FATE OF NITROUS OXIDE (N₂O) IN FINNISH BOREAL FOREST AND PEATLAND SOILS

Sankari Rajasakaren

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University of Eastern Finland (Kuopio), Faculty of Science and Forestry

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Abstract

Recent studies show that nitrous oxide (N_2O) has a missing sink in soils but the potential of this sink of N_2O is poorly understood. Denitrification has been shown to be the major consumption process of N_2O and could result as a sink potential for N_2O if the process is highly efficient. However, the process of denitrification is influenced by several factors such as oxygen, pH, temperature, nutrient availability, soil moisture content and soil type. The main aim of this thesis was to understand the mechanisms that influence N_2O concentrations in boreal forest soils and peatlands and to evaluate their potential to act as sinks for N_2O. The boreal forest study site selected for this study is located in Heinävesi and the peatland site in Salmisuo. Soil samples from the organic and mineral layer of the boreal forest and the upper 0-10cm and lower 10-20cm of the peatlands were collected to understand the differences in N_2O concentrations within different soil layers. Furthermore, the factors affecting N_2O concentrations were investigated, namely; the role of oxygen and substrate availability (more specifically, NH_4^+, NO_3^-). The effect of different carbon substrates namely succinate, butyrate, formate, propionate and lactate on N_2O concentrations were also investigated to understand if they stimulated distinct microorganisms which influenced net N_2O consumption/production potential.

In anoxic treatment both boreal peat (Salmisuo) and forest (Heinävesi) soil showed a net consumption of N_2O. However, there were differences in net N_2O consumption potential between forest soils and peat as well as between the different layers within each site. In oxic treatment forest soils showed a net production of N_2O in organic soil but near negligible production of N_2O in mineral soils. However in peat, there was a net consumption of N_2O in both peat layers. The net consumption potential of N_2O in peat was smaller in oxic conditions than in anoxic conditions. The addition of different carbon substrates to the mineral (Heinävesi) forest soil and the lower 10-20cm peat (Salmisuo) soil resulted in similar trends with exception of formate. Although there were similar trends observed in both soils, the consumption or production potential depending on the relevant carbon substrate was comparatively larger in the lower 10-20cm (Salmisuo) peat.

This study indicates that the effect of oxygen on denitrification plays a crucial role in regulating N_2O fluxes in boreal soils and their potential to act as sinks for N_2O. These results

also indicate that boreal soils are sensitive to aerobic/anaerobic conditions which can influence their potential to act as a sink for N_2O.

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Contents

1. Introduction

1.1 Nitrous oxide

Nitrous oxide (N_2O) is a potent greenhouse gas (GHG) contributing to climate change with an atmospheric lifetime of 114 years (Forster *et al.,* 2007). With its ozone-depleting properties (Ravishankara *et al.,* 2009) and a global warming potential 300 times that of carbon dioxide (CO_2), N_2O is attributed to be the third most important contributor to radiative forcing (Forster *et al.,* 2007). As N_2O plays an important role in the atmosphere, identifying the sources and sinks of this greenhouse gas is critical for understanding its implications across ecosystems. Global N_2O emissions are estimated to be 17.71 Tg N yr^{-1} (Schlesinger, 2013). The main sources of N_2O to the atmosphere are agricultural (2.8 Tg N yr^{-1}) and tropical rain forest soils (4.0 Tg N yr^{-1}) contributing around 40% of the total emissions. Since the pre-industrial era, global atmospheric N_2O concentrations have increased by about 20%, which has been attributed largely to the increased use of nitrogen-based fertilizers (IPCC, 2013). It has also been predicted that with continuous increase of N_2O in the atmosphere, the significance of N_2O to greenhouse effect and ozone depletion will be more severe in the twenty-first century (Ravishankara *et al.,* 2009).

The most important and main sink for N_2O is the photochemical reactions (12.5 Tg N yr^{-1}), which take place in the stratosphere (Crutzen and Oppenheimer, 2008). However, there is growing evidence that soils can also behave as sinks for N_2O (Chapuis-Lardy *et al.,* 2007; Schlesinger, 2013). Nevertheless, further understanding is necessary to elucidate the potential of soils as a sink of N_2O. Soils prone to high N_2O sink activity are mostly located in northern regions (Kroeze *et al.,* 2007). Although Schlesinger (2013) has estimated global N_2O uptake in soils to be not higher than 0.3 Tg N yr^{-1} which corresponds to global sinks being no greater than 2% of estimated sources of atmospheric N_2O, the exact potential of soils as N_2O sinks have yet to be exactly determined. Hence, by understanding the mechanisms that influence N_2O uptake in soils we can ascertain the atmospheric importance of this N_2O sink.

