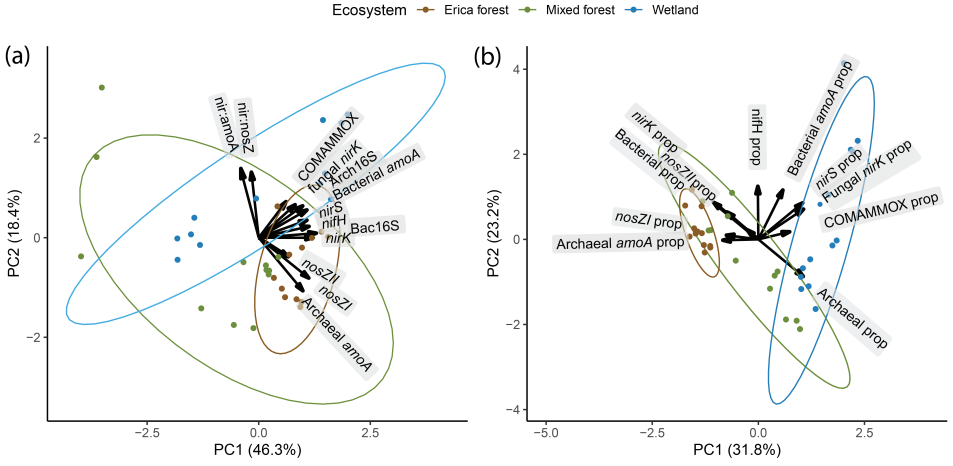

Figure 11. Principal component analysis (PCA) biplots of nitrogen cycling-related **(a)** gene abundances and **(b)** the gene proportions in the total prokaryotic abundances in different ecosystems as groups. The x and y axes represent the first two principal components (PCs), capturing the most significant variation in the data. The length of vectors represents the importance of that gene in explaining the variance captured by the PCs. The ellipses in different colors represent the distribution of samples from different ecosystems (95% confidence intervals) (article IV).

In PCA, Erica forest samples made distinct clusters for gene abundance and relative proportions in the total prokaryotic abundance, showing less variability within the forest. Many key genes also showed their dominance in this cluster (for example, the archaeal *amoA*, *nirK*, as well as the *nosZ* genes). As compared to the wetland and erica forest soil, the mixed forest soils showed intermediate characteristics regarding the abundance as well as the relative proportions of the key functional genes.

3.2 Methane cycle dynamics

3.2.1 Temperate peatland forests

In the Agali's drained peatland forest, a temporal study on CH4 fluxes from soils has shown that this forest is a net CH_4 sink with a mean flux value of −32.2 ± 1.2 (μg C m^{-2} h^{-1}) when considered for one year (Ranniku et al., 2024). Drier periods mark high uptake, while wetter periods also tend to be net sinks according to this study. In article III, the transition time from moderately wet to dry period (during spring) was considered to study the soil CH_4 fluxes and related microbial genes to investigate the shift in the microbial potentials in this crucial time. In this study, which continued for a little over a month, a continuous drop in WTD and SWC was observed, showing the shift from wet to dry period. However, the CH_4 fluxes increased from zero at the beginning of measurements to a maximum after two weeks and then decreased (article III). There was no correlation found between CH_4 fluxes and any physical-chemical and microbial gene parameters. There was an increase in the dissolved CH_4 in both water and the sap of birch trees. This indicates that CH_4 was being produced in deeper soil levels and subsequently absorbed by the roots. The stem CH_4 fluxes exhibited a similar trend. In the final week of measurements, the topsoil showed a significant increase in the abundance of the *pmoA* gene, following a decrease observed in the middle of the study (Fig. 12a). This suggests that while the deeper soils were still producing CH_4, the decline in the net soil flux in the last week was due to the increased activity of aerobic methane oxidizers in the topsoil. The abundance of n-DAMO microbes increased during the middle of the measurement period before dropping again; however, their overall abundance remained low (Fig. 12b). At the same time, the abundance of the *mcrA* gene in the topsoil decreased, leading to a reduced ratio of producers to consumers. This implies that while CH_4 production was occurring in the deeper soils, the topsoil was preparing to act as a CH_4 sink. Under these conditions, CH_4 was channeled through the roots and stems in the form of dissolved flux and was eventually released from the surfaces of the tree stems.

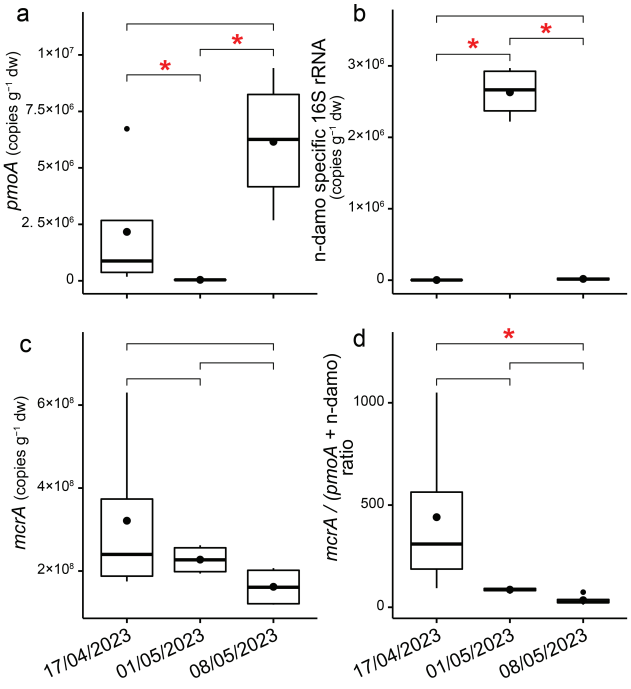

Figure 12. The abundance of methane-cycling genes in the soils of temperate drained peatland forest (article III).

3.2.2 Tropical peatland cloud forests

The peat soil in both cloud forests was a net sink of atmospheric CH_4 (article V). Mean soil CH_4 flux in the Erica forest was measured at −22.4 ± 4.20 (mean ± SE μg C m^{-2} h^{-1}). At the location in the mixed forest, where *Erica reunionensis* predominated, the uptake was recorded at −33.7 ± 15 μg C m^{-2} h^{-1}, while the mean CH_4 flux in the location dominated by tree fern *Alsophila glaucifolia* was −16.7 ± 9.88 μg C m^{-2} h^{-1}. The metagenomic analyses show that key genes involved in CH_4 oxidation, *pmoAB*, encoding pMMO, were observed in soil samples, irrespective of the forest type, while *mmoC,* encoding soluble methane monooxygenase (sMMO), were present across all soil samples (Fig. 13). Furthermore, among key genes for methanol oxidation, *xoxF* encoding lanthanide-dependent methanol dehydrogenase, was found to be the most abundant in all soil samples. These situations are likely associated with the abundance of n-DAMO 16S rRNA quantified from qPCR. However, the Erica forest soil showed significantly higher abundance of n-DAMO-related 16S rRNA gene.

