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Master's thesis in Geoinformatics for Urbanised Society (30 ECTS)

Dynamics of soil microbial nitrogen cycle during a year-long study in a drained peatland forest

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Abstract:

Drained peatland forests are a significant source of nitrous oxide (N_2O), which is a potent greenhouse gas. These environments are complicated to restore due to the greenhouse gas emissions associated with rewetting. Studying the emissions of N_2O from a drained peatland forest is important in understanding how to restore these environments. Soil samples from 12 sites within a drained peatland forest in south-eastern Estonia were collected over a year and analyzed for their physical and chemical properties and the abundance of genes associated with nitrogen cycling. This data was paired with N_2O flux data collected in automatic dynamic gas chambers throughout the study period. Spatial patterns visible in the soil's chemical and physical makeup indicate different vegetation and microbial communities throughout the site. Bacterial and archaeal 16S rRNA and four genes associated with nitrogen cycling, *nirK, nirS,* archaeal *amoA,* and COMAMMOX *amoA,* were all found to correlate with N_2O emissions. Archaeal 16S rRNA and archaeal *amoA* both positively correlated with N_2O, and most strongly related to phosphorous and potassium concentrations in the soil. The other significant genes correlated negatively with N_2O emissions but were all strongly linked with water table depth. Over the year, the water table and volumetric water content of the soil were significant factors in the emissions of N_2O and the abundance of nitrogen cycling genes. Due to subzero temperatures in the winter preventing water from entering the ground, two periods of drying and rewetting of the soil are recorded. The site has both hot spots, where N_2O emissions are consistently greater, and hot moments, when N_2O emissions are periodically greater, due to the combination of physical, chemical, and genetic characteristics in the peatland soil.

Keywords: Nitrous Oxide (N_2O), Nitrogen Cycle, Peatland Forest, Microbiome

CERCS: B230 Microbiology, bacteriology, virology, mycology; T270 Environmental technology, pollution control

Annotatsioon:

Kuivendatud turvasmuldadel kasvavad metsad on märkimisväärsed dilämmastikoksiidi (N_2O) allikad. See on tugev kasvuhoonegaas, mille voogusid on turbaalade veerežiimi taastamisega võimalik vähendada. Kuivendatud turbaalade taastamiseks on oluline neid komplekssеid keskkondi uurida, et mõista neis toimuvaid protsesse. Kuivendatud turvasmullaga metsas mulla mikroobikoosluste, füüsikaliste ja keemiliste omaduste ning N_2O voogude vaheliste ajaliste ja ruumiliste seoste analüüsimiseks koguti aastase uurimisperioodi vältel Kagu-Eestis asuval uurimisalal mullaproove kokku 12 kohast. Analüüsiti nii mulla füüsikalisi ja keemilisi omadusi kui ka lämmastikuringega seotud geenide arvukust, mis seoti kogu uurimisperioodi vältel automaatse dünaamilise kambri meetodil kogutud N_2O voogudega. Varieeruvad mulla keemilised ja füüsikalised parameetrid viitasid proovialal erinevatele taimestiku ja mikroobide kooslustele. Leiti, et N_2O vooga korreleerusid nii bakterite ja arhede arvukused kui ka neli lämmastikuringega seotud geenide arvukust (*nirK*, *nirS*, arhede *amoA* ja COMAMMOX *amoA*). Arhede ja arhede *amoA* geenide arvukused korreleerusid mõlemad N_2O vooga positiivselt ning nende arvukused olid tugevas korrelatsioonis ka mulla fosfori ja kaaliumi kontsentratsiooniga. Teised statistiliselt olulised korrelatsioonid olid geenide arvukuste ja N_2O voo vahel negatiivsed, kuid nende geenide arvukused olid tugevalt sõltuvad veetaseme muutustest. Kogu uurimisperioodi vältel olid N_2O voogude ja lämmastikuringega seotud geenide arvukuse peamised mõjutajad veetase ja mullaniiskus. Talvised temperatuurimuutused takistasid vee liikumist ning muld külmus ja sulas korduvalt, mis oli seotud ka kõrgete N_2O emissioonidega. Uurimisalal esines kõrgema N_2O vooga alasid ruumiliselt kui ka ajaliselt. Olid alad, kus N_2O vood olid pidevalt kõrgemad, ka alad, kus N_2O vood olid perioodiliselt mõjutatud mulla füüsikalistest ja keemilistest omaduste ja mikroobikoosluse muutustest.

Märksõnad: dilämmastikoksiid (N_2O), lämmastikuringe, metsad kuivendatud turvasmullal, mikrobioom

CERCS: B230 Mikrobioloogia, bakterioloogia, viroloogia, mükoloogia; T270 Keskkonnatehnoloogia, reostuskontroll

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1 – Introduction:

Peatlands are wetland environments created through the accumulation of plant matter which grows faster than it can decay. Although peatlands take up 3% of the land surface, they are responsible for storing 21% of the world's carbon stocks (Leifeld & Menichetti, 2018) and about one-tenth of the world's soil nitrogen (Batjes, 2014). Despite their importance as carbon sinks, peatlands have been historically ignored environments and continue to be degraded – 10% of the world's peatlands are being actively drained or mined at the present time (Leifeld & Menichetti, 2018). Restoring peatlands is important for biodiversity and environmental health, but the situation is nuanced. Peatlands are also a globally significant source of greenhouse gas emissions such as methane (CH_4) and nitrous oxide (N_2O) (Butterbach-Bahl et al., 2013; Lamers et al., 2015). Peatlands are drained to make use of the land, for example in Northern Europe peatlands have been drained to grow lumber (Huttunen et al., 2003a). This change in land use has negative effects on peatland bird populations, and loss of biodiversity is common to drained peatlands globally (Yule, 2010; Fraixedas et al., 2017). A drained peatland ceases being a carbon sink, and when water is returned to the peatland in attempt to restore it, the peatland becomes a significant source of N_2O (Rudaz et al., 1991; Pärn et al., 2018). N_2O is ~310 times more effective than carbon dioxide (CO_2) as a greenhouse gas, and restoration of peatlands can take over a decade and a half before the peatland stops being a source of N_2O (Nugent et al., 2019).

N_2O is produced by microorganisms in the soil mainly as a product of nitrification and denitrification, processes responsible for roughly 70% of global N_2O emissions (Syakila & Kroeze, 2011). During denitrification, where reactive nitrogen is turned into dinitrogen gas (N_2), N_2O is produced as an intermediary step. Some species of denitrifiers, however, produce N_2O as the end product (Kuypers et al., 2018). Moreover, the final step of reducing N_2O into N_2 can be suppressed under the specific soil conditions (Klemedtsson et al., 1988). N_2O can also be produced as part of nitrification, where nitrogen compounds are oxidized into nitrite and nitrate, but usually under specific conditions (Bremner & Blackmer, 1978; Anderson & Levine, 1986; Butterbach-Bahl et al., 2013). The current study aims to investigate the relationship between the different microbial life and emissions of N_2O in a drained peatland forest to estimate the effectiveness of peatlands' current and future management practices.

1.1 – Aims and research questions:

Aims: This master's thesis aims to explore the relationship between soil microbial communities and N_2O gaseous fluxes in the Agali II drained peatland forest over the course of a year.

Research question 1: How are the genes responsible for nitrogen cycling in the peatland spatially distributed?

Research question 2: How are the genes responsible for nitrogen cycling in the peatland temporally distributed?

Research question 3: What patterns are there between soil characteristics (e.g. soil water content, soil temperature and pH) and nitrogen cycling gene abundances?

2 – Theoretical overview:

2.1 – Climate change and impacts:

Anthropogenic climate change poses an existential threat to environments, ecosystems, and societies globally, posing ethical dilemmas and spurring on both climate action and denial (Myers, 2014; Stollberg & Jonas, 2021). Greenhouse gases such as carbon dioxide (CO_2), nitrous oxide (N_2O), methane (CH_4), and water vapor contribute to increased global temperatures by trapping solar radiation (Lashof and Ahuja 1990; Darkwah et al. 2018). Global increases in temperatures due to greenhouse gases can create feedbacks loops that further magnify the effects of climate change, such as the release of the greenhouse gas CH_4 due to melting permafrost (Podesta & Ogden, 2008). Anthropogenic climate change is both an environmental and humanitarian crisis and confronting the possibility that there may not be solutions to every concern may lead to anxieties and denial – another sort of feedback loop (Podesta & Ogden, 2008; Myers, 2014).

Climate change will affect the daily lives and livelihoods of billions of people. Coastal communities will be faced with rising seawaters, forcing the migration of hundreds of millions of people (Mimura & Horikawa, 2013). In many arid regions such as Saudi Arabia and northwestern China, however, climate change and unsustainable water usage will cause water scarcity issues (Deng & Zhao, 2015; DeNicola et al., 2015). In more extreme situations, the arid climate paired with human activity can cause desertification in areas such as the Sahel, the western US, and Central Asia (Huang et al., 2020; le Houérou, 1996). In boreal environments, the more arid climate brought on by increased temperatures can also promote wildfires (Flannigan et al., 2006; Wotton et al., 2010). Rising seawaters, water accessibility issues, desertification, and wildfires are all capable of causing mass migrations, and all require different methods of risk assessment and mitigation. Population centers such as cities and suburbs are also affected by climate change. Cities create pockets of hot air known as heat islands, due to myriad factors such as the shape of buildings stifling airflow, and the materials of buildings retaining solar energy throughout the day. As populations and cities grow, heat islands will only get more intense unless steps are taken to make cities less contiguous to aid in airflow and to use more reflective materials to reduce heat retention

(Kleerekoper et al., 2012; Debbage & Shepherd, 2015; Yang et al., 2015). There are few, if any, places where people will not be affected by climate change.

The impacts of climate change upon our environment are ubiquitous. As sea and air temperatures increase, the polar ice melts. The resulting reduction in solar reflectance in the poles creates a feedback loop that may become increasingly rapid (Winton, 2008). The water once stored in polar ice can cause sea level rises globally (Mimura & Horikawa, 2013). High concentrations of CH_4 are released from melting arctic permafrost – particularly in frozen loess and peat (Kvenvolden & Lorenson, 1993). The arctic is not the only area of concern with regards to climate change and greenhouse gas emissions. Because of anaerobic conditions, the pristine peatlands are the sources of CH4, but at the same time, they accumulate carbon into the soil through the increasing biomass (Drösler et al., 2008). The carbon stored in the biomass of the peatland is also at risk of being released back into the atmosphere if the peatlands are allowed to degrade. Unfortunately, it is predicted that 25% of the world's peatlands are expected to degrade by 2050 (Urák et al., 2017). One form of degradation common to peatlands is when the water from the environment is drained, letting the peatland dry out. Drained peatlands are an even more significant source of greenhouse gases because changing conditions in these environments can also make them sources of N_2O (Couwenberg et al., 2011).

2.2 – Peatlands and peatland forests:

Peatlands are an important, yet often overlooked environment that are becoming more relevant as their role in climate change is increasingly acknowledged. Defined by waterlogged organic soils with low nutrient content but high carbon content, peatlands are formed by the accumulation of dead plants which grow quicker than they can decay. Historically, peatlands have not been highly valued, and have instead been left damaged and degraded by their use as fuel or for forestry. However, they provide important ecosystem services and should be preserved. Peatlands are the largest terrestrial carbon sinks but may also be significant sources of N_2O in certain conditions (Butterbach-Bahl et al., 2013; Viru et al., 2020). Restoring these environments is of utmost importance to act as a carbon sink and to promote microbial communities which more efficiently use the reactive nitrogen in the soil, releasing less N_2O into the atmosphere. Bacteria

and archaea which fix atmospheric dinitrogen gas are diverse but rare, so most microbial life has to rely on more reactive nitrogen sources (Kuypers et al., 2018). While N_2O has been recognized for a long time as an important greenhouse gas, this was mostly in the context of agricultural nitrogen added to the soil; measuring *in situ* N_2O is not as simple, and the relation of microbial N_2O to the biotic and abiotic factors are not fully understood (H.-W. Hu et al., 2015; Bahram et al., 2022).

The flooding of peatland environments has been studied as a method for ecosystem restoration for decades (Molles et al., 1998; Sprenger et al., 2002). When a flooded area dries out and is subsequently rewetted, the soil becomes a significant source of N_2O (Rudaz et al., 1991). Since N_2O is an important greenhouse gas, the implications of such wetting practices are of particular note. Mature peatlands can act as important carbon sinks; restoration can take over a decade, but a degraded peatland acts as a significant source of CO_2 (Strack & Zuback, 2013; Nugent et al., 2019). Restoration of such peatlands with flooding also improves nutrient cycling between plants – particularly the peat, which after 10 years can be comparable to pristine peatlands (Haapalehto et al., 2010). However, the effects of extraction are not all remediated by restoration efforts: vegetation communities may remain in an intermediate state between the degraded and natural (Strack & Zuback, 2013). Additionally, the short term flooding of a drained peatland can increase the emissions of N_2O and methane (Schindler et al., 2020). Degraded peatlands release gigatons of CO_2-equivalent greenhouse gases annually (Leifeld & Menichetti, 2018). As peatlands are the largest natural carbon stores on land, when degraded or damaged, they have a great capacity to emit greenhouse gases (Viru et al., 2020). Moreover, the drainage of a peatland negatively affects surrounding water bodies as high amounts of nutrients are washed out of the wetland (Haapalehto et al., 2014; Viru et al., 2020).

The priority of peatland restoration and the greenhouse gases associated with drying soils require a sort of delicate balance: too much flooding can create anoxic conditions which prevent the primary production of plants; no flooding at all will see the peatland continue to be a source of greenhouse gases; and intermittent flooding can exacerbate the problem (Strack & Zuback, 2013; Lamers et al., 2015; Nugent et al., 2019). Therefore, other methods have been researched in connection to the restoration of drained peatland ecosystems. Peatlands in Northern Europe were drained as a practice for forestry starting in the 20th century (Huttunen et al., 2003a). Drainage is not unique to Europe, and, for example, an estimated 80% of peatlands in South-East Asia have

been drained or deforested (Mishra et al., 2021). This activity contributes to biodiversity loss, such as decreasing peatland bird species in Northern Europe (Fraixedas et al., 2017). The loss of biodiversity is not limited to birds, though, and in South-East Asia the degradation of peatlands has negatively affected everything from microbial communities to orangutans (Yule, 2010). While draining these peatlands for forestry can result in the area becoming a carbon sink for CH_4, in the long term they are less efficient than a natural peatland for climate-relevant emissions (Kimmel & Mander, 2010; Minkkinen & Laine, 2017).