In a review investigating 100 different studies on natural or recovering ecosystems, measured uptake potentials of N_2O in soils ranged from 1.0µg $Nm^{-2}h^{-1}$ to 207µg $Nm^{-2}h^{-1}$ with a median value of 4µg $Nm^{-2}h^{-1}$ with the highest consumption of N_2O observed in wetland and peatland ecosystems (Schlesinger, 2013). The net fluxes of N_2O indicate whether soils are a net source or sink with N_2O production and consumption primarily related to the processes of nitrification and denitrification, respectively. These in turn depend on environmental factors

regulating consumption and production. Recent studies show that N_2O has a missing sink in soils but the potential of sinks of N_2O have been poorly delineated. It is pertinent to note that denitrification process can result as a sink potential for N_2O if the process is efficient.

For sink activity to occur, the ambient N_2O concentration in the atmosphere has to exceed that in the soil gas phase. The N_2O exchange between soils and the atmosphere can be explained by the equilibrium concept (Conrad and Dentener, 1999). At N_2O compensation point, the concentration of N_2O in the soil gas phase results in no net exchange of N_2O between ambient atmosphere and soil gas phase. Hence, it can be concluded that the compensation point determines the direction of the flux at a given ambient atmospheric N_2O concentration. Compensation concentrations of N_2O are typically significantly higher than the ambient atmospheric concentrations which correlate with the observation that most soils are sources of atmospheric N_2O. Studies that indicate consumption of N_2O by soils suggest that during uptake the compensation concentrations are lower than ambient concentrations, i.e. <310ppbv (Kroeze *et al.,* 2007). It has also been suggested that the compensation concentration for N_2O increases with other factors such as temperature, soil moisture and nitrogen availability (Conrad and Dentener, 1999).

1.2 Research Objectives and Hypotheses

In this study, the main objective was to quantify the N_2O fluxes at a boreal spruce forest and pristine peatland in Finland in anaerobic and aerobic conditions. Two main hypotheses were tested. The first was that aerobic conditions in forest soils would result in markedly increased N_2O fluxes in organic soils compared to mineral soils; but in both peat layers would result in consistent N_2O fluxes. The second hypothesis was that the addition of different carbon sources to both forest soils and peat would affect N_2O fluxes because of the resulting preference for certain carbon sources by microbes in the soils. To test these hypotheses experimental anaerobic and aerobic conditions and manipulation of carbon sources were arranged in flask microcosms and following N_2O fluxes were determined. Furthermore, mineral nitrogen (nitrate and ammonium) concentrations in soil were quantified.