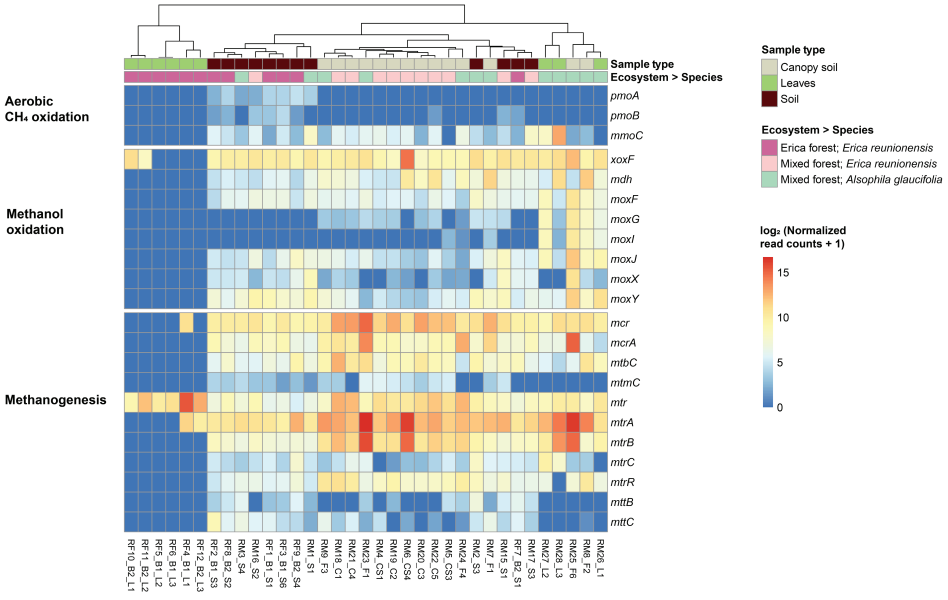

Figure 13. Functional gene abundance across soil, canopy soil, and leaf samples. The row-scaled heatmap represents the log_2-transformed normalized read counts for key functional genes for methanogenesis, aerobic methanotrophy, and methanol oxidation (article V).

In the cloud forests' soils, the ratio of the quantified *mcrA* gene to the product of *pmoA* and n-DAMO 16S rRNA genes was found to be less than one, indicating a relatively higher abundance of methanotrophic genes in the soil as compared to the methanogenic gene *mcrA*. Methanogenic communities (*mcrA*) are highly sensitive to the inundation and drying of tropical soils (Hernández et al., 2019), which also happened in this instance. In soils of cloud forest, the n-DAMO 16S rRNA gene showed a strong positive correlation with SWC and NO_3^- levels. The relative abundance of the NC-10 phylum also had a positive relationship with soil NH_4^+ and NO_3^- levels, indicating the potential for nitrate-dependent methanotrophy in soil. The peat soils of Erica forest contained a significantly higher abundance of the 16S rRNA gene and relative abundance of NC-10 phylum bacteria (Fig. 14a), meanwhile, the mixed forest peat soils had *pmoA*-containing verrucomicrobial MOB and alphaproteobacterial MOB as their dominant methanotrophic groups in different samples, followed by methylotrophs. The relative abundance of NC-10 bacteria also correlated with the soil NO_3^- values (Fig. 14b).

Structural Equation Model analysis was employed to understand the processes explaining CH_4 fluxes in the cloud forest soils, particularly focusing on the contributions of aerobic methanotrophy and n-DAMO in CH_4 oxidation. In general, the *pmoA* gene-driven methanotrophy is considered the main player for CH_4 oxidation, but due to recent research on the n-DAMO process's contribution to CH_4 oxidation in peat soils (Shi et al., 2022), it was intended to explore the relationship between CH_4 fluxes and the abundances of both candidate genes of methanotrophy. There was a weak negative correlation between n-DAMO gene abundance and CH_4 flux values (Fig. 14c), while a significant positive correlation with soil CO_2 flux values, indicating the potential for CH_4 oxidation. The corre-

lation was also significantly positive between NO_3^- and n-DAMO gene abundances, showing that the main source of oxygen was NO_3^- in this CH_4 oxidation pathway. A negative correlation between the abundance of *pmoA* and CH_4 flux was also observed, but it did not show any effect on the CO_2 production via oxidation. This suggested that n-DAMO was a major contributor to the overall CH_4 oxidation process, followed by the aerobic CH_4 oxidation in the peat soils of cloud forests.