As a peatland is drained, the depth of the oxic peat layer increase leading to the peatland being a net source of carbon – if trees are not grown on the land to sequester carbon (Bjarnadottir et al., 2021). However, while growing forests on drained peatlands may temporarily be a carbon sink, these forests are intended for forestry and will eventually be cut. Clear-cutting of drained peatland in Finland resulted in a raised water table in the peatland, but also significantly more N_2O emissions from the soils (Huttunenet al., 2003a), though mostly in the summer months. Drained peatland forests are also significant sources of N_2O throughout the winter when compared to merely abandoned peat extraction sites, found in research in southeastern Estonia (Viru et al., 2020), so the importance of drained peatlands as sources of N_2O is not limited to only warm months or countries. In fact, some studies find a bimodal behavior of N_2O fluxes over the course of the year, with higher emissions in the summer and winter (Korkiakoski et al., 2020). The authors explain it may be because of N_2O accumulation under an ice layer until it thaws. Because of the delicate balance between drained and rewetted peatlands with regards to climate, some research suggests that the most environmentally sustainable method should be to keep the groundwater at a steady level as opposed to restoring it to a previous state or draining it further (von Arnold et al., 2005). Kimmel and Mander (2010) argue, however, that while in the short term wetlands produce greenhouse gases, in the long term, they compensate for the greenhouse effect. Therefore, restoration efforts should be carefully designed to balance CH_4 and CO_2 exchange. While rewetting a drained peatland will lower CO_2 and N_2O in exchange for higher CH_4 emissions in the short term, over time the rewetting process leads to a net reduction in greenhouse gases emissions compared to the previously drained baseline.

Microbial activity is a key component of the behavior of greenhouse gas fluxes in drained peatlands and is an increasingly important focus for further research. Extracting peat, draining peatlands, flooding peatlands, and clearing drained peatland forests all impact the soil microbial

life. The different nitrogen cycling genes found in various types of peatland soils can be extracted and compared to see their relationship to the greenhouse gas fluxes in the area. This type of analysis has been done on tropical peatlands in French Guiana, where it was found that natural and drained sites had significantly different assemblages of nitrogen cycling genes (Espenberg et al., 2018). Their research found especially important the impact of archaea on nitrogen cycling in these environments. In the context of boreal drained peatland forests of Estonia, the bacterial and archaeal communities appear to be associated with the type of trees which grow there (Truu et al., 2020). Even when the soil conditions in these forest types were initially similar, the microbial communities changed over time. Furthermore, higher N_2O production was associated with more abundant microbial communities. In the drained tropical peatlands and boreal pine-dominant drained peatland forests, the *nirK* gene was found to be more abundant, however in birch dominant drained peatland forests, the *nirS* gene was found to be more abundant (Espenberg et al., 2018; Truu et al., 2020). The *nirK* and *nirS* genes reduce NO_2- into NO, which is thereafter turned into N_2O.

2.3 – Nitrogen cycle in peatland forests:

The nitrogen cycle is a complex web of interactions between many life forms and natural processes. As a component of proteins and nucleic acids, nitrogen is an essential element for all living organisms (X. Zhang et al., 2020). For living organisms, the role of nitrogen can be either assimilatory – being taken up into the organism's biomass, or dissimilatory – where nitrogen plays a part in the organism's extraction of energy from the environment (Thamdrup, 2012). While some parts of the cycle are well studied, other parts have only recently been discovered or are yet to be fully understood (Moeller et al., 2021; Thamdrup, 2012). Different new metabolic pathways, e.g. complete ammonia-oxidation (COMAMMOX) (Daims et al., 2015) shows that our understanding of the nitrogen cycle is still evolving. Furthermore, one nitrogen transforming microorganism can play different roles in the nitrogen cycle depending on its living environment, further underlining the complexity of the nitrogen cycle's interactions (Daims et al., 2016; Stein & Klotz, 2016). To understand the nitrogen cycle in even a single environment is to understand the sum of many parts which are individually as complex as the whole.

2.3.1 – Denitrification:

Denitrification is a three or four-step process where nitrate (NO_3-N) is reduced to N_2O or N_2, an ability commonly found in microbial life in many low oxygen environments (Philippot et al., 2007; Maia & Moura, 2014). The main genes used for studying denitrification are nitrite reductase genes, (*nirK* and *nirS*) and N_2O reductase genes (*nosZI* and *nosZII*). The *nirK* gene encodes a copper containing nitrite reductase, while *nirS* encodes a haem-containing nitrite reductase.

N_2 is finally produced by N_2O reductase, an enzyme encoded by *nosZ* genes, of which there are two: Clade 1 (typical) *nosZ* and Clade 2 (atypical) *nosZ*. (Chee-Sanford et al., 2020). Typical *nosZ*, or *nosZ I*, is found in denitrifiers, but Atypical *nosZ II* can be found in both denitrifiers and non-denitrifiers, is more diverse, and is abundant in soil ecosystems (Chee-Sanford et al., 2020). Soils with high levels of *nirK*, *nirS*, and *nosZ* may be associated with high N_2O production and emissions, however it's estimated that 30-80% of N_2O is reduced to N_2 before it leaves the soil (Hu et al., 2015). In some microorganisms, however, N_2O is the end product of denitrification (Kuypers et al., 2018). N_2O produced by denitrification is almost exclusively an anaerobic process (Anderson & Levine, 1986; Butterbach-Bahl et al., 2013). The rate of denitrification in peatlands may have multiple limiting factors, including lack of free nitrate from peat extracts, substrate availability, pH, and levels of oxygen (Hayden & Ross, 2005). Soil moisture is a key driver of oxygen levels which are significant in the production of N_2O in denitrification and nitrification (Rubol et al., 2012). While the *nosZ* gene had a greater frequency of co-occurrence with *nirS* than with *nirK* in microorganisms, up to 30% of microorganisms with the *nosZ* gene did not have either *nir* gene. This highlights the importance of community structure in the process of denitrification, since denitrification can be the result of communities of microorganisms working in tandem (Graf et al., 2014).

2.3.2 – Nitrification:

Nitrification is an aerobic process whereby ammonia is oxidized into nitrate via nitrite (Hu et al., 2015). This is accomplished by the *amoA* gene which encodes the ammonia-monooxygenase

found in ammonia-oxidizing bacteria and archaea. Ammonia oxidized by ammonia-monooxygenase is turned into nitrite, which is later further processed into nitrate. The *amoA* gene found in some proteobacteria is shown to be evolutionarily similar to the *pmoA* gene of methanotrophs which produce methane-monooxygenase (Holmes et al., 1995). Ammonia-oxidizing bacteria (AOB) have been known for a long time and live on a wide variety of substrates (including CH_4), but ammonia-oxidizing archaea (AOA) are a more recent discovery and, while ubiquitous in many environments, are not as well studied (Hatzenpichler, 2012). Members of the lineage from which the first nitrifying archaea were found are extremely abundant, making up 20-30% of marine microbes, and have been found in soil and freshwater environments as well (Hatzenpichler, 2012).

While for the most part nitrification is a two-step process where ammonia is oxidized into nitrite, and then is turned into nitrate by nitrite-oxidizing bacteria (NOB), there exists as well a complete ammonia-oxidation pathway known as COMAMMOX, found within the genus *Nitrospira* (Daims et al., 2015; Hu & He, 2017). The usual two-part nature of nitrification results in tight interaction between ammonia-oxidizing microorganisms (AOM) and NOB, but a complete nitrification has a higher energy yield. Although in many environments the AOM and NOB consortia can hypothetically outcompete COMAMMOX bacteria, there's the possibility that complete nitrifiers would be competitive in environments where growth yield is more advantageous than growth rate (Daims et al., 2015). Despite this, complete nitrifiers can be found in abundance in many natural and man-made habitats (Hu & He, 2017). Nitrifiers produce both NO and N_2O under aerobic conditions but will only produce N_2O when there is sufficient soil moisture (Anderson & Levine, 1986). The abundance of nitrification as a process in peatlands may be related to the quality of organic matter in the soil and may be subject to significant spatial variability due to varying vegetation throughout a peatland (Devito et al., 1999).

2.3.3 – DNRA:

Although NOB turn nitrite into nitrate during nitrification, many microorganisms, including most bacterial lineages and CH_4-oxidizing archaea, can grow through a process known as Dissimilatory Nitrate Reduction to Ammonium (DNRA) (Kuypers et al., 2018). DNRA is found to be more common in aquatic sediments where there is an excess of electron donor compared to

nitrate (Kuypers et al., 2018). Unlike denitrification, which removes reactive nitrogen from the environment through gas loss as either N_2 or the greenhouse gas N_2O, DNRA usually retains this nitrogen and has been found to account for as much as 30% of the nitrate reduction in some coastal habitats (Giblin et al., 2013; Putz et al., 2018). However, depending on the environmental conditions, DNRA microbes may release N_2O as a by-product of the reduction process or may reduce available N_2O (Mania et al., 2014). DNRA is often achieved through a nitrite reductase which is encoded by the *nrfA* gene, common in *Proteobacteria*, *Protomycetes*, and *Bacteroides*. However, some species such as some members of *Epsilonproteobacteria* are capable of DNRA without *nrfA* (Song et al., 2014). DNRA has been found to possibly function in peatlands, sometimes as a significant competitor to denitrification (Hayden & Ross, 2005; Kull et al., 2008).

2.3.4 – Nitrogen fixation:

Nitrogen transforming microbial life must have a source of nitrogen, and while reactive nitrogen is easily transformed it is not as readily available as atmospheric N_2. Microbes with the widespread nitrogenase gene *nifH* are capable of fixing atmospheric nitrogen, making them competitive in environments where nitrogen is a limiting factor (Kuypers et al., 2018). Although nitrogenase is oxygen-sensitive, nitrogen-fixing prokaryotes are found in aerobic and anaerobic environments, and some species can survive in both (Barney, 2020; Klawonn et al., 2015; Minamisawa et al., 2004). The wide variety of environments in which nitrogen-fixing prokaryotes live essentially ensures that the nitrogen cycle progresses. Peatlands can contain nitrogen fixing cyanobacteria if they are neutral to alkaline, and bacteria and symbiotic actinomycetes fix nitrogen in acidic peatlands (Limpens et al., 2006). Peatland nitrogen fixation is limited by pH, but moisture availability, potassium and calcium concentrates also limit nitrogen fixation.

2.4 – Temporal and spatial distribution of nitrogen cycle:

The complex web of interactions found throughout the nitrogen cycle, particularly in peatlands, are all subject to spatial and temporal variability: aspects that are impossible to examine in a lab setting. Peatlands are very heterogeneous, making extrapolating catchment scale greenhouse gas fluxes from chamber measurements challenging (Dinsmore et al., 2009). While

peatlands are a significant source of CH_4 and N_2O, seasonal effects such as plant activity, soil temperature, and the height of the water table all have impacts on the scale and timing of greenhouse gas fluxes (Huttunen, et al., 2003b; Dinsmore et al., 2009; Anthony & Silver, 2021).

In some cases, the spatial variability in gas emissions can be very localized: phenomena such as frost heaves can push parts of the peatland above the water table that can cause N_2O emissions (Marushchak et al., 2011). In addition to that, quick changes in soil water content can influence N_2O emissions in riparian forests, and thus, they may be hotspots and can produce hot moments in which emissions are greater (Mander et al., 2021). Short-lived hot moments throughout different seasons are a significant factor in temporal fluctuations of N_2O emissions. Temporal variation in emissions is also a matter of scale, with patterns in peatland gas fluxes visible at both daily and annually (Maljanen et al., 2002; Arn Teh et al., 2017). Conversely, peatland research must also consider the immense scales at which gas fluxes and nitrogen cycling take place. Peatlands grow slowly, and so just as one can detect changes in nitrogen within a day, it is also possible to find patterns over the course of a century, such as with the buildup of nitrogen deposited upon British peatlands (Payne, 2014). Research on peatlands must consider the various dimensions and scales at which the environment exists and processes happen.

3 – Data and methods:

3.1 – Site description and soil sampling

The Agali II research site (58˚17’ N; 27˚19’ E) is located in a drained peatland forest near the town of Agali in Tartu County, Estonia (Figure 1). The studied *Oxalis* site type forest is managed for lumber and is surrounded by forest where lumber is still being actively extracted, the peatland having been drained roughly 60 years ago. The trees are mostly Downy birch (*Betula pubescens*) and Norway spruce (*Picea abies*) spaced roughly a meter apart. Bordering two edges of the research area is a drainage ditch, and on a third edge is a small pond that is kept filled by local beavers. Potentially due to the drainage around the edge of the research site, Agali II has a water table that sits close to the surface. In the spring and autumn months, the high-water levels of the pond and drainage ditch are reflected in the ground water of the forest. However, in the peak

of the summer, the drainage enables significant drying within the forest. The depth of the peat layer was ca 40-60 cm.