2. Literature Review

2.1 Boreal Region

The boreal region is the biggest terrestrial biome in the world forming a distinct band of vegetation. It comprises nearly 14.5% of the Earth's terrestrial ecosystems (Berner *et al.,* 2015). The boreal region (Figure 1) is a circumpolar range throughout the entire Northern Hemisphere from Alaska and Canada; across the Atlantic to Norway, Sweden and Finland, through vast areas of Russia, and into parts of China, Mongolia, the Korean Peninsula and northern Japan (Miyawaki and Tüxen, 1977). It is characterized by harsh winters with mean temperatures below freezing for up to six months where much of boreal life comes to a halt (Runesson, 2011). Meanwhile, summers in boreal forests are characterized by long, warm periods of sunlight which leads to swift growth in boreal forests (Rumney, 1968 and Larsen, 1980). In Finland, the biogeography is composed of extensive boreal forests, mires and peatlands.
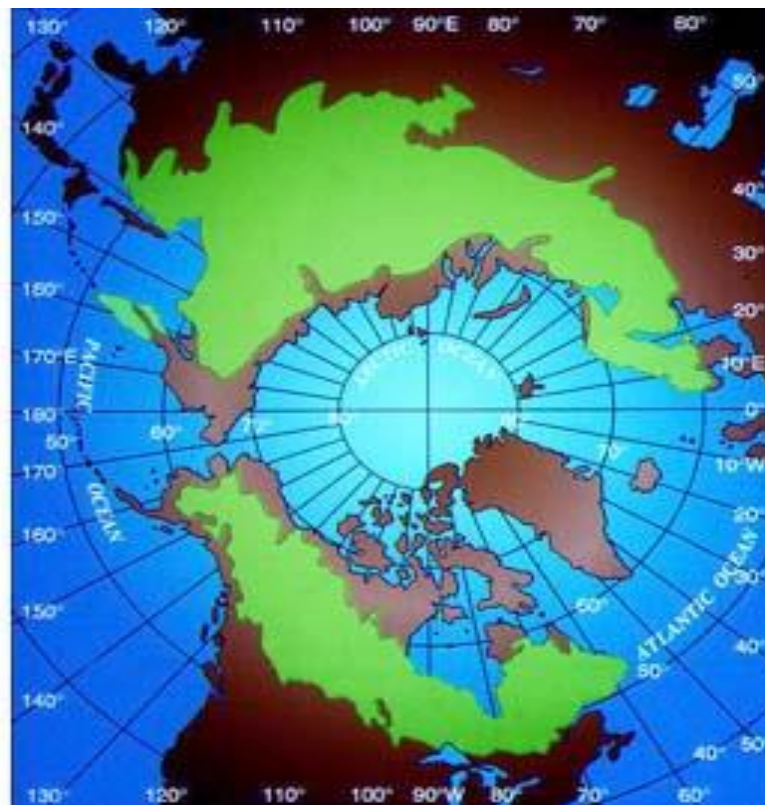

Figure 1: Circumpolar Range of Boreal Region (**indicated in green**) (Hare and Ritchie, 1972)

The boreal forests that dominate Finland are characterized by *Pinus Silvestris* (Pine), *Picea abies* (Spruce) and *Betula pendula* and *B. Pubescens* (Birch) which together encompass 73% of Finnish land surface (METLA, 2013). The boreal forests are dominated by nutrient-poor

soils, with slow nutrient cycling. This is because of limited availability of nitrogen due to low mineralization rate and the high demand for available nitrogen for growth (Näsholm *et al.,* 1998). Although the growth of boreal forests is generally N-limited, wide ranges in nutrient availability and interaction between elements have been observed (Vitousek *et al.,* 1997). Hence, it is important to understand nitrogen cycling processes thoroughly.

Peatlands are defined as a subset of wetlands containing peat-covered terrain having a minimum depth of peat of 30cm (Joosten and Clarke, 2002). Peat is a soil type, consisting of partially decomposed organic matter accumulating under water-saturated conditions due to incomplete decomposition. Peat forms as a result of anoxic condition, low decomposability of plant material and other factors. This accumulation of peat results in more acidic and nutrient-poor conditions because the influence of cations derived from mineral soil decreases over time (Vitt *et al.,* 2005)

There are two main types of peatlands, minerotrophic and ombrotrophic. In minerotrophic peatlands, also known as fens, water in the peat surface is connected with, or has passed over or through surrounding mineral soil (Rydin and Jeglum, 2013). Fens have slightly acidic to slightly basic mineral-rich waters from groundwater discharge and seepage. They may be flat or gently sloping and are dominated by sedges, grasses, and non-sphagnum mosses. Fens can be classified in poor fen, intermediate-moderately rich fen and extremely rich fen based on their vegetation composition, local geology, chemical and nutrient status of the water supply among others. Poor fens have a pH of 4-5.5, while intermediate-moderately rich fens have a pH of 5-7, and rich fens have a pH of 6.8-8. On the other hand, ombrotrophic peatlands, also known as bogs, are isolated from mineral soil water and only receive their water and nutrients from precipitation. Bogs are nutrient poor and usually have a pH range of 3.5-4.2. They are dominated by the species of moss genus *Sphagnum* and heath shrubs (Rydin and Jeglum, 2013). Fens have a broader range of nutrient and pH variation and therefore more abiotic and biotic variation than bogs (Rydin and Jeglum, 2013).