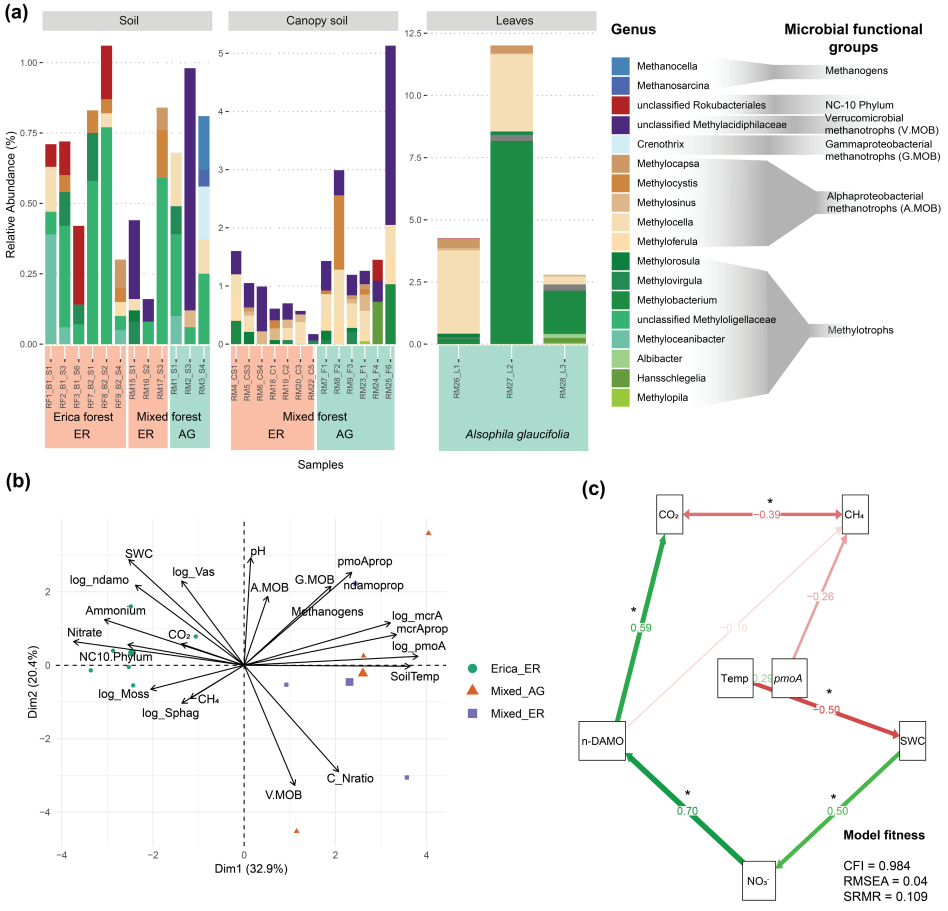

Figure 14. (a) Relative abundance of small subunit rRNA (SSU) sequence of key CH_4 cycling microbial genera (including methanogenic archaea, methanotrophs, and methylotrophs) in soil, canopy soil, and leaves across tropical cloud forests: Erica Forest dominated by *E. reunionensis* (Erica_ER), Mixed Forest dominated by *E. reunionensis* (Mixed_ER), and dominated by *A. glaucifolia* (Mixed_AG). **(b)** Clustering of the forest sites based on principal component analysis biplot of soil variables, including physicochemical properties, gene copy numbers, and microbial functional groups. Big triangles represent the mean values, and colors represent the forest sites. **(c)** Structural Equation Model (SEM) for the soil CH_4 and CO_2 fluxes, including key controlling factors, i.e., SWC, soil NO_3^-, n-DAMO, and *pmoA* gene abundances. Red lines show negative correlations, while green lines show positive correlations. Asterisks represent significant relationships (article V).

3.3 Role of above-ground forest compartments in biogeochemical cycles

Since the above-ground forest compartments in temperate as well as tropical ecosystems also play a crucial role in biogeochemical cycles in the respective ecosystems, they were also studied for this dissertation. In the case of Agali's drained peatland forest (article III), the tree stem fluxes were considered in the springtime, due to fluctuating WTD and SWC, which can also be reflected in the tree stem fluxes. Post-spring-thaw was marked with minimal N_2O and CH_4 fluxes, which rose as WTD dropped. The reason behind the increased stem N_2O fluxes was the elevated *nir:nosZ* ratio in the topsoil in the later weeks of the measurement. CH_4 emissions from birch stems in peatland forests have been shown to stay low but detectable in the winter and increase in the spring following the rising water table after snowmelt (Ranniku et al., 2024). In our study (article III), we had hypothesized that elevated WTD would be correlated with the high CH_4 fluxes from the tree stems. However, this was not supported by our observations. As shifts in WTD and SWC during the study period were relatively small, soil hydrological conditions may be more important for long-term CH_4 flux dynamics, for example, with changes between dry and wet periods, and are not direct governing factors in the short term. Birch trees have low-reaching root systems (Bachofen et al., 2024), hence, stem fluxes may be due to CH_4 originating from deeper soil layers where methanogenesis prevails, as it is taken up by tree roots and moves up the xylem. This was reflected in the measurements of dissolved fluxes of birch sap as well as water from the ground (article III). The reason why stem CH_4 fluxes did not relate to soil microbial genes is because of the sampling strategy. The samples were obtained only from topsoil, which suggested its readiness to function as a potential CH_4 sink, while CH_4 was only being produced in the deeper soil, which was not tested for *mcrA* gene abundance.

In the temperate forest, the microbial analyses of the above-ground components were not performed. This limits our discussion about any independent role of these components in the biogeochemical cycles. However, in the case of tropical cloud forests, the above-ground components (canopy soil, tree stems, and leaves) were also included for the microbial analyses in addition to the tree stem fluxes. The tree stems in the tropical cloud forests varied from weak sources to weak sinks of N_2O (article IV). The mean N_2O fluxes from the stems of *Erica reunionensis* in the Erica forest were −0.267 μg N m^{-2} h^{-1}, while in the mixed forest they were 0.843 μg N m^{-2} h^{-1}. The mean N_2O fluxes from *Alsophila glaucifolia* stems in the mixed forest were −0.016 μg N m^{-2} h^{-1}. Overall, the *E. reunionensis* stems showed more variation in the stem N_2O fluxes from weak sinks to weak sources of N_2O. Although there were no *nifH* genes detected in the above-ground samples, archaeal *amoA* genes were detected in the individual, but the abundance was too small (for example, the leaf of *A. glaucifolia* had $1.05\times10^5 \pm 4.86\times10^3$ copies/g). The denitrification genes were detected in most above-ground samples (Fig. 15). The *nirS* gene abundance was found to be significantly higher in the canopy soil as compared to other above-ground samples ($p < 0.001$). The *nirS* genes were not detected in the leaves of *E. reunionensis* and stalks of *T. domingensis* (from the wetland). Meanwhile, the *nirK* genes were abundant

in all canopy samples, with canopy soil showing the highest mean value of $2.09\times10^8 \pm 8.30\times10^7$ copies/g, which was significantly higher than leaves and stem cores ($p < 0.01$). Fungal *nirK* was only detected in the canopy soil. The *nosZ*-I was detected in all the samples taken from the canopy. The highest abundance was found in the canopy soil ($1.20\times10^6 \pm 3.42\times10^5$ copies/g, $p < 0.01$). The canopy soil from *E. reunionensis* had a higher abundance of *nirS*, *nirK,* and *nosZ*-I genes than *A. glaucifolia's* canopy soil (Fig. 15).

The absence of the *nifH* gene in the above-ground samples in tropical cloud forests indicated a lack of microbial N fixation potential in these ecosystems, contrasting with previous studies in a similar ecosystem, which estimated high N_2 fixation in the canopy soil during the dry season (Matson et al., 2015; Shi et al., 2023; Stanton et al., 2019). However, the atmospheric NH_3 converted to NH_4^+ in the presence of cloud droplets or precipitation can be deposited in the canopy (Behera et al., 2013) which can become part of the above-ground nitrogen cycle in these forests. The minor detection of archaeal *amoA* in above-ground samples indicated a potential for nitrification in the canopy of cloud forests. The NO_3^- yielded through canopy nitrification can also become available for the epiphytes growing on the canopy soil. Meanwhile, the detection of denitrifiers in the canopy revealed potential for above-ground denitrification in these forests.