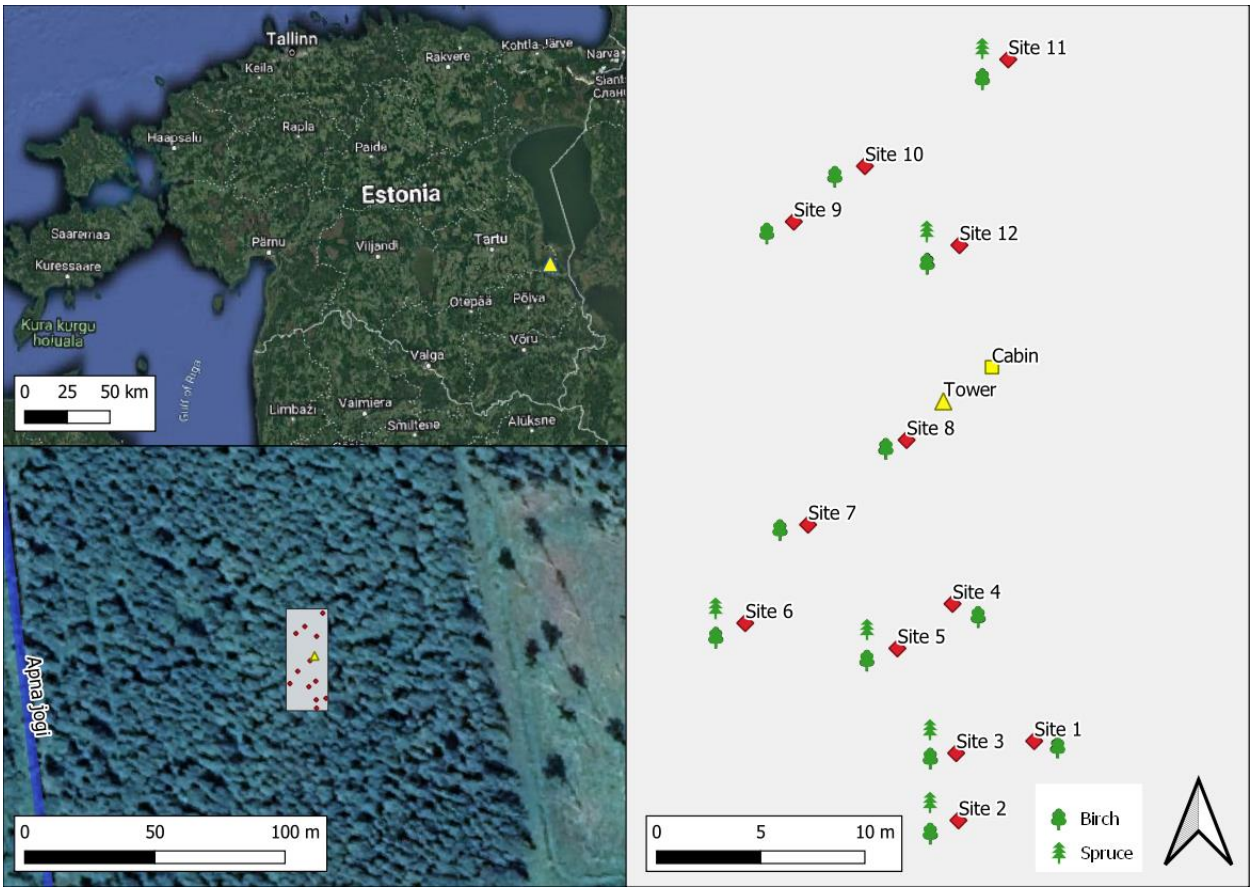

Figure 1: Location of Agali II Station and layout of data collection sites

Monthly expeditions to the site were made starting in November 2020 and continued until October 2021. One day each month, topsoil cores (0–10 cm layer) were taken from the proximity of each of the 12 automatic dynamic gas chambers. Three soil cores from each sampling point were collected and pooled to form a composite sample. Ancillary measurements taken at each soil sampling site include soil temperature at 10 cm (Ts 10cm C°), water table depth (WTD), volumetric water content (VWC) and electrical conductivity (EC). In total 144 composite soil samples were collected. Samples were split into two bags: ca 10 g was separated for microbial analysis and ca 200 g was used for chemical analyses. The soil samples were stored at +4 °C and –20 °C until chemical and microbiological analyses, respectively. The pH, dry matter (DM, %), organic matter (OM, %), nitrogen (N, %), nitrate (NO_3-N, mg kg^{-1}), ammonium (NH_4-N, mg kg^{-}

^{1}), phosphorous (P, mg kg^{-1}), potassium (K, mg kg^{-1}), calcium (Ca, mg kg^{-1}), magnesium (Mg, mg kg^{-1}) contents were determined in the soil samples using standard procedures (APHA-AWWA-WEF, 2005).

3.2 – Soil DNA extraction and concentration measurements

The DNA was extracted from 0.25 g of the wet soil samples using the DNeasy® PowerSoil® Pro Kit (Qiagen, USA), following the manufacturer's instructions. A Precellys24 (Bertin Instruments, Montigny-le-Bretonneux, France) was used to homogenize the samples at 5000 rpm for 20 s. The extracted DNA was analyzed for their concentrations and quality using the Infinite M200 spectrophotometer (Tecan AG, Grodig, Austria). The DNA was stored at −20 °C until further analyses.

3.3 – Quantitative PCR

With DNA from each site and month, qPCR was used to evaluate the bacterial and archaeal community abundances by quantifying the abundances of specific 16S rRNA genes and to evaluating the abundances of 9 genes associated with nitrogen cycling: denitrification (*nirS*, *nirK*, *nosZ* clade I, and *nosZ* clade II), nitrification (bacterial, archaeal, and COMAMMOX *amoA*), DNRA (*nrfA*), and nitrogen fixation (*nifH*).

Real-time quantitative polymerase chain reaction (qPCR) assays were performed using a RotorGene® Q equipment (Qiagen, Valencia, CA, USA). Amplifications were carried out in 10 μL reaction solutions containing 5 μL Maxima SYBR Green Master Mix (Thermo Fisher Scientific Inc., Waltham, MA, USA), with an optimized concentration of forward and reverse primers, 1 μL of template DNA and sterile distilled water. The gene-specific primer sets, optimized primer concentrations, and thermal cycling conditions for each target gene are shown in Table 1. All qPCR measurements were performed in triplicate and the absence of contaminations was verified against negative controls. Standard curves for each target gene were prepared from serially diluted stock solutions of target sequences (Eurofins MWG Operon, Ebersberg, Germany).

Quantitative data were analysed with RotorGene Series Software v. 2.0.2 (Qiagen) and the LinRegPCR program v. 2021.2 (Ruijter et al., 2009). Gene abundances were calculated as the mean fold differences between samples and corresponding 10-fold standard dilution in respective standards, as recommended by Ruijter et al., 2009; gene abundances were reported as gene copy numbers per gram of dry soil (copies/g dw).

Table 1. Characteristics of qPCR primer pairs and programs used.

Target gene	Primer	Primer reference	Amplicon size (bp)	Primer concentration (µM)	qPCR program
Bacterial 16S rRNA	Bact517F	(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[a]
	nirSR3cd				
nirK	nirK876	(Henry et al., 2004)	165	0.8	95°C 10 min; 45 cycles: 95°C 15 s; 58°C 30 s; 72°C 30s, 80°C 30 s[a]
	nirK1040				
nosZI	nosZ2F	(Henry et al., 2006)	267	0.8	95°C 10 min; 45 cycles: 95°C 15 s, 60°C 30 s, 72°C 30 s, 80°C 30 s[a]
	nosZ2R				
nosZII	nosZIIF	(Jones et al., 2012)	~700	1.2	95°C 10 min; 45 cycles: 95°C 30 s, 54°C 45 s, 72°C 45 s, 80°C 45 s[a]
	nosZIIR				
Bacterial *amoA*	amoA-1F	(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
	amoA-2R				
Archaeal *amoA*	CrenamoA 23F	(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
	CrenamoA 616R				
COMAMMOX *amoA*	comamoA AF	(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
	comamoA SR				
nrfA	6F	(Takeuchi, 2007)	222	0.8	95°C 10 min; 45 cycles: 95°C 15 s, 55°C 30 s, 72°C 30 s
	6R				
nifH	Ueda19F	(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
	Ueda407R				

a Fluorescence signal was read after the second extension step (80 ˚C)

3.4 – Soil N_2O flux measurements and calculations

Soil fluxes were measured using 12 automatic dynamic chambers located close to each measurement tree and installed in Spring 2020. Every polyvinyl chloride (PVC) made soil chamber covered a 0.16 m^2 soil surface, containing a volume of 0.032 m^3. Air with a constant flow rate of 1.8 L/min was circulated within a closed loop between the chamber and the gas analyzer unit during the measurements by a diaphragm pump to avoid gas stratification inside the chamber. The air sample was taken from the top of the chamber headspace and pumped back by distributing it to each side of the chamber. The soil chambers were closed automatically for 9 minutes each for

the measurements. The flushing time of the whole system with ambient air between measurement periods was 1 minute. Thus, there were ca 12 measurements per chamber per day. A Picarro G2508 (Picarro Inc., United States) gas analyzer using cavity ring-down spectroscopy (CRDS) technology was used to monitor N_2O gas concentrations at the frequency of approximately 1.17 measurements per second. The chambers were connected to the gas analyzer using a multiplexer, allowing a continuous sequential measurement (see Mander et al., 2021 for details).

Fluxes were quantified on a linear approach according to the change of N_2O concentrations in the chamber headspace over time, using the equation according to Livingston and Hutchison (1995).

3.5 – Ancillary measurements

The dynamics of the water table depth (WTD) was measured using perforated polypropene pipes (Ø 7.5 cm) as monitoring wells. At a depth of 5 cm, soil temperature was measured using a handheld temperature logger (Comet Systems Ltd., Rožnov pod Radhoštem, Czech Republic), and soil volumetric water content (VWC, m^3/m^3) and electrical conductivity (EC, mS/cm) were recorded using a handheld soil moisture sensor (model GS3, Decagon Devices Inc., Pullman, WA, USA) during each sampling campaign.

3.6 – Statistical analysis

Statistical, spatial, and temporal analyses were performed in Python (version 3.8.12), R (version 4.0.4) and QGIS software (version 3.14). For statistical analyses, Principal Component Analyses (PCA) were used to detect differences in physiochemical parameters throughout seasons. Spearman's rank correlation coefficient was used to evaluate the relationships between soil microbial communities and environmental factors. For spatial and temporal analysis, QGIS was used to map the change in environmental factors and soil microbial communities over time. Multiple methods of interpolation were used, including Inverse Distance Weighting (IDW), Radial Basis Functions (RBF), Ordinary Kriging (OK), and Splines. Kriging is found to generally have

the best performance in geospatial interpolation, but due to lack of fitting variogram, IDW was chosen (Li & Heap, 2011, 2014).

4 – Results:

4.1 – Soil physicochemical characteristics and N_2O emissions temporally and spatially

Visualizing the chemical and physical properties of the soil over the course of the year shows that some soil characteristics exhibit clear temporal patterns, while other characteristics are not as clear (Figure 2 and Figure 3). For example, soil temperature at 10 cm deep oscillates throughout the year, with lower soil temperatures in January and higher in July. Many characteristics show a bimodal distribution, such as EC and pH, which peak in late spring and late autumn. On the other hand, organic matter does not appear to have such a pattern and stays relatively stable throughout the year.

The regression line for soil temperature, WTD, VWC, EC, K and Ca all have p values below 0.05. The R^2 of NH_4-N could not be calculated.