In Finland, peatlands typically contain large amounts of carbon (C) and nitrogen (N) in organic matter in soil and vegetation (Gorham, 1991). Hence, they play an important role in the global C and N cycles particularly with processes related to producing and consuming greenhouse gases (GHG) (Khalil, 1999).

2.2 Processes that result in production and consumption of N_2O in soils

N_2O emissions from the soil can be largely attributed to bacterial and fungal respiratory processes in soils, namely denitrification and nitrification (Lassey and Harvey, 2007). About 70% of global N_2O emission have been attributed to microbial processes of nitrification and denitrification in soils (Braker and Conrad, 2011; Syakila and Kroeze, 2011). However, it is impossible to solely ascribe the sources of N_2O to microbial nitrification and denitrification because several biogeochemical pathways and abiotic mechanism produce or consume N_2O (Butterbach-Bahl *et al.,* 2013). Overall, soils are a complex heterogeneous matrix where N_2O production can occur through different processes simultaneously in different microsites of the same soil (Butterbach-Bahl *et al.,* 2013).

During the process of nitrification, there is microbial oxidation of ammonium (NH_4^+) to nitrate (NO_3^-) by chemoautotrophic nitrifying bacteria in aerobic conditions. This process is affected by numerous factors including NH_4^+, nitrite (NO_2^-), phosphate (PO_4^{3-}) concentrations, oxygen (O_2), pH, temperature, water potential and possible allelopathic compounds (Haynes, 1975). In aerobic autotrophic nitrification, there is stepwise oxidation of ammonia (NH_3) to hydroxylamine (NH_2OH) and subsequently to NO_2^- and NO_3^- (Kowalchuk and Stephen, 2001). N_2O production from these processes can result because of enzymatic decompositions of the substrates NH_2OH and NO_2^- (Arp and Stein, 2003; Baggs, 2011). Biosynthesis of the intermediate NO_2^- as substrate for soil N_2O production can occur during the process of nitrate ammonification and nitrifier denitrification (Poth and Focht, 1985; Baggs, 2011; Wrage *et al.,* 2001). Chemodenitrification also contributes to N_2O production when nitrite ion is chemically reduced to N_2O by compounds such as amines present in soil organic matter, and by inorganic ions (Fe^{2+}, Cu^{2+}), particularly in subsoils (Granli and Bøckman, 1994).

Denitrification is a form of anaerobic respiration whereby the sequential dissimilatory reduction of NO_3^- to NO_2^-, nitric oxide (NO), N_2O and dinitrogen (N_2) is catalyzed by nitrate reductases (NaR or Nap), nitrite reductase (NiR), nitric oxide reductase (NoR) and nitrous oxide reductase (N_2OR) respectively (Zumft, 1997). Denitrification is traditionally considered to require completely anoxic conditions because O_2 is seen as a competing electron acceptor for NO_3^- respiration and key enzymes of the denitrification pathways are inhibited by relatively small amounts of O_2 (Zumft, 1997; Shapleigh, 2006). It is dominated by heterotrophic bacteria and fungi with the process considered to be the main N_2O producing process (Skiba and Ball, 2002). It is mainly affected by the presence of bacteria possessing

metabolic capacity; the availability of suitable reductants such as organic carbon; the restriction of O_2 availability and the availability of N oxides such as NO_3^-, NO_2^-, NO or N_2O. Closely coupled with denitrification, microbial co-denitrification can utilize N from organic co-substrates for increased N_2O production (Kim *et al.,* 2012).

In recent greenhouse gas models, the final step of denitrification ($N_2O \rightarrow N_2$) has been assumed to be the major controlling mechanism reducing N_2O fluxes to the atmosphere (Sanford *et al.,* 2012). Most N_2O is produced when there is incomplete denitrification in suboxic and anoxic conditions whereby anaerobic microbial bacteria reduce nitrate to nitrite and subsequently to gaseous nitrogen compounds nitric oxide (NO) and N_2O. In table 1, adapted from Firestone and Davidson (1989), the factors that affect the proportion of N_2O produced relative to N_2 in denitrifying cells and soils are shown.

Table 1: Factors affecting the proportion of N_2O and N_2 produced during denitrification as adapted from Firestone and Davidson (1989)

Factor	Will increase N_2O/N_2
$[NO_