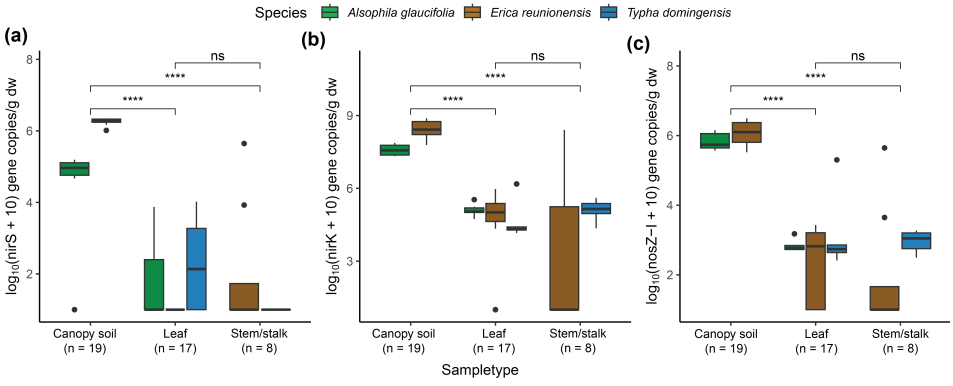

Figure 15. Boxplots of **(a)** *nirS*, **(b)** *nirK,* and **(c)** *nosZ*-I gene abundances in the above-ground samples from tropical cloud forests and a tropical wetland (article IV). The colors represent different plant species. The line within the box shows the median. Dots outside the whiskers represent outliers. The whiskers show the 95% confidence intervals. The significance of the pairwise relationships is indicated by ns (not significant), * ($p \leq 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$), and **** ($p \leq 0.0001$) (article IV).

The NO_3^- from canopy nitrification (Guerrieri et al., 2024), assimilation by plants, and foliar uptake (Bourgeois et al., 2019) can be used by these denitrifiers on the surface (canopy soil) or inside the anoxic parts of leaves. Meta-transcriptomics study suggests that if denitrifiers are given anoxic conditions after long periods of oxic conditions, *nirK*-containing microbes exhibit faster rates of NO_3^- reduction. However, during a short-term anoxic situation (e.g., created by a brief spell of rain or by cloud condensation), only the *nosZ*-II is able to complete the denitrification (N_2O-reduction) in the given time (Sennett et al., 2024). Our study did not detect the *nosZ*-II gene clade in the above-ground samples, and only

nosZ-I was detected, which requires long-term anoxic conditions to reduce the N_2O, according to the same study. In cloud forests, cloud condensation can induce short-lived anoxic conditions in canopy soils or in the phyllosphere. This could lead to incomplete denitrification and N_2O release. We found stems of *E. reunionensis* as weak sources of N_2O in the mixed forest where these trees were covered with canopy soil. The *E. reunionensis* stems in the Erica forest without any canopy soil were found to be the weak sinks of N_2O. The presence of *nosZ*-I in the stem cores of the cloud forest trees can also explain the N_2O uptake by *E. reunionensis* stems in our study. The *nosZ*-I was found in the leaf samples of all plant species under study. The N_2O, which is transported from the soil to different plant compartments (Machacova et al., 2016), can be reduced within the leaves. The *nosZ*-I-type denitrifying microbes in the above-ground compartments during the wet season, when anoxic conditions prevail for longer periods on plant surfaces, can reduce the N_2O emitted by soil and/or canopy soil.

The tree stems in both tropical cloud forests were weak sinks for CH_4 (article V). The stems of *E. reunionensis* in the Erica forest showed mean CH_4 fluxes of −0.31 ± 0.17 and −0.96 ± 0.67 μg C m^{-2} h^{-1} in the mixed forest, while the mean CH_4 flux from the tree fern *A. glaucifolia* stem was −1.53 ± 1.10 μg C m^{-2} h^{-1}. However, for CO_2, the mean flux from *E. reunionensis* in the Erica forest was 20.9 ± 8.30 mg C m^{-2} h^{-1}, while in the mixed forest, it was 10.5 ± 2.59 mg C m^{-2} h^{-1}. The CO_2 fluxes from the *A. glaucifolia* stems were lower than the rest of the trees and recorded at −1.10 ± 2.47 mg C m^{-2} h^{-1}. The difference between the two forests was statistically insignificant regarding the overall stem CH_4 ($p = 0.165$) and CO_2 flux ($p = 0.140$). In the mixed forest, a high abundance of the *mcrA* gene was found in canopy soil samples. The *pmoA* gene was also found in the canopy soils, but in less abundance than the *mcrA* and n-DAMO 16S rRNA genes. The gene abundances were higher in canopy soils than in leaves and stems for *mcrA*, *pmoA*, and n-DAMO 16S rRNA (Fig. 16a–c).

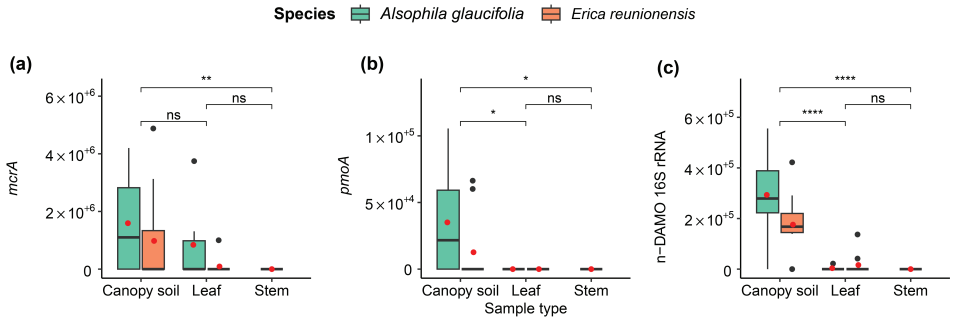

Figure 16. Abundances of genes (g⁻ dry weight) involved in the processes of **(a)** methanogenesis (*mcrA*) and methanotrophy; **(b)** *pmoA* and **(c)** n-DAMO 16S rRNA) in plant samples quantified by qPCR. Colors represent the species related to the samples. The box depicts the interquartile range (IQR), which captures the 25th and 75th percentiles of the data. The whiskers that extend from the box represent the data range within 1.5 times the IQR. Inside the box, a line indicates the median value of the data set, while the red dots represent the mean values. Black dots are outliers. The pairwise relationships are indicated by *ns* (not significant), * ($p \leq 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$), and **** ($p \leq 0.0001$) (article V).

The tree stems of both species in the cloud forests functioned as weak sinks for CH_4. However, there was no correlation between the flux values and the gene abundances in terms of the stem samples. Despite the absence of methanotrophic genes in their core samples, stems of *A. glaucifolia* absorbed more CH_4 than those of *E. reunionensis*. This may indicate that the microorganisms living on the surface of stems or bark are responsible for the active methanotrophy instead of those living inside of stems. Previous research has indicated that microbial communities residing on stem surfaces or within wood crevices can oxidize atmospheric CH_4 (Gauci et al., 2024; Jeffrey et al., 2021). The leaves of *A. glaucifolia* showed a high abundance of the *mcrA* gene (Fig. 16a) in addition to the relative abundance of methylotrophs (Fig. 14a). The metagenomics analyses of the above-ground samples revealed that the leaves of *E. reunionensis* had a higher relative abundance of the *mtr* gene as compared to the leaves of *A. glaucifolia*. However, the leaves of *A. glaucifolia* contained a higher abundance of *mtrA* and *mtrB* genes (Fig. 13). This finding suggested that the phyllosphere of *A. glaucifolia* has microbial potential to emit CH_4.