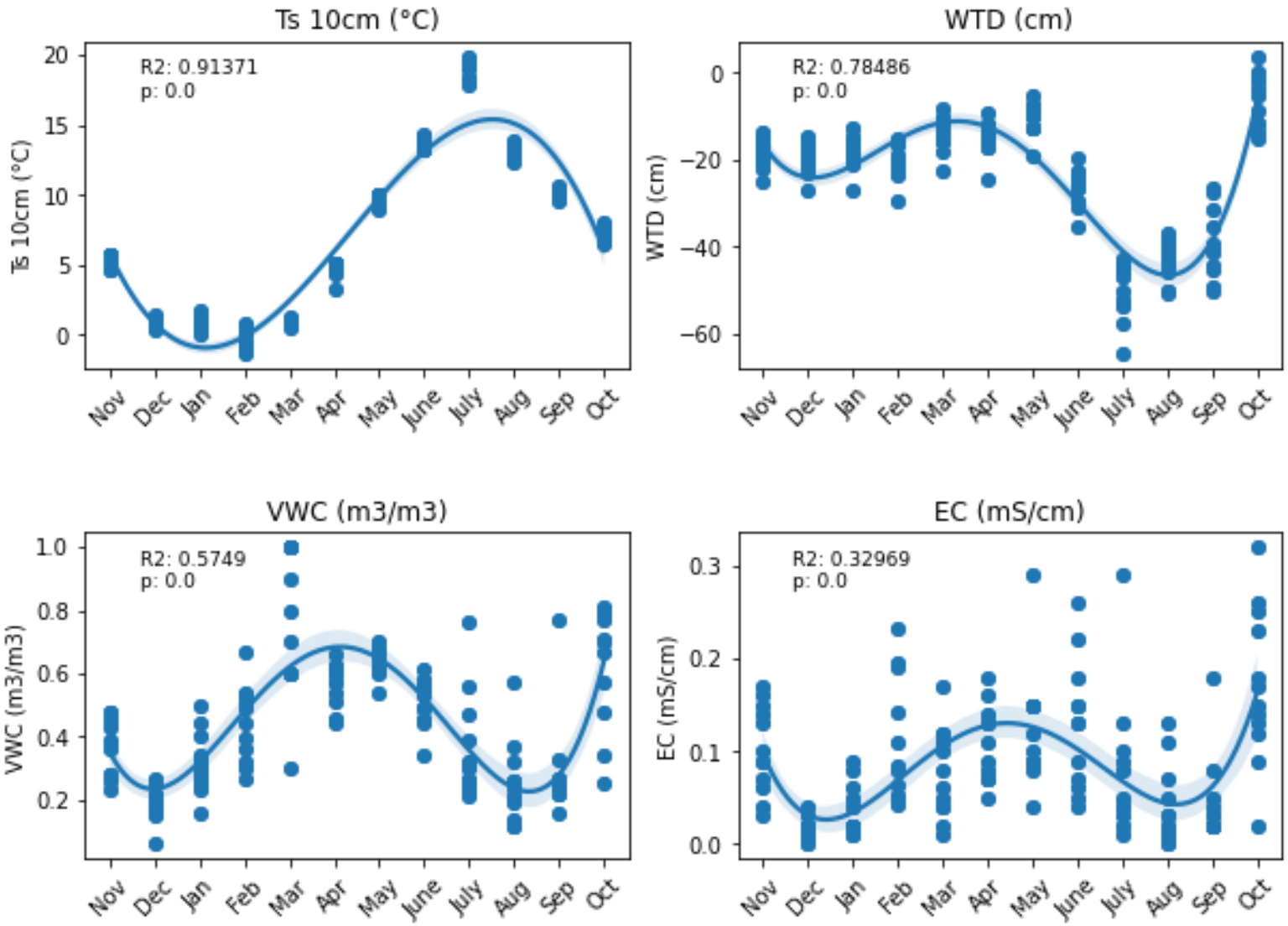

Figure 2: Soil physical characteristics throughout the year

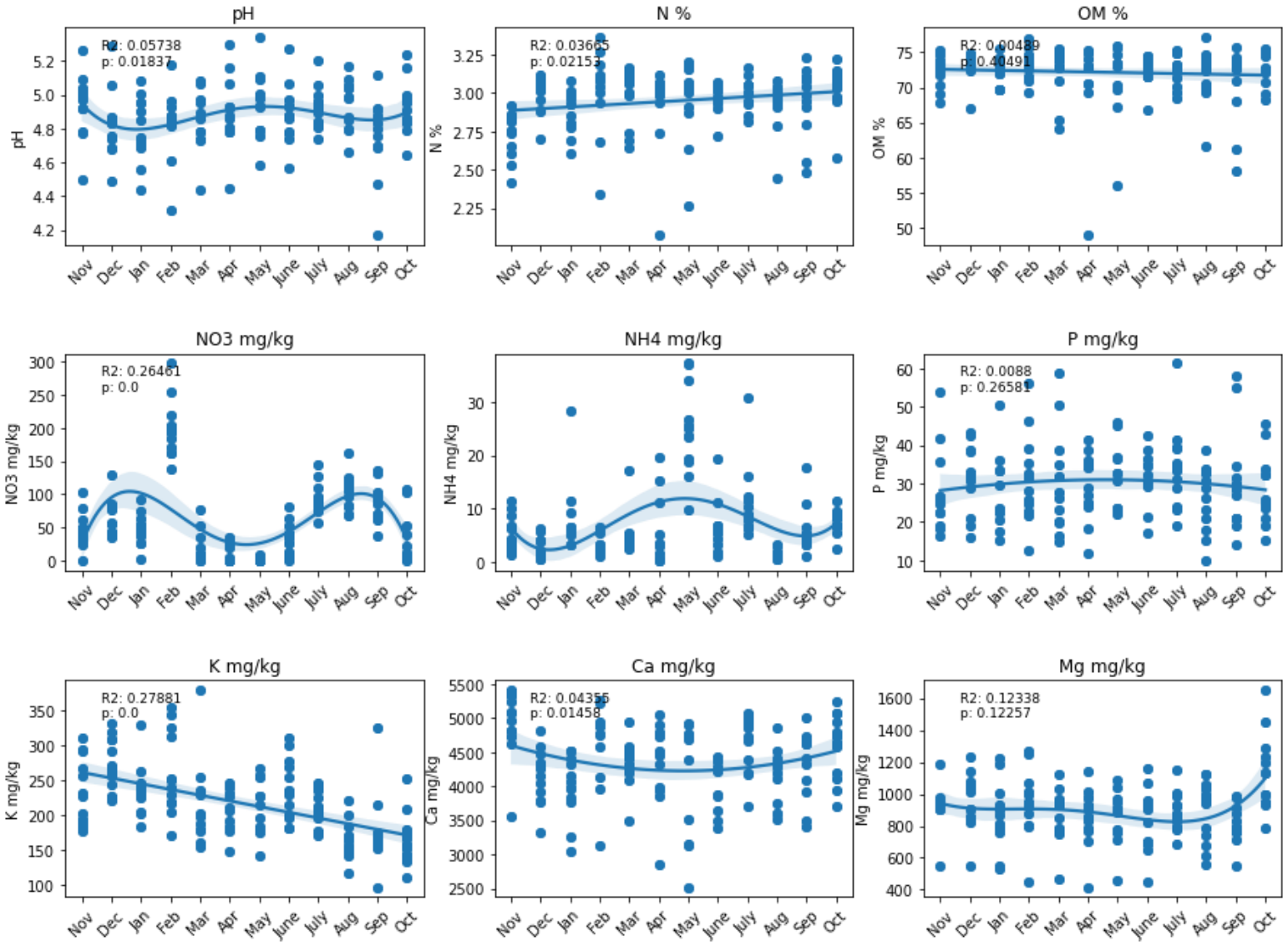

Figure 3: Soil chemical characteristics throughout the year

The spatial distribution of the soil's physical and chemical properties also shows some notable patterns (Figure 4 and Figure 5, respectively). Site 6 differs from the other sites in many of its qualities, such as overall higher pH, lower N and OM, and a lower WTD. Conversely, and to a lesser extent, Site 11 has a low pH and EC, high upper range for N, OM, P and Ca concentrations. Some soil characteristics, such as temperature and WTD (with the exception of site 6), are relatively constant across the sites. N_2O levels varied across sites, with Site 10 having the highest average N_2O emissions – possibly due to the comparatively high outlier. Grouping the properties by tree type shows relatively little difference between sites near just birch and sites near both birch and spruce. For soil chemical properties there seems to be more variability in sites with both birch and spruce.

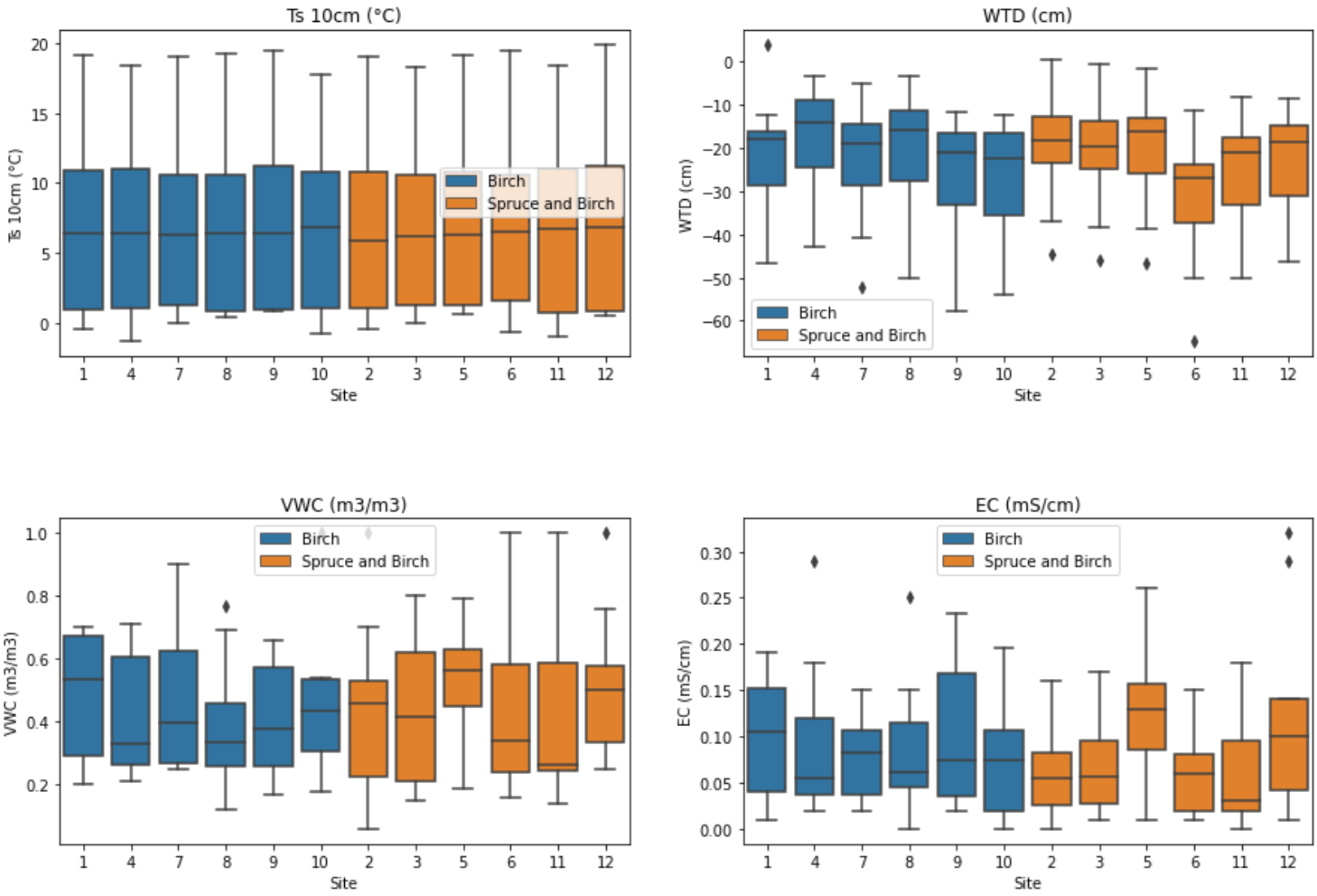

Figure 4: Physical characteristics of sample sites, grouped by nearby trees

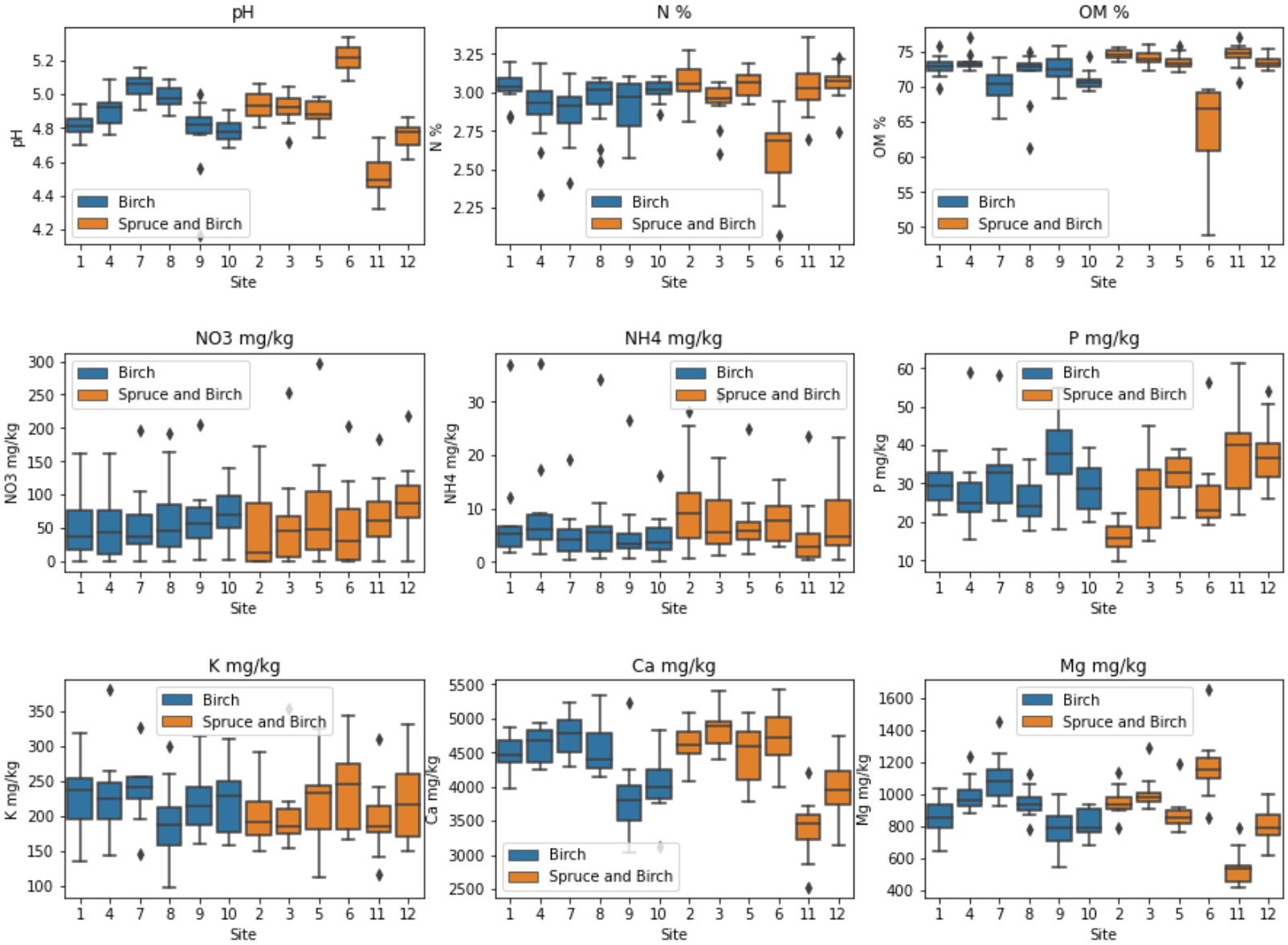

Figure 5: Chemical characteristics of sample sites, grouped by nearby trees

Conducting a PCA on the soil characteristics with groupings by season show little obvious groupings, however some are visible (Figure 6). The seasons of summer and spring appear to be opposite each other on the axes of WTD and VWC. Winter is relatively closer to summer, while fall is closer to spring. Fall, however, has large variance, while spring does not greatly intersect with winter and summer.