4. SYNTHESIS

The underlying microbial processes driving the fluxes of GHGs like N_2O and CH_4 are well understood; however, in different soil types and ecosystems, especially the peatland forest ecosystems, the specific processes responsible for the emission or consumption of these GHGs are not fully studied. This dissertation has targeted two different forest ecosystems in temperate and tropical regions to compare the microbial potential of different GHG-related processes in the peat soils.

In the peat soils of temperate forests, the temporal study suggested that the nitrification process, mainly driven by archaea, is the main driver of N_2O fluxes. Meanwhile, freeze-thaw events in the temperate forest are the major contributors of annual N_2O fluxes (Fig. 6). This was due to changing soil moisture levels within a narrow range, which promotes a shift in microbial processes. This was observed in the freeze-thaw experiment, and N_2O fluxes during the thawing phase were found to be driven by *nirK*-type denitrifiers mainly (Fig. 8). Springtime in the temperate forest was marked with a high *nosZ*:*nir* ratio with minimal N_2O fluxes. This dominance of N_2O-reducers occurs because of inundated conditions, followed by the complete thaw of frozen soil as well as the snow. Like most temperate forests, the drained peatland forest is also a net CH_4 sink, and the process of methanotrophy in the peat soils is mainly driven by aerobic methanotrophs containing *pmoA* genes. In a transition time from wet to dry (lowering of groundwater table), the *mcrA* abundance dropped in the topsoil, while the fluxes of CH_4 increased from the tree stems, showing that with lowering groundwater table, methanogenesis occurred in the deeper anoxic parts of soils, while the topsoil shifted to function as the CH_4 sink.

Archaeal nitrification was an active process in the peat soils of tropical cloud forests, similar to that in the temperate forest, leading to accumulation of high levels of soil NO_3^-. Interestingly, even with the dominant presence of *nir*-type denitrifiers over the nitrifiers, the fluxes of N_2O were minimal. Meanwhile, the N_2 fluxes from these peat soils were high. Complete denitrification could not solely account for the high N_2 fluxes observed in these peat soils, particularly due to the low pH, which restricts the rates of the denitrification process. A high abundance and proportion of *nosZ*-I can be considered influential in N_2 fluxes; however, the primary process driving these N_2 fluxes in the peat soils was n-DAMO. This was supported by the significant presence of NC-10 bacteria, which are responsible for the n-DAMO process. The n-DAMO 16S rRNA marker gene showed a positive correlation with soil NO_3^- levels and CO_2 fluxes, while exhibiting a weak negative relationship with CH_4. This suggests that the main process responsible for CH_4 consumption in these soils is n-DAMO, followed by aerobic methanotrophy. Since n-DAMO is an anaerobic process, it can also take place in deeper soil layers or during inundated conditions, where CH_4 production is likely. As a result, the overall net flux in these forests can remain negative (Fig. 17).

The above-ground components of cloud forests, such as canopy soil and the leaves, which are often overlooked in many studies, were found to possess significant microbial potential to play a crucial role in the biogeochemical cycles within cloud forests. The microbial genes *nirK* and *nosZ*-I were detected in the canopy soils as well as in the leaf samples. This shows that within these forests, a biogeochemical cycle independent of soils can also function. This may involve the denitrification and N_2O reduction in the canopies of these forests. In addition, the methanogenic gene *mcrA* was also detected in the leaf samples, with metagenomics also revealing the presence of methylotrophs. Verrucomicrobial methanotrophs were also detected in the canopy soils. All this shows that the canopies of these forests are capable of producing and consuming CH_4.

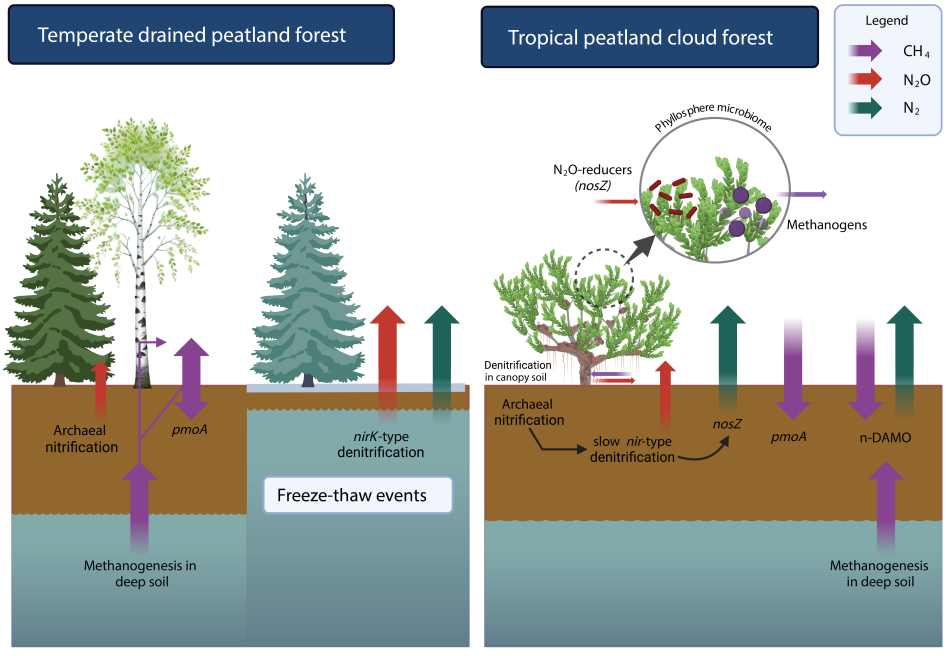

Figure 17. Microbial processes related to methane (CH_4) and nitrous oxide (N_2O) flux dynamics in the temperate drained peatland forest and tropical peatland cloud forests (during the dry season). Trees represent the species in temperate forest (*Picea abies*, *Betula pubescens*) and tropical cloud forest (*Erica reunionensis* with canopy soil). The arrows represent the direction of fluxes, the large size arrows represent the magnitude ≥ 20 µg m^{-2} h^{-1} while the medium size represent the fluxes between 5–20 µg m^{-2} h^{-1}, while the smallest arrows represent the minimal fluxes (≤ 5 µg m^{-2} h^{-1}). The arrows in the phyllosphere microbiome represent only the potential flux directions. Methanogens are shown as round-shaped microbes, while the N_2O-reducers are shown as rod-shaped bacilli.

5. CONCLUSIONS

In the temperate forest, the temporal variability, driven by seasonal fluctuations in temperature, soil moisture, water table depth, NO_3, NH_4, and potential N_2 emissions, influenced overall N_2O emissions, with peaks observed during the spring freeze-thaw period. These emissions were driven by the variation between aerobic and anaerobic conditions, which triggered nitrification and denitrification processes within a narrow range of soil moisture. The fluctuation in soil NH_4^+ and NO_3^- levels showed that the microbial processes were active during thawing phases, and the fluxes were *de novo*. Temporal fluxes correlated with archaeal *amoA* gene as well as with the prokaryotic and fungal *nirK* genes; however, the high N_2O fluxes during the freeze-thaw events were found to be correlated only with prokaryotic *nirK* gene abundance and proportions. The increased abundance of *nirK*-type denitrifiers, with the unchanging abundance of *nosZ*-containing N_2O-reducers, showed that incomplete denitrification is a dominant process during the thawing of frozen soil. The fall in freeze-thaw N_2O spike during springtime was caused by the increased *nosZ* abundance over time and low soil NO_3^- content, potentially because of slow nitrification in inundated conditions. The hypotheses I and II regarding the N_2O flux processes were supported by our results. During springtime, the peat soils shifted from minimal to an N_2O source because of the dropping water table and increasing *nir*:*nosZ* ratio. This was reflected in both soil and tree stem fluxes. The same period was also marked by a change in the methanogenic potential. While the topsoil's methanogenic potential, determined by the *mcrA*:(*pmoA* + nDAMO) ratio, decreased, the soil and tree stem CH_4 increased, possibly because of deep soil methanogenesis. Thus the hypothesis III was also supported.

In the tropical cloud forests, the levels of NH_4^+-N were significantly correlated with the *nifH* gene abundance in the peat soils, showing that these soils are nitrogen-fixing hotspots. The high soil NO_3^--N was correlated with the archaeal *amoA* abundance and proportions. However, the N_2O fluxes were minimal due to low pH, which potentially slowed *nir*-type denitrification, along with a high abundance and proportion of *nosZ* genes. High N_2 fluxes were found in these forest soils, which can be attributed to *nosZ* activity as well as the n-DAMO process. The peat soil and tree stems in cloud forests acted as a CH_4 sink and exhibited high methanotrophic potential, mainly driven by NC-10 bacteria (n-DAMO), followed by the alphaproteobacterial methanotrophs.

Microbes possessing *nirK* and *nosZ*-I genes were detected in the above-ground components of tropical cloud forests, including canopy soil and the phyllosphere. The canopies of these forests showed microbial potential for denitrification and N_2O reduction. Similarly, the leaf samples, especially from the endemic fern species *A. glaucifolia*, possessed the methanogenic gene *mcrA*, while the other species also showed methanogenic potential in metagenomics analyses. Canopy soils in the mixed forests possessed verrucomicrobial methanotrophs. Canopies of the cloud forests showed the microbial potential of producing and consuming

CH_4. While the soil had high methanotrophic potential, above-ground components showed high methanogenic potential, supporting hypothesis IV partially. The detection of the microbial genes involved in crucial GHG-related processes in the above-ground compartments of a tropical forest is unprecedented and warrants further research, especially by incorporating the shoot fluxes in the forest GHG flux studies.

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SUMMARY

Peatlands are a substantial carbon stock, and, in their pristine state, they act as net carbon sinks. However, the drained peatlands can become sources of greenhouse gases (GHG), nitrous oxide (N_2O) and carbon dioxide (CO_2), in addition to sporadic methane (CH_4) emissions. N_2O and CH_4 are ~298 and ~25 times stronger than CO_2, respectively, in terms of their global warming potential. The release of these gases can impact the global climate through global warming and can cause ozone depletion (N_2O). Peatland forests have their own dynamics of biogeochemical cycles, determined by the dominating plant species and other physicochemical factors. Fluctuations in groundwater levels, substrate availability, and soil moisture levels, along with high temporal variability, lead to changes in the microbial processes occurring in these ecosystems. In addition to soils, the above-ground compartments of forests can also play a crucial role in biogeochemical processes impacting the net exchange of greenhouse gases from these ecosystems. There is a dearth of research focusing on the temporal variations in the N_2O-related microbiome within temperate peatland forests. Most microbial research on significant N_2O flux events, such as freeze-thaw cycles, has primarily been conducted in agricultural ecosystems. Furthermore, microbiomes and GHG exchange in tropical forests that grow on peat soils have not been fully studied. This is especially true for tropical cloud forests, which are rare ecosystems characterized by their endemism and highly variable moisture levels due to cloud condensation.

The objective of this dissertation was to focus on the GHG fluxes and examine microbial processes in the peat soils of temperate and tropical peatland forests. Additionally, the aim was to understand the microbial processes related to the fluxes based on their relationships with the carbon and nitrogen cycling functional genes' abundance and the soil's physicochemical characteristics, such as soil water content (SWC), quantities of different substrates, and potential gas fluxes obtained through incubations. For this purpose, soil and tree stem fluxes were determined using gas chromatography and portable trace gas analyzers. Soil fluxes in the long-term study in the temperate peatland forest were measured with automated chambers connected to a Picarro gas analyzer. The functional genes associated with N-transforming processes (nitrogen fixation, nitrification, denitrification, and N_2O reduction) and those related to methanogenesis and methanotrophy were quantified using qPCR. Metagenomic analyses were performed additionally for the tropical cloud forests to observe the CH_4-related microbiome structure in soil and above-ground forest compartments. The studies were conducted in a temperate peatland forest (articles I–III) located in Southeast Estonia (58.290184° N, 27.31725° E), and two tropical cloud forests (articles IV, V) on Réunion Island; one in Plaine des Cafres in Le Tampon municipality (21.145343° S, 55.569692° E) and other in in Forêt de Bébour region of Saint-Benoît commune (21.097139° S, 55.548028° E).

In the temperate drained peatland forest temporal study showed that the forest was a net N_2O source (mean: 33.1 ± 4.70 μg N m^{-2} h^{-1}). The majority of the N_2O emissions occurred during the freeze-thaw period. The emissions during the thawing of topsoil increased significantly and peaked (128.5 ± 17.1 μg N m^{-2} h^{-1}) within a narrow range of soil moisture (0.7−0.8 m^3 m^{-3}). The temporal N_2O fluxes were correlated with the abundance of the archaeal *amoA* gene, as well as with fungal and prokaryotic *nirK* genes, indicating that both nitrification and denitrification influenced the N_2O fluxes. However, during the freeze-thaw events, a significant correlation was only observed between N_2O and the prokaryotic *nirK* gene. During the springtime this spike of N_2O dropped to 13.2 ± 1.4 μg N m^{-2} h^{-1} which was attributed to the heightened *nosZ*: *nir* ratio, and the relationship between N_2O and *nosZ*-II was found significantly negative (−0.78, $p < 0.05$). Meanwhile, springtime CH_4 fluxes from the drained peatland forest were also low (5.08 ± 1.38 μg CH_4-C m^{-2} h^{-1}). During the spring period, the methanogenic potential of the peat soil (*mcrA*: (*pmoA* + n-DAMO 16S rRNA) ratio) decreased significantly, changing topsoil from a potential source to a sink. However, the CH_4 from tree stems increased, suggesting the deep soil methanogenesis.