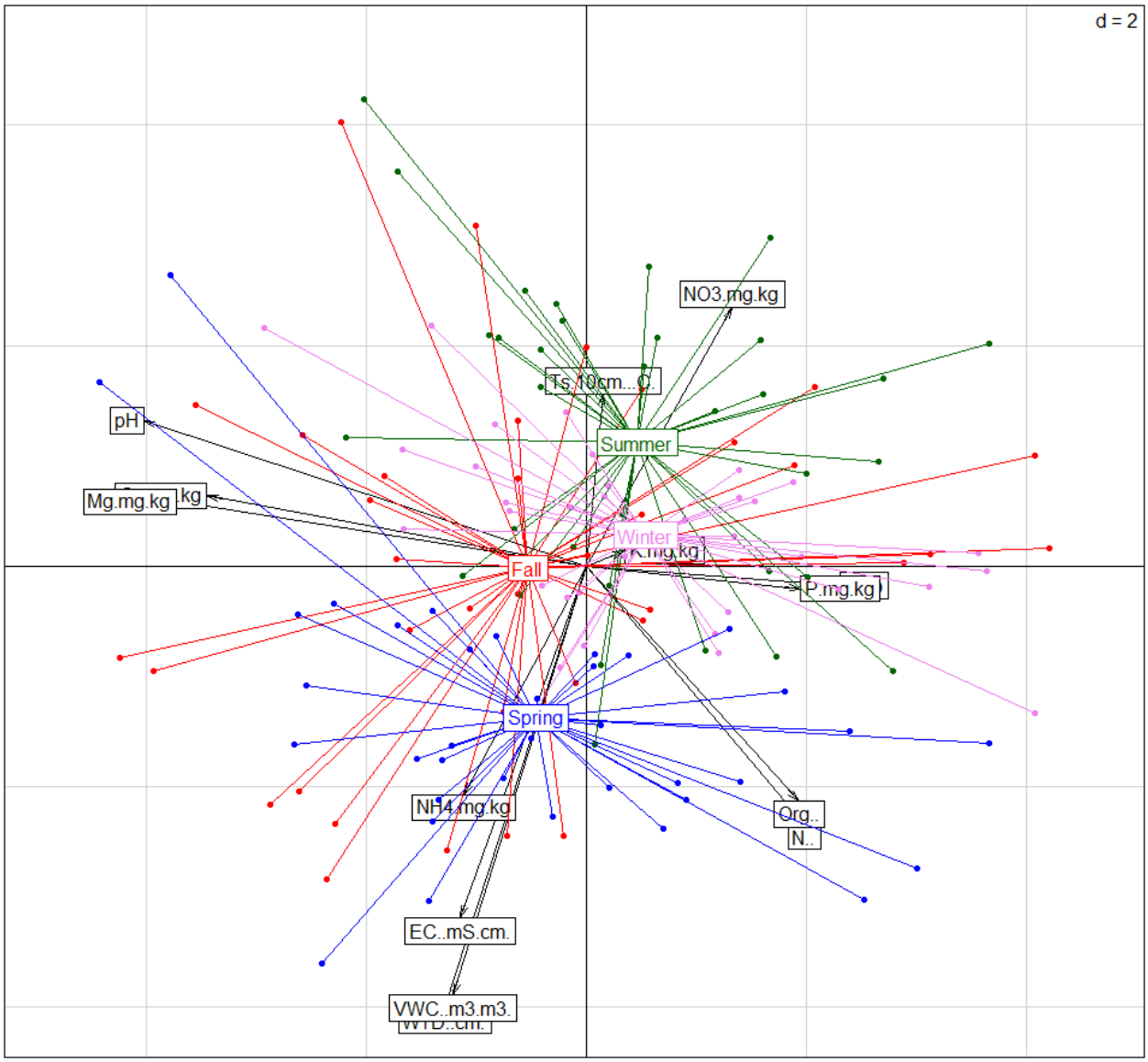

Figure 6: PCA of soil characteristics by season. Axis 1 of the PCAs accounted for 23.3% and axis 2 for 17.3% of the overall data variation.

Graphing the fluxes of N_2O over time shows monthly spikes in the N_2O levels (Figure 7). Site 10 has the highest peaks for monthly emissions of N_2O. Furthermore, site 10 has a large peak occurring in the middle of March 2021, and site 11 similarly has a peak in the middle of April 2021: both of these are outside of the otherwise consistent pattern of N_2O. N_2O fluxes have stayed roughly the same or have even gotten less extreme over time.

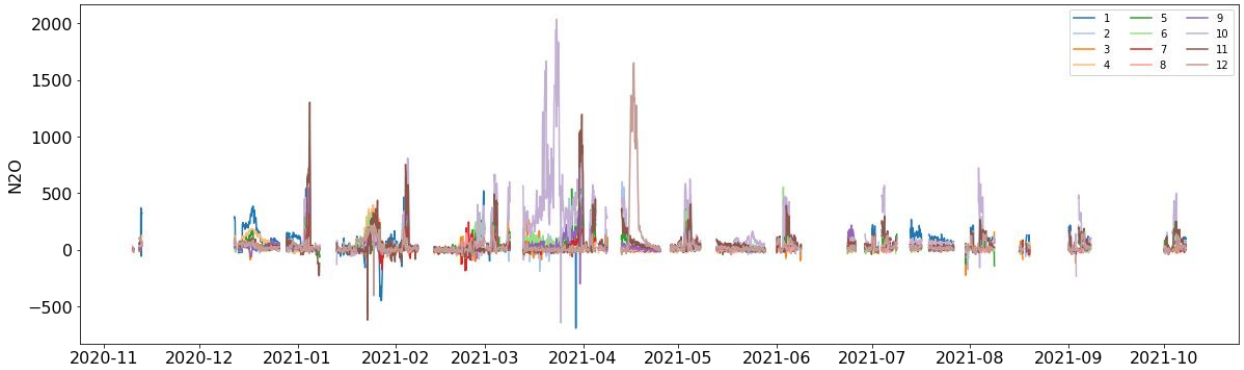

Figure 7: Gas fluxes over time for N_2O (µg N m^{-2} h^{-1})

4.2 – Nitrogen cycling genes temporally and spatially

Visualizing the abundance of genes associated with the cycling of nitrogen over time shows that the abundance changes throughout the year (Figure 8 and Figure 9). The abundances of genes such as COMAMMOX *amoA* and *nrfA* tended to change uniformly across all sites throughout the year, while other abundances such as *nirS* and *nosZI* changed mostly uniformly with the exception of a couple sites; abundances of archaeal 16S rRNA and bacterial *amoA* did not appear to have any uniformity across sites over time and showed significant variation at all points in the year. Gene abundance for *nirS* and COMAMMOX *amoA* went up in October but did not appear to be highly abundant a year prior in November. Conversely, archaeal *amoA* and bacterial 16S rRNA appear to have high abundance in November but no indication of increasing levels by October 2021. The abundances of genes *nirK*, bacterial *amoA*, and archaeal 16S rRNA seemed to have high points in both Autumn periods. The abundances of genes *nrfA* and *nifH* appear to have a high point in June, although this pattern is stronger in *nrfA*.

Early to late spring saw spikes in abundance levels for most genes at varying intensities. There appear to be two spring moments where gene abundances went up – late winter to early spring (January – February) and middle to late spring (March – May). Bacterial 16S rRNA, archaeal *amoA*, and COMAMMOX *amoA* genes abundances all increase in February. Bacterial 16S rRNA (continuing from February), *nirS*, and *nosZI* all have peak gene abundances in March.

Grouping the genes by nearby tree type shows that sites with different tree communities have high gene abundance at different times of the year (Figure 9). Archaeal 16S rRNA and bacterial *amoA* have a large range in abundances and no clear pattern can be observed. However, with the other genes, there are months where tree community appears to correspond with high or low abundances throughout the year.

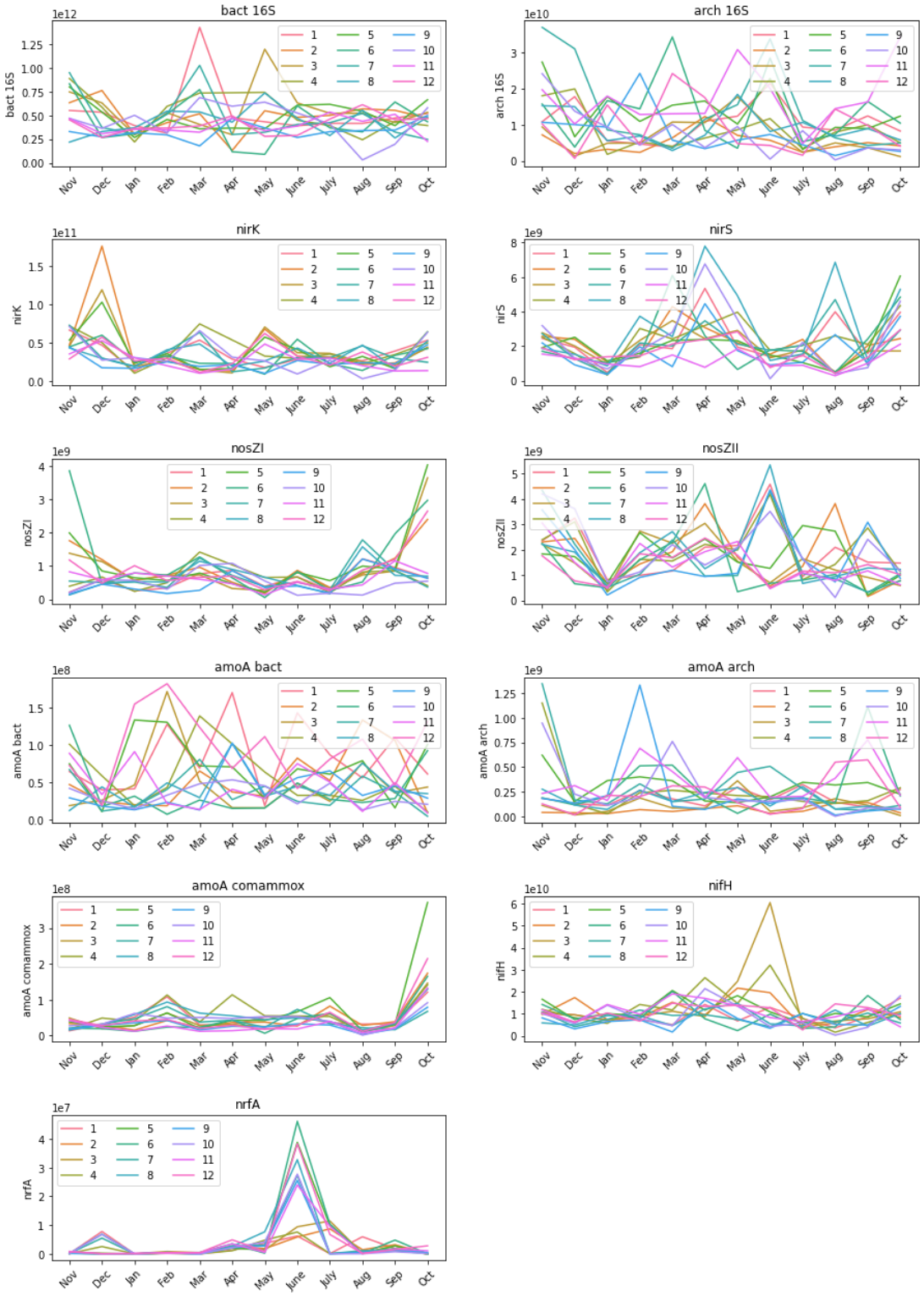

Figure 1: Nitrogen cycling gene abundance over time

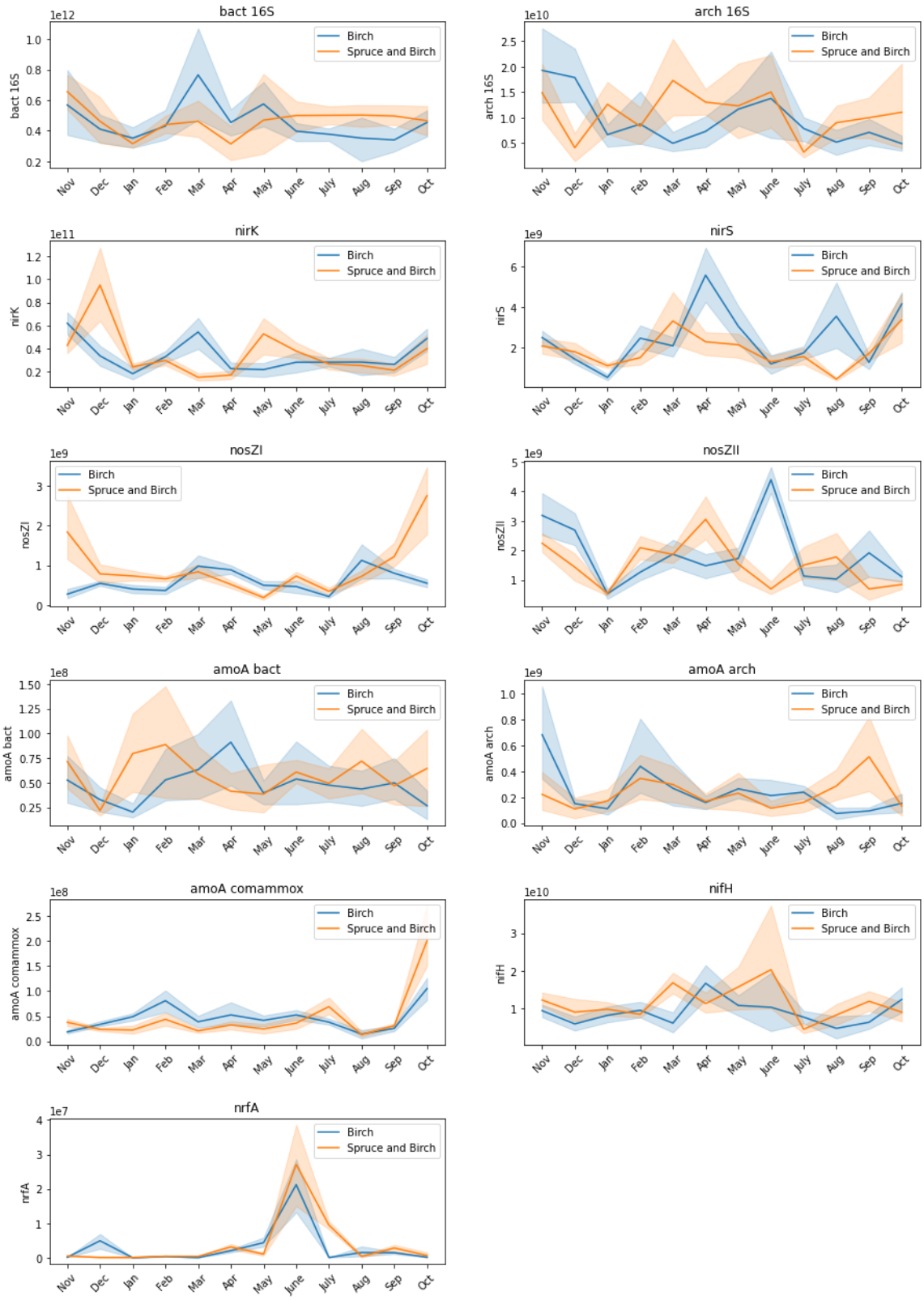

Figure 9: Nitrogen cycling gene abundance over time, grouped by nearby trees

Figure 10 shows interpolated average abundances of nitrogen cycling genes at each site. Abundances were averaged from values over the course of the year. The gene abundances of bacterial 16S rRNA, *nirK*, and to a lesser extent *nifH* have similar patterns in their distribution. The abundances of archaeal 16S rRNA and *amoA* also had similar distributions, particularly with hotspots at sites 5 and 11, but archaeal *amoA* appears to have higher abundances because of the comparatively low levels at sites 1, 2 and 3. COMAMMOX *amoA* abundances appears in a pattern similar to *nifH.* Bacterial *amoA* genes are particularly abundant at site 12, which is unique among the genes.