In the tropical cloud forests, peat soil exhibited minimal soil N_2O emissions (1.06 μg N m^{-2} h^{-1} in the mixed forest, 0.37 μg N m^{-2} h^{-1} in the Erica forest). The nitrogen-fixing *nifH* gene abundance was positively correlated with soil NH_4^+-N levels in the forest soils dominated by endemic shrub species *E. reunionensis*. Meanwhile, the nitrification process was mainly driven by archaea. Despite having high NO_3^--N levels, the N_2O fluxes were minimal, which can be attributed to the low pH in these peat soils, as low pH potentially slowed down the *nir*-type denitrification. High abundance and proportions of both clades of the *nosZ* gene indicated the potential of N_2O reduction in these soils. Peat soil in the cloud forests acted as a CH_4 sink (-23.8 ± 4.84 μg C m^{-2} h^{-1}) and CO_2 source (55.5 ± 5.51 μg C m^{-2} h^{-1}), with higher CH_4 uptake in sites dominated by *E. reunionensis*. A high abundance of n-DAMO 16S rRNA gene ($3.42 \times 10^7 \pm 7 \times 10^6$ copies/g dw) was associated with soil NO_3^--N levels, CH_4 uptake, and CO_2 emissions indicating a high anaerobic methane oxidation potential. Meanwhile, the above-ground components of these forests (canopy soils and leaves) contained denitrification genes *nirK* and *nosZ*-I, in addition to the methanogenic gene *mcrA*. This indicates that the above-ground components of cloud forests, particularly the phyllosphere, can play a significant role in the biogeochemical processes in tropical forest ecosystems, independent of the soil.

These findings underscore the importance of conducting long-term studies on microbiomes related to GHG fluxes in various global forest ecosystems, particularly examining the hot moments of these fluxes. This must involve a deep analysis of the dynamics of microbial processes, focusing on the microbial communities and their activity during periods of increased GHG release. Furthermore, this dissertation highlights the significance of above-ground forest components (canopy soil, tree stem, and phyllosphere) for future studies on global GHG modeling and the associated microbiomes within forest ecosystems.

SUMMARY (ESTONIAN)

Mikrobioomi seosed metaani ja naerugaasi voogudega parasvöötme ja troopika soometsades

Turbaaladesse on talletunud märkimisväärne süsinikuvaru ja need alad on looduslikud süsiniku (C) sidujad. Siiski muutuvad kuivendatud turbaalad kasvuhoonegaaside (KHG) – dilämmastikoksiidi (N_2O) ja süsinikdioksiidi (CO_2) – allikateks, millele lisanduvad episoodilised metaani (CH_4) emissioonid, kui tekivad anaeroobsed tingimused üleujutusel. N_2O ja CH_4 kliimasoojendamise potentsiaal on vastavalt umbes 298 ja 25 korda tugevam kui süsihappegaasil (CO_2). Nende gaaside vabanemine atmosfääri põhjustab globaalset kliimasoojenemist ning lisaks lõhub N_2O stratosfäärset osoonikihti. Soometsade biogeokeemilistes aineringetes esineb dünaamika, mida mõjutavad metsade domineerivad taimeliigid ja mitmed füüsikalis-keemilised tegurid. Põhjaveetaseme, toitainete kättesaadavuse ja mullaniiskuse suur ajaline muutlikkus viivad mikroobikoosluse ja sellega seotud mikroobsete protsesside muutusteni neis ökosüsteemides. Lisaks mullas toimuvatele protsessidele võivad ka taimede maapealsed osad mängida metsas olulist rolli biogeokeemilistes protsessides, mis mõjutavad KHG vooge neis ökosüsteemides. N_2O-ga seotud ajalist varieeruvust parasvöötme soometsade mikroobikooslustes on senini vähe uuritud. Enamik mikrobioloogilisi uuringuid, mis käsitlevad olulisi N_2O emissiooni sündmusi nagu näiteks külmumis-sulamisperioodid, on seni keskendunud põllumajandussüsteemidele. Samuti on troopilistel turbaaladel kasvavate metsade mikroobikooslusi ja KHG vooge veel vähe uuritud, eriti troopilisi pilvemetsi, mis on haruldased ökosüsteemid, mida iseloomustab kõrge endeemilisus ja tugevalt varieeruv niiskustase pilvekondensatsiooni tõttu.

Käesoleva väitekirja eesmärk oli keskenduda KHG voogudele ning uurida mikroobiprotsesse parasvöötme ja troopiliste alade soometsades. Lisaks KHG mõõtmistele uuriti nendega seotud mikroobiprotsesse, seostades lämmastiku- (N) ja süsinikuringe funktsionaalsete geenide arvukust mulla füüsikalis-keemiliste parameetritega, näiteks mulla veesisalduse, erinevate toitainete sisalduse ja inkubatsiooni meetodil määratud mulla potentsiaalse gaasivooga. Mulla ja puutüvede gaasivood määrati gaaskromatograafiliselt ning kaasaskantavate gaasianalüsaatoritega. Parasvöötme soometsa pikaajalises uuringus kasutati mulla gaasivoogude määramiseks Picarro gaasianalüsaatoriga ühendatud mõõtekambreid. N-ringe funktsionaalsed markergeenid (N sidumine, nitrifikatsioon, denitrifikatsioon ja N_2O redutseerimine) ning metanogeneesi ja metanotroofia geenid kvantifitseeriti kvantitatiivse polümeraasi ahelreaktsiooni (qPCR) meetodil. Troopilistes pilvemetsades viidi lisaks läbi mikroobikoosluse metagenoomi analüüs, et iseloomustada CH_4-ga seotud mikroobikooslust nii pinnases kui ka metsa maapealsetes osades. Uuringud viidi läbi parasvöötme soometsas Kagu-Eestis (58.290184° N, 27.31725° E) (artiklid I–III) ning kahes troopilises pilvemetsas (artiklid IV, V) Réunioni saarel: Plaine des Cafres'is Le Tamponi piirkonnas (21.145343° S,

55.569692° E) ja Forêt de Bébour piirkonnas Saint-Benoît'is (21.097139° S, 55.548028° E).