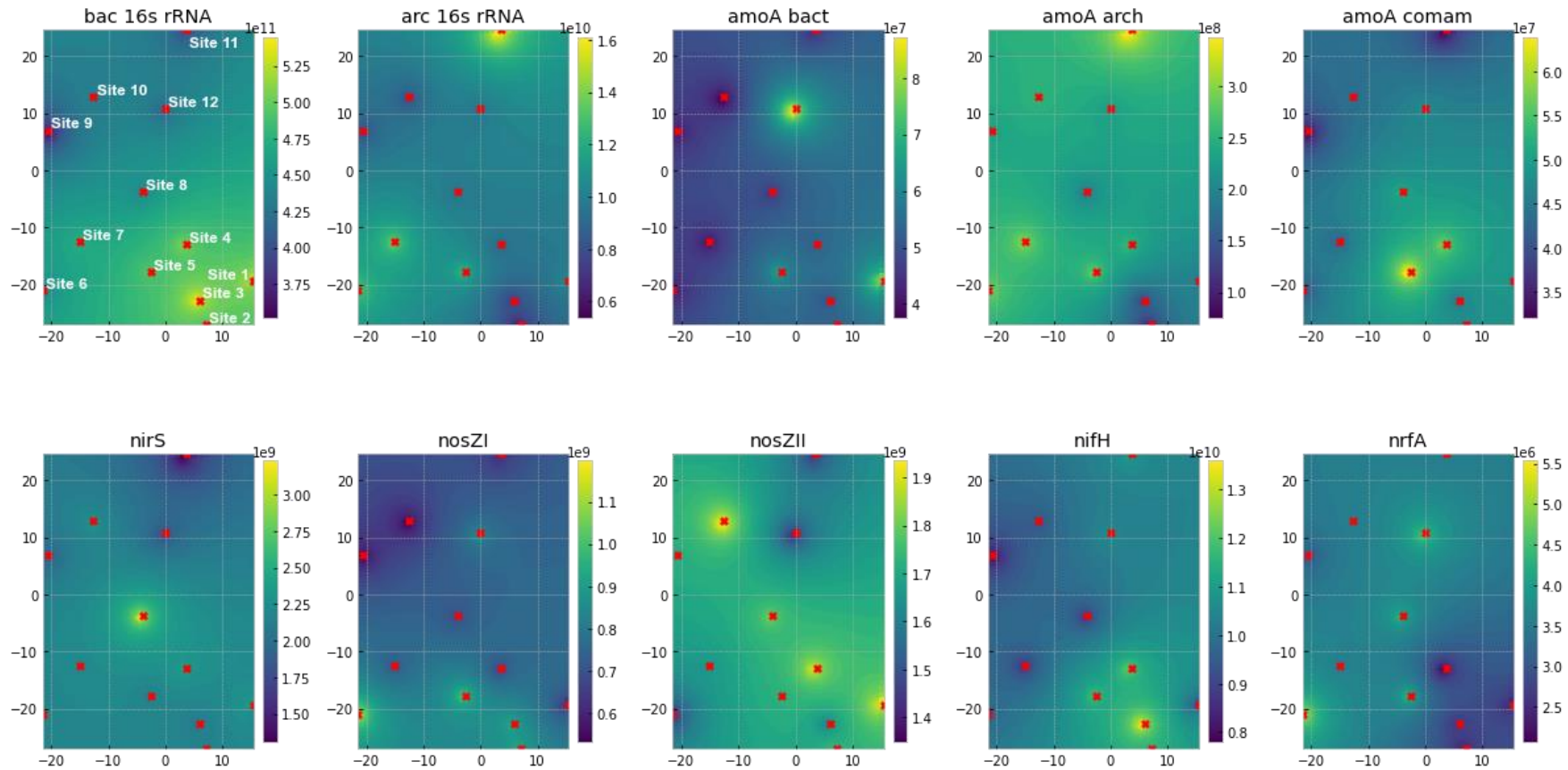

Figure 10: Interpolated average abundance of nitrogen cycling genes

Interpolating average N_2O emissions throughout the entire area show sites 10 and 11 as a hotspot (Figure 11). The southern half of the research area has comparatively low emissions at sites 3, 4, 7, and 8.

Figure 12 shows that for all months of the year except November, December, March, and April, site 10 is the largest hotspot for N_2O emissions. For November and December, site 1 is producing the most N_2O. For March, the N_2O emissions seem relatively spread out, concentrating more on the western edge of the research area. In April, site 12 is the largest emitter of N_2O.

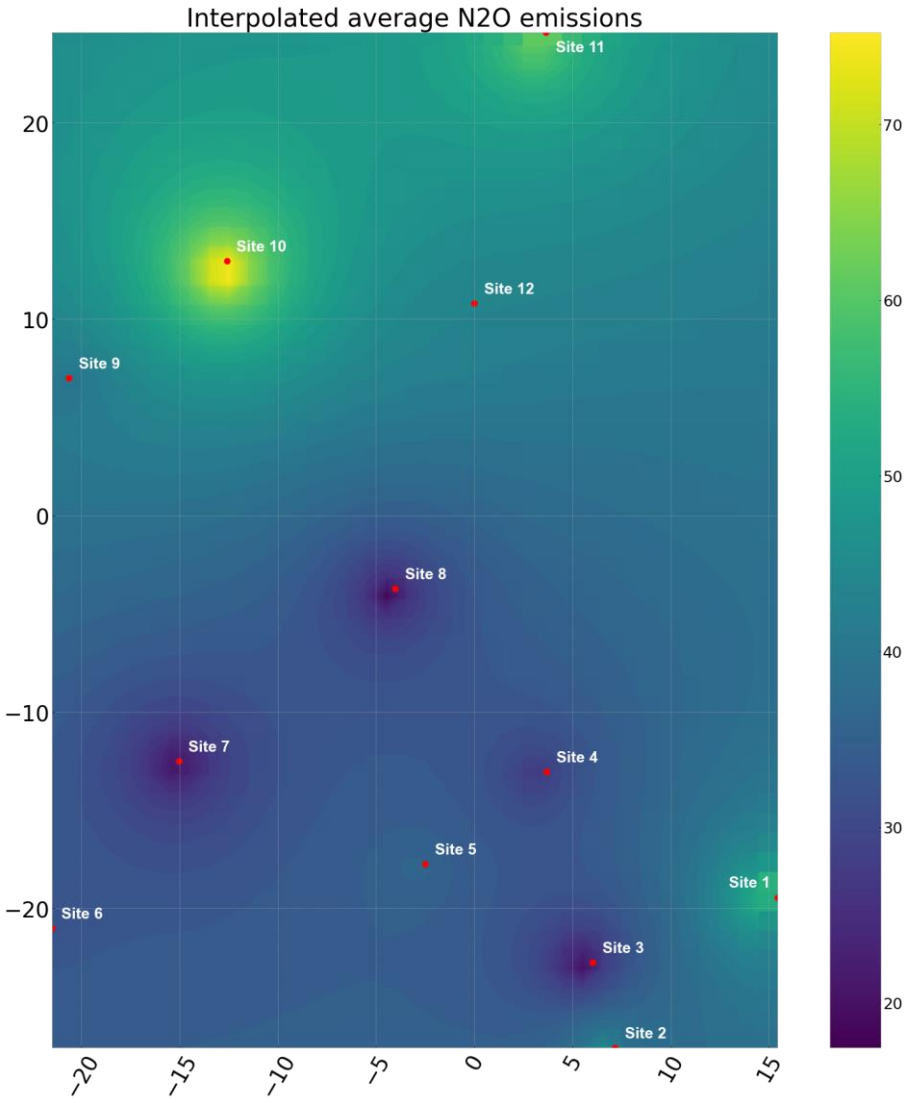

Figure 11: Interpolated average N_2O emissions (µg N m^{-2} h^{-1})

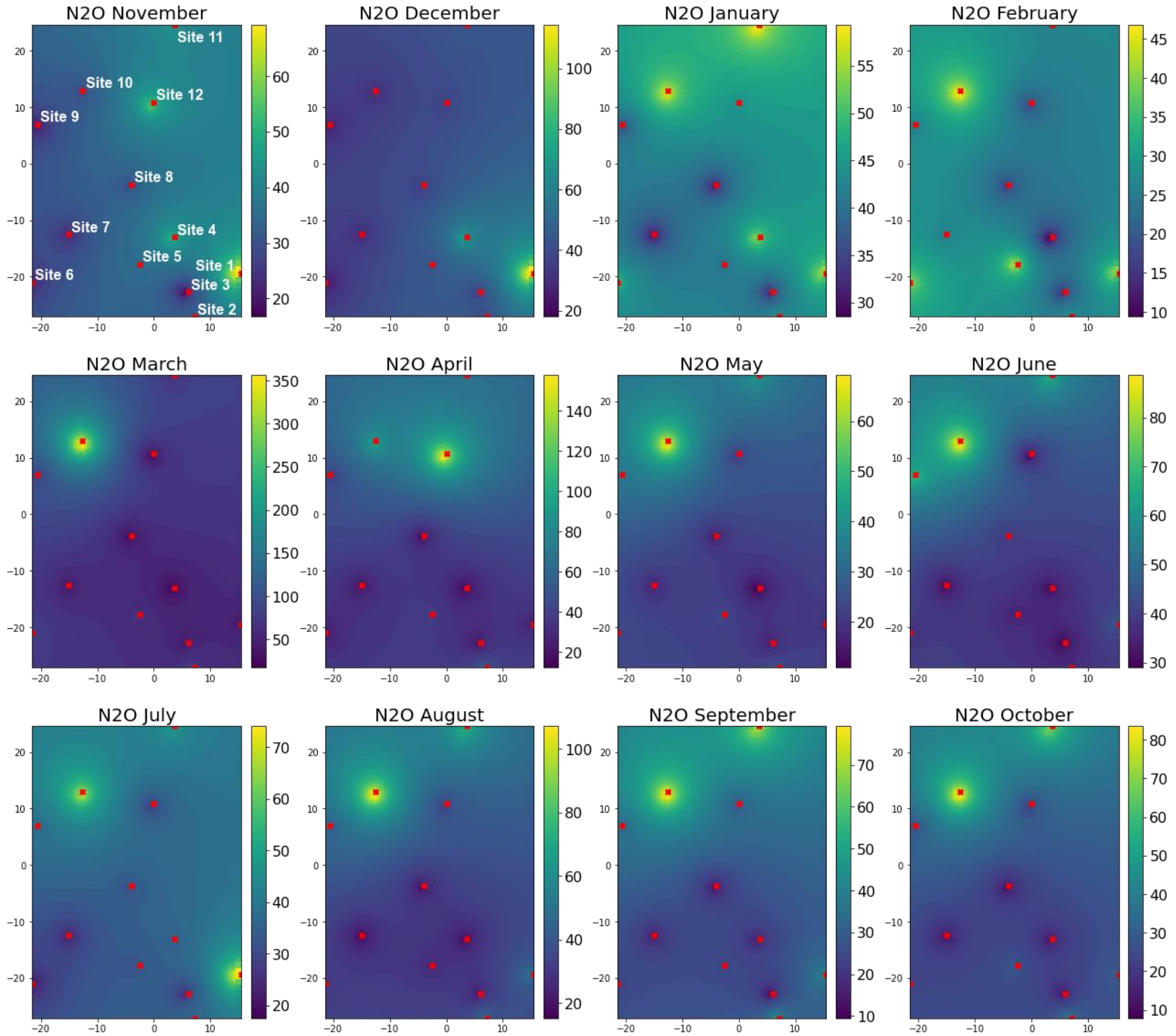

Figure 12: Interpolated N_2O emissions throughout the year

Interpolating the WTD between sites over the course of the year shows that, although WTD changes throughout the year, the depth tends to change relatively uniformly across the research area (Figure 13). From January to July, the same general pattern of WTD appears with few changes until August when the water table starts to rise again. There are periods where the WTD fluctuates in depth – such as from April to June – but the pattern is overall the same. The deepest point in May is as deep as the shallowest point in June. From August to October, there is again little change in the pattern of the WTD.

Sites 1, 2, 3, and 4 have the shallowest water table throughout the year. Site 4 is the shallowest from November to July. Sites 1, 2, and 3 are similarly shallow from August to October. Site 6 has a deep water table year round.

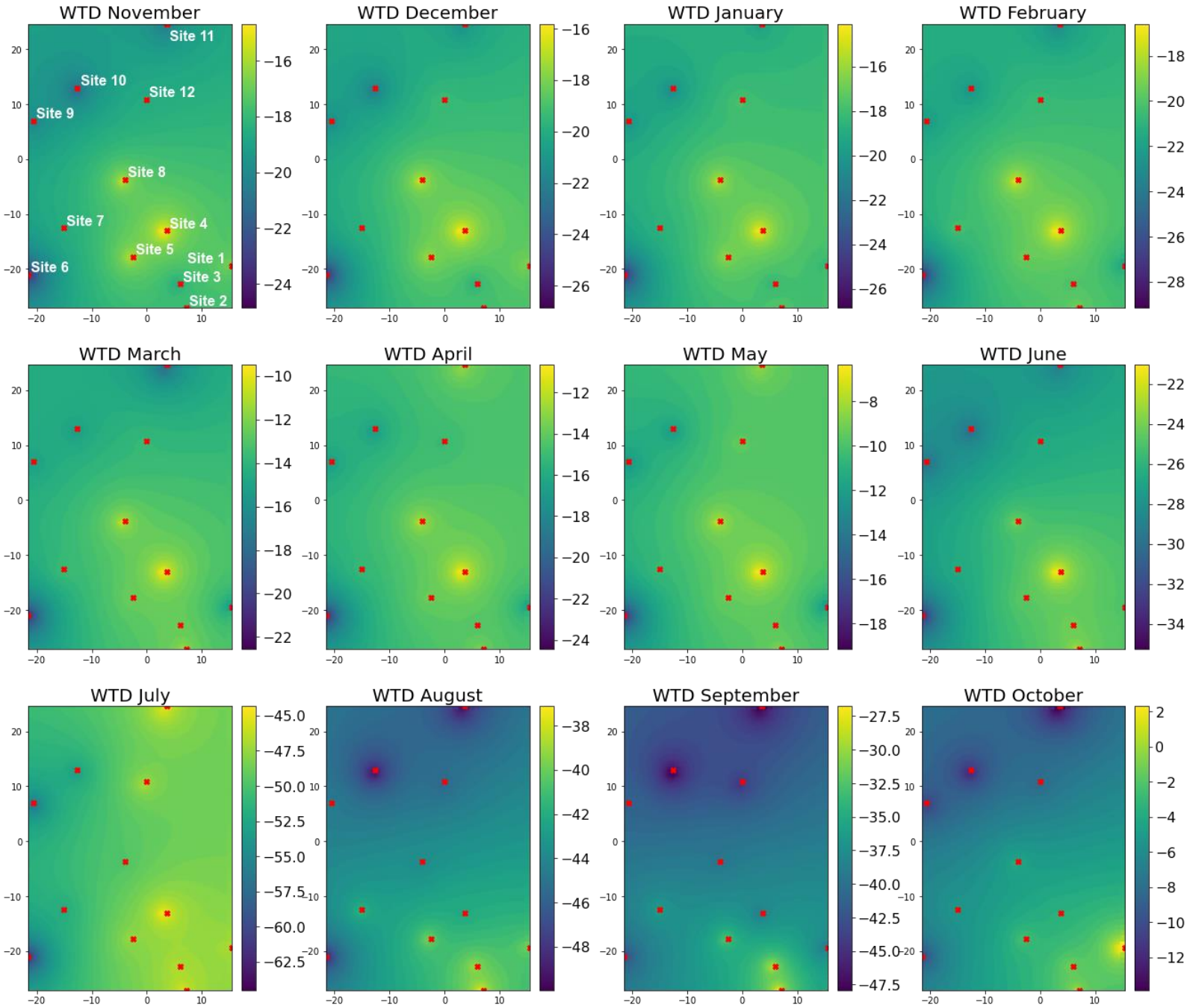

Figure 13: Water table depth (WTD) interpolated throughout the year

Figure 14 shows the correlation between the various soil physiochemical characteristics and nitrogen cycling genes, with correlations with a significant p-value (> 0.05) colored in. WTD correlated with NO_3-N, NH_4-N and Ca concentrations as well as soil temperature, VWC, and EC in the soil. The strongest correlation for physiochemical characteristics was between WTD and EC. Chemical concentrations, except for K were correlated to pH (Figure 3). OM also correlated with pH. Soil organic matter correlated with magnesium and calcium. The second strongest correlation was between Mg concentrations and pH, with the correlations between Mg and Ca and pH and Ca also high. NO_3-N correlated most strongly negatively with WTD and VWC. N_2O correlated with pH and all chemical concentrations other than K. It did not, however, correlate with N in the soil, but instead with OM (Org %). N_2O levels in the soil correlated most strongly with pH.

The abundances of nitrogen cycling gene were found to mostly correlate positively to each other (Figure 14). The abundance of the gene *nirK* correlated positively to Ca and Mg concentrations and negatively to soil temperature and N_2O emissions. The genes whose abundances correlated with N_2O levels were archaeal and bacterial 16S rRNA, *nirK*, *nirS, archaeal amoA,* and COMAMMOX *amoA*. Archaeal 16S rRNA and *amoA* abundances both correlated positively with N_2O. The rest correlated negatively, and *nirS* abundances correlated strongest. The abundances of the genes *nirS* and COMAMMOX *amoA* correlated most strongly with factors relating to soil water content: correlating positively with WTD and VWC. Archaeal 16S rRNA and archaeal *amoA* abundances correlated very strongly with each other.

The location of the site also correlates with multiple factors. The north axis positively correlated with N_2O, NO_3-N, archaeal 16S rRNA and *amoA* abundances, and negatively with WTD, NH_4-N, bacterial 16S rRNA, *nirS*, *nirK nosZI,* and COMAMMOX *amoA* abundances. Sites that were more east had and higher OM and N concentrations, and location east correlated positively with *nifH* and *nosZI* abundances.

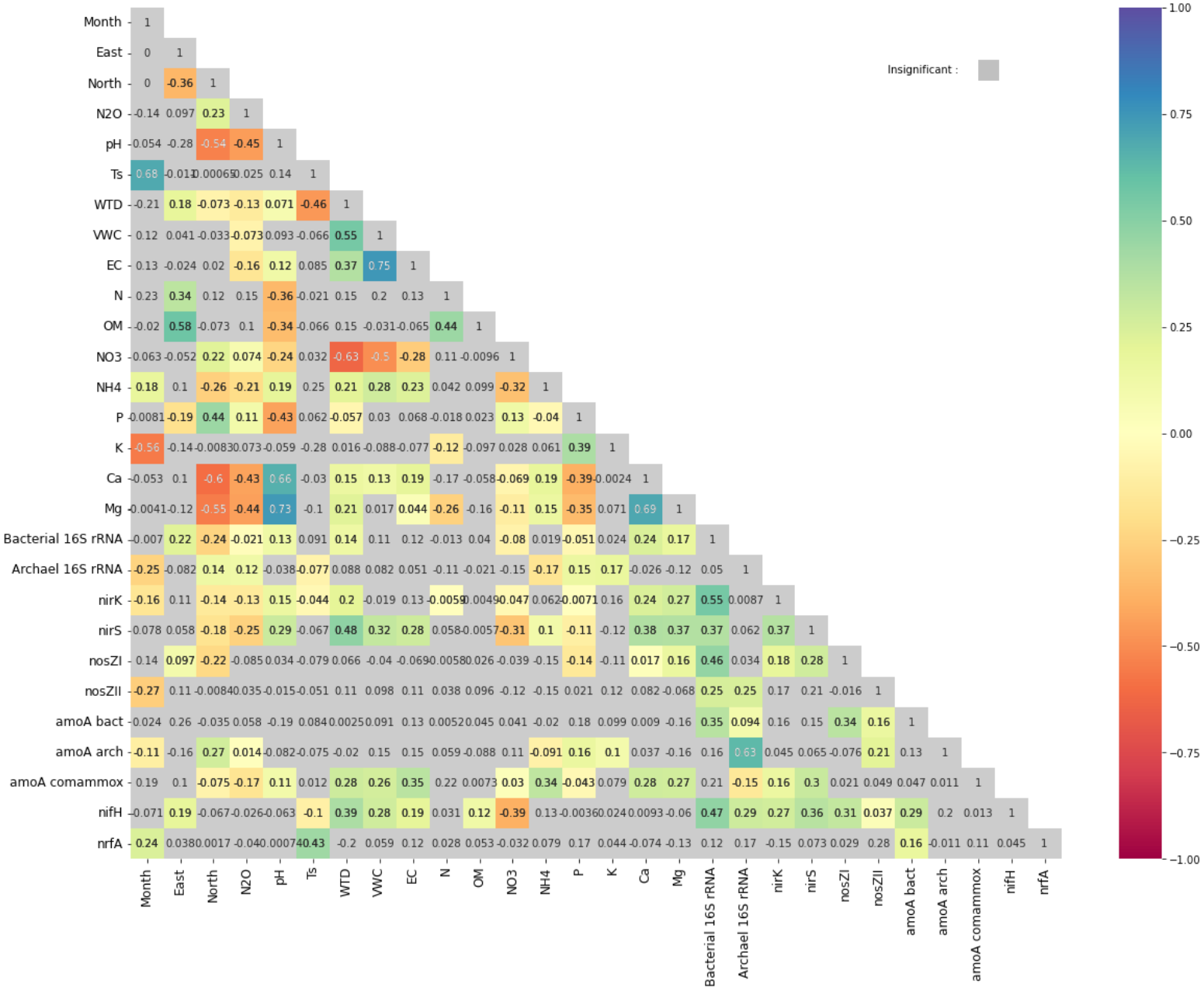

Figure 2: Correlations between soil physiochemical characteristics and nitrogen cycling gene abundances. Grey indicates a statistically insignificant correlation of p >0.05

Figure 15 shows the frequency of statistically significant correlations, both positive and negative, across all sites. Hashed marks indicate a lack of significance for the whole research area and correspond to grey areas in Figure 14. There are multiple cases where correlations between factors are statistically significant for more than half of the sites but insignificant for the whole. This is due to a wide range in the level of correlation: in one site, two variables may be significantly positively correlated, and in another, they may be negatively correlated. This means the relationship is significant locally but may not mean too much when considering a larger area.

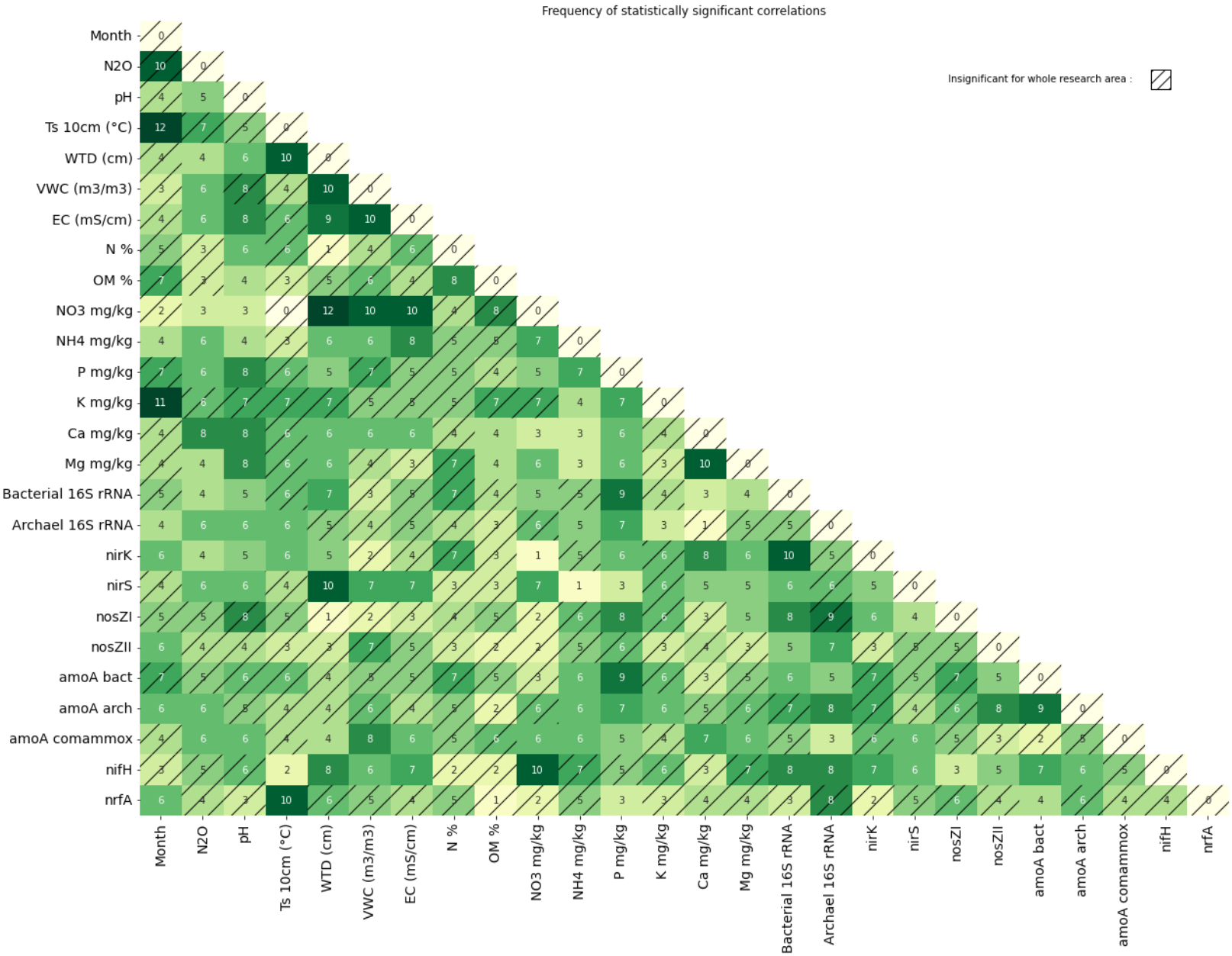

Figure 3: Frequency of statistically significant correlations across all sites. Numbers indicate the number of sites where a correlation was statistically significant ($p < 0.05$). Hashed lines indicate a lack of significance when looking at whole research area

5 – Discussion:

5.1 – Temporal Patterns in the Drained Peatland Soil

The temporal changes in the environment are the most easily observable. The temperature of the soil oscillates throughout the year, with colder winters and warmer summers being reflected in the data (Figure 2). Similarly, some soil characteristics are mostly stable throughout the year, such as OM and P (Figure 3). Although OM is not likely to be deposited evenly throughout the year, OM deposition does not create a pattern at the 10 cm depth of the soil cores.