Pika-ajalisel analüüsil ilmnes, et parasvöötme soomets oli N_2O allikas (aasta keskmine: 33.1 ± 4.70 μg N m^{-2} h−1), kusjuures enamik N_2O emissioonidest paiskus atmosfääri maapinna külmumis-sulamisperioodil. Maapinna ülemise kihi sulamise ajal suurenes emissioon märkimisväärselt ja saavutas kõrgeima taseme (128.5 ± 17.1 μg N m^{-2} h^{-1}) mulla niiskuse kitsas vahemikus (0.7–0.8 m^3 m^{-3}). Pika-ajalised N_2O vood korreleerusid arhede *amoA* geeni, samuti seente ja prokarüootide *nirK* geenide arvukusega, viidates sellele, et nii nitrifikatsioon kui ka denitrifikatsioon mõjutasid N_2O emissioone. Siiski ilmnes külmumis-sulamisperioodil oluline korrelatsioon vaid prokarüootse *nirK* geeniga. Kevadel N_2O emissioon langes tasemele 13.2 ± 1.4 μg N m^{-2} h^{-1}, mida seostati kõrgenenud *nosZ*:nir suhtarvuga; N_2O ja *nosZ*II vahel leiti negatiivne korrelatsioon (−0.78, $p < 0.05$). Samal ajal olid CH_4 vood kevadisel perioodil madalad (5.08 ± 1.38 μg CH_4-C m^{-2} h^{-1}). Metanogeensete geenide suhteline potentsiaal (*mcrA*:(*pmoA* + n-DAMO 16S rRNA)) langes märkimisväärselt, muutes pinnase CH_4 sidujaks. Samas suurenes puutüvede kaudu eralduv CH_4, viidates mulla sügavamates kihtides toimuvale metanogeneesile.

Troopilistes pilvemetsades oli N_2O emissioon minimaalne (1.06 μg N m^{-2} h^{-1} segametsas ja 0.37 μg N m^{-2} h^{-1} metsas, mille domineeriv taimeliik oli *Erica reunionensis*). *E. reunionensis* enamusega metsades oli lämmastikku siduva *nifH* geeni arvukus positiivses korrelatsioonis NH_4^+-N sisaldusega mullas. Nitrifikatsiooni protsessis osalesid peamiselt arhed. Vaatamata kõrgele NO_3^--N tasemele olid N_2O vood minimaalsed, mis võib olla seotud nende turvasmuldade madala pH tasemega, kuna madal pH aeglustab *nir*-tüüpi denitrifikatsiooni. *NosZ* geen klaad I ja klaad II kõrge arvukus ja osakaal prokarüootide koguhulgast näitasid geneetilist potentsiaali N_2O redutseerimiseks. Turvasmuld troopilistes pilvemetsades oli CH_4 siduja (−23.8 ± μg C m^{-2} h^{-1}) ja CO_2 allikas (55.5 ± 5.51 μg C m^{-2} h^{-1}), kusjuures CH_4 sidumine oli suurem *E. reunionensis'e* puuliigi enamusega aladel. Kõrge n-DAMO 16S rRNA geeni arvukus (3.42×10^7 ± 7×10^6 geenikoopiat/g kuivaine kohta) korreleerus NO_3^--N sisalduse, CH_4 sidumise ja CO_2 emissiooniga, viidates tugevale anaeroobsele CH_4 oksüdatsiooni potentsiaalile. Lisaks leiti metsa maapealsetes osades (puuvõra muld ja lehed) denitrifikatsiooni markergeenid *nirK* ja *nosZ*-I ning metanogeneesiga seotud *mcrA* geen, mis viitab, et lisaks mullale võib ka troopiliste pilvemetsade füllosfääril olla oluline roll biogeokeemilistes protsessides, rõhutades metsa maapealsete osade uurimise tähtsust tulevastes mikroobikoosluste ja KHG uuringutes.

Käesoleva doktoritöö tulemused rõhutavad pikaajaliste mikrobioomiuuringute tähtsust erinevate globaalsete metsaökosüsteemide KHG uurimisel, eriti arvestades lühikesi kõrgete gaasiemissioonidega perioode. Selleks on vaja mikroobiprotsesside dünaamika põhjalikku analüüsi, keskendudes mikroobikooslustele ja nende aktiivsusele perioodidel, mil KHG emissioon on suurenenud. Lisaks toonitab käesolev doktoritöö metsa maapealsete osade (puutüved, puuvõra muld ja füllosfäär) olulisust tulevaste globaalsete KHG modelleerimise ja sellega seotud mikrobioomiuuringute jaoks metsaökosüsteemides.

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PUBLICATIONS

CURRICULUM VITAE

Name: Fahad Ali Kazmi
Date of birth: 07 December 1995
Nationality: Pakistani
Phone: +372 5405 7149
E-mail: fahadalikazmi@outlook.com

Education:

Since 2021	University of Tartu, Faculty of Science and Technology, Institute of Ecology and Earth Sciences, Tartu (Estonia), PhD (Environmental Technology)
2018–2021	Norwegian University of Life Sciences, Faculty of Environmental Sciences and Natural Resource Management, Ås (Norway), M.Sc. (Ecology)
2012–2016	University of Azad Jammu & Kashmir, Department of Zoology, Muzaffarabad (Pakistan), B.Sc. & M.Sc. (Zoology)

Professional employment:

Since 2022	University of Tartu, Faculty of Science and Technology, Institute of Ecology and Earth Sciences, Junior Research Fellow

Publications:

Kazmi, F.A., Espenberg, M., Pärn, J., Masta, M., Ranniku, R., Thayamkottu, S., Mander, Ü. 2025. Meltwater of freeze-thaw cycles drives N_2O-governing microbial communities in a drained peatland forest soil. *Biology and Fertility of Soils* 61, 667–680. https://doi.org/10.1007/s00374-023-01790-w

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ELULOOKIRJELDUS

Nimi: Fahad Ali Kazmi
Sünniaeg: 07 Detsember 1995
Kodakondsus: Pakistanlane
Telefon: +372 5405 7149
E-post: fahadalikazmi@outlook.com

Haridus:

Alates 2021	Tartu Ülikool, Loodus- ja täppisteaduste valdkond, Ökoloogia ja Maateaduste Instituut, Tartu (Eesti), keskkonnatehnoloogia doktorantuur
2018–2021	Norra Maaülikool, Keskkonnateaduste ja Loodusvarade Halduse Teaduskond, Ås (Norra), magistrikraad (ökoloogia)
2012–2016	Azad Jammu ja Kashmiri Ülikool, Zooloogia osakond, Muzaffarabad (Pakistan), bakalaureuse- ja magistrikraad (zooloogia)

Teenistuskäik:

Alates 2022	Tartu Ülikool, Loodus- ja täppisteaduste valdkond, Ökoloogia ja Maateaduste Instituut, nooremteadur

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