The bimodal distribution of many soil characteristics is noteworthy. Figures 2 and 3 shows that NO_3-N, NH_4-N, VWC, EC, and WTD all have bimodal distributions of varying intensities. These characteristics all significantly correlate to WTD (Figure 14). WTD is unique among these bimodal distributions in its strong skew towards the summer months; the WTD is dependent upon the amount of precipitation entering the soil (Novakowski & Gillham, 1988). The soil's VWC at a shallow depth of 10 centimeters is higher when the WTD is less than 10 centimeters deep. As water is a conductor of electricity, the EC of the soil is connected to the soil's water content. These qualities all decrease with the drier soils of the middle of winter (December, January, and February) and late spring and summer (May – September). The sub-zero winter months prevent precipitation from entering the soil, where it instead gathers upon the ground as snow. Snow melts in the spring and raises the water table for a time, but the water table goes deeper again in the dryer months of the summer. The soil therefore experiences two periods of dry soil – once in the winter when water is frozen above ground, and then in summer with less precipitation and hotter temperatures drying the soil – and two subsequent periods of rewetting. The PCA in Figure 6 shows that winter and summer are close together on the axis of WTD, meaning that these seasons are both drier than spring and fall. Fall is also relatively drier than spring, mirroring the skewed bimodal pattern of WTD in Figure 2.

The chemical characteristics of the soil change in relation to the biannual wetting and drying of the soil. NH_4-N concentrations appear to follow a pattern similar to WTD, while NO_3-N concentrations have an inverse pattern where the lowest amounts are found in the autumn and spring. NO_3-N levels negatively correlated with WTD and VWC, indicating that soil moisture was a key driver for NO_3-N production. As soil moisture levels increase, oxygen concentrations and

redox potential in the soil decrease (Rubol et al., 2012). In addition, Rubol et al. found that NH_4-N levels also correlated with O_2 and redox potential, as reflected by the positive correlation between NH_4-N and VWC and WTD (Figure 14). NO_3-N also has a significant outlier in February. The data from February 2021 was sampled on a particularly cold day, reaching −28 degrees and causing significant problems for the research team. The speed at which the soil froze when taking samples was extraordinary and may lead to overall some differences in the NO_3-N levels in February.

The positive correlation between N_2O emissions and NO_3-N levels, and the negative correlation between N_2O and WTD and NH_4-N means that wetter periods are influencing the nitrogen cycle. Both NO_3-N and N_2O can be produced in nitrification; while denitrification uses NO_3-N to produce N_2O, it's possible that nitrification is more responsible for N_2O emissions than denitrification (Hu et al., 2015). Overall, abundances of both denitrification associated genes – *nirS* and *nirK* – correlated negatively with N_2O (Figure 14), which may also mean that the denitrification was complete and the end product was N_2 instead of N_2O.

N_2O positively correlated with abundances of the two archaea genes: archaeal *amoA* and archaeal 16S rRNA (Figure 14). These were the only genes that had a positive correlation with N_2O levels and are also notable for not being correlated with WTD. Instead, these two genes positively correlated with P and K, and negatively with NH_4-N. In strongly acidic soils of a pH less than 4.5, ammonia-oxidizing archaea have been found to be more influential in nitrification than ammonia-oxidizing bacteria (L. M. Zhang et al., 2011). While pH throughout the year fluctuates between 4.8 and 5, there are sites which have pH lower than 4.5 (Figure 3). AOA have been found to be a stronger control on nitrification than AOB in some acidic soils with pH values ranging from 4.5-6 (Gubry-Rangin et al., 2010). However, abundances of neither of the two archaea genes correlated with pH in the soil, possibly because the fluctuations in pH levels were within suitable ranges for AOA. This relationship between AOA, NH_4-N, pH, and WTD has been studied before, and AOA have been found to be more competitive in environments with low pH and NH_4-N levels (Zheng et al., 2017).

N_2O over time shows a period of increased emissions in the spring, starting in late February and continuing through to May (Figure 7). Abundances of the genes *nirS, nosZII,* bacterial *amoA,* COMAMMOX *amoA,* and archaeal *amoA* all spike in abundance during February (Figure 8). In

March, *nirK, nirS, nosZII, nosZII,* bacterial *amoA,* COMAMMOX *amoA,* and *nifH* are all present in greater abundances. The largest and most sustained emissions were from site 10 throughout March (Figure 7). This site was a hotspot for the gene *nosZII*, but also contained comparatively high amounts of archaeal *amoA* (Figure 10). Unlike *nosZII*, archaeal *amoA* abundances spike at site 10 during March, implying some correlation (Figure 7). The abundance of archaeal 16S rRNA at sites near spruce and birch also increased during March through May, but at sites near birch only increased in June (Figure 8). Conversely, bacterial 16S rRNA showed increased abundance during the springtime for only sites near birch trees. Soil near spruce trees may have conditions which promoted archaea or inhibited bacteria, leading to increased abundances of the gene archaeal *amoA.*

5.2 – Spatial Patterns in the Drained Peatland Soil

The sites at Agali II had some level of variation in almost every soil characteristic except soil temperature. Sites 6 and 11 both stand out as different from the whole. Site 6 has lower OM and N, WTD, and a higher pH than the other sites (Figure 5). This site had much higher clay content – particularly in the 30-40 centimeter range – and was at the base of a tree at the edge of an opening in the forest canopy. The soil of this area was often significantly different from the rest of the site, as reflected by the different characteristics. Site 11 also stood out, as it was in an area which was populated mostly by coniferous trees and had a correspondingly high amount of humus with needles. It's possible that the different vegetation in this site is responsible for the decreased pH, calcium, and magnesium levels in the soil. Site 11 also is a hotspot for archaeal 16S rRNA and archaeal *amoA* (Figure 10). Both sites 6 and 11 were near spruce and birch trees.

While all sites followed the same pattern with regards to WTD over time (Figure 2), WTD was only significantly correlated to N_2O emissions in five of the 12 sites (Figure 15). WTD depth correlated negatively with N_2O, meaning that in dryer months, N_2O emissions were higher (Figure 14). The relationship between a site's location along the north-south axis and WTD, pH, NO_3-N, NH_4-N, N_2O, Ca, P, Mg, archaeal 16S rRNA abundance, and archaeal *amoA* abundance imply that there is a gradient in soil composition and ammonia oxidizing archaea. The more northern parts of the peatland have shallower water tables and more AOA alongside decreased pH and NH_4-N. Although the WTD depth changes throughout the year, a similar pattern of depths is present from

the beginning of the study period until August (Figure 13). From August on, the WTD appears to have a different distribution in the research area. The north axis' negative correlation with pH is potentially due to the change in vegetation. Increased presence of coniferous trees and deposited needles could be creating more acidic soil substrates in the northern parts of the research area. The WTD may impact oxygen availability in the soil, affecting denitrification (Rubol et al., 2012).

AOA have been found to correlate positively with P levels in aquatic environments (Erguder et al., 2009). Moreover, in experiments on alpine steppes, AOB abundance was negatively affected by P levels, while AOA were not affected (Dong et al., 2020). The north-south axis positively correlated with P (Figure 14). This is possibly due to greater abundance of spruce in the northern parts of the research area. The waterlogging of pine and spruce trees has found to reduce Ca and Mg concentrations in needles (Palomäki et al., 1994). Pine needles were found to have higher levels of P, while spruce needles were found to have slightly lower levels of P after waterlogging. The north-south axis likely corresponds to a change in vegetation communities from more deciduous trees in the south and coniferous trees in the north. AOA levels may be greater in the northern parts of the research area due to the higher concentrations of P inhibiting AOB abundances.

Site 10 is notable for having produced the most N_2O, and is responsible for a large, extended period of emissions in late March (Figures 7, 11). However, site 10 was not the biggest N_2O producer throughout the year (Figure 12). Site 1 is recorded to be the biggest N_2O producer in November and December, but there are many holes in the data for these months (Figures 7, 12). In April, site 12 emitted more N_2O than site 10, but both could be still considered hotspots. The gene *nosZII* had highest abundances at site 10 and was the only gene to be most abundant at this site (Figure 10).

The east-west axis of the research area was not as important for N_2O emissions, but showed strong correlations for different soil make up, with more easterly sites having increase OM and N. The east axis correlated with abundances of bacterial 16S rRNA, *nosZI*, and *nifH*. Although sites more to the east did not record significantly different N_2O emissions, the variation in the soil makeup influenced bacterial 16S rRNA abundances. N_2O emissions are impacted by multiple different factors in the peatland. Water table depth, nearby tree types, and pH of the soil in the

northern parts of the research area impacted nitrification by archaea, and potentially inhibited nitrification by bacteria.

6 – Conclusions:

The drained peatland forest in the Agali II research site varies across the whole research area and differences in the soil and nitrogen cycling genes are evident in this spatial variation. Different gradients in change can be measured in both north and east axes. The north axis contains a gradient in WTD, pH, P, Ca, Mg, N_2O emissions, and the abundance of Archaeal 16S rRNA and archaeal *amoA* genes. This axis is one which is significant for the emissions of N_2O, and likely corresponds to a change in vegetation communities. Sites near spruce trees had larger archaea communities and smaller bacterial communities during the spring when the greatest N_2O emissions were recorded. The east axis contains a gradient in soil composition and the abundance of N_2O-correlated Bacterial 16S rRNA, although this axis was not significant for gas emissions itself. Separate from north and east axes, specific sites within the research area varied from the whole of Agali II, indicating high local variations. Site 6 had the highest variations in soil composition. Site 10 had the highest N_2O emissions, possibly being a hotspot where local conditions support N_2O production via nitrification. This site had the greatest abundances of the gene *nosZII*, but also had a spike in abundance of archaeal *amoA* during a hot moment. Site 11 had the highest representation of Archaeal 16S rRNA and archaeal *amoA*. Environmental conditions may promote archaea growth or inhibit bacteria communities, leading to an abundance of nitrifying archaea and greater N_2O emissions.

The temporal variations in the site appear mostly related to fluctuations in the water table and periods of drying and rewetting. There are potentially two periods of drying – once in winter when precipitation is held as snow above ground and again in summer with heat and lack of precipitation – and two periods of rewetting – after snow melts, and after autumn sets in. Many soil characteristics, notably pH, also follow WTD and fluctuate during spring and autumn. This is mirrored by two periods of greater abundance in nitrogen cycling genes, particularly archaea at sites near spruce and birch trees. The combination of increased NO_3-N, decreased NH_4-N, and

greater N_2O emissions during these periods indicates that nitrification by ammonia oxidizing archaea is playing a greater role than denitrification in the emission of N_2O.

7 – Kokkuvõte:

Lämmastikuringega seotud mikrobioloogiliste protsesside muutused kuivendatud turvasmullal kasvavas metsas üheaastase uurimisperioodi vältel

Zane Ferch

Kuivendatud turvasmuldadel kasvavad metsad on märkimisväärsed N_2O allikad. See on tugev kasvuhoonegaas, mille voogusid on turbaalade veerežiimi taastamisega võimalik vähendada. Kuigi looduslikud turbaalad on süsinikusidujad, siis kuivendamisest mõjutatuna on need ulatuslikud süsihappegaasi (CO_2) ja N_2O allikad ning nende taastamine võib võtta aastakümneid, et turba degradeerumine lakkaks. Kuivendatud turbaalade taastamine tähendab nende veerežiimi taastamist. Mõistmaks, kuidas veerežiimi muutmine neid komplekssseid keskkondi mõjutab, on kuivendatud turbaalade kasvuhoonegaaside voogude uurimine oluline. Käesoleva magistritöö eesmärk oli uurida mulla mikroobikoosluste, füüsikaliste ja keemiliste omaduste ning N_2O voogude vahelisi ruumilisi ja ajalisi seoseid Agali II uurimisjaama kuivendatud turvasmullaga metsas ühe aasta jooksul.

Kuivendatud turvasmullaga metsas mulla mikroobikoosluse, füüsikaliste ja keemiliste omaduste ning N_2O voogude vaheliste ajaliste ja ruumiliste seoste analüüsimiseks koguti aastase uurimisperioodi vältel Kagu-Eestis asuval uurimisalal mullaproove 12 kohast. Analüüsiti mulla füüsikalisi ja keemilisi omadusi ning üheksa geeni arvukust lämmastikuringega seotud protsessides: denitrifikatsioon (*nirS, nirK, nosZ* klaad I, and *nosZ* klaad II), nitrifikatsioon (*amoA* geeni omavad bakterid, arhed ja commamox-i organismid), dissimilatoorne nitraadi redutseerimine ammooniumiks (*Dissimilatory Nitrate Reduction to Ammonium* - DNRA) (*nrfA*) ja lämmastiku sidumine (*nifH*). Mullaparameetreid seoti kogu uurimisperioodi vältel automaatse dünaamilise kambri meetodil kogutud N_2O voogudega. Kvantitatiivse polümeraasi ahelreaktsiooni abil (*quantitative Polymerase Chain Reaction* – qPCR) määrati geenide arvukus. Erinevate uuritud parameetrite omavaheliste seoste testimiseks rakendati Spearman`i korrelatsioonikoefitsenti ning

ajaliste ja ruumiliste seoste analüüsiks kasutati interpoleerimist deterministliku meetodiga (*Inverse Distance Weighing* - IDW).

Varieeruvad mulla keemilised ja füüsikalised parameetrid viitasid proovialal erinevatele taimestiku ja mikroobide kooslustele. Leiti, et N_2O vooga korreleerusid nii bakterite ja arhede 16S rRNA geenide arvukused kui ka lämmastikuringega seotud geenide *nirK*, *nirS* ning arhede *amoA* ja COMAMMOX *amoA* arvukused. Arhede 16S rRNA ja arhede *amoA* geenide arvukused korreleerusid mõlemad N_2O vooga positiivselt ning nende arvukused olid tugevas korrelatsioonis ka mulla fosfori ja kaaliumi kontsentratsiooniga. Teised statistiliselt olulised korrelatsioonid olid geenide ja N_2O voo vahel negatiivsed, kuid nende geenide arvukused olid tugevalt sõltuvad veetaseme muutustest. Kogu uurimisperioodi vältel olid N_2O voogude ja lämmastikuringega seotud geenide arvukuse peamised mõjutajad veetase ja mullaniiskus. Talvised temperatuurimuutused takistasid vee liikumist ning muld külmus ja sulas korduvalt, mis oli seotud ka kõrgete N_2O emissioonidega. Uurimisalal esines kõrgema N_2O vooga alasid ruumiliselt kui ka ajaliselt. Olid alad, kus N_2O vood olid pidevalt kõrgemad, ning need, kus N_2O vood olid perioodiliselt mõjutatud mulla füüsikalistest ja keemilistest omadustest ja mikroobikooslusest.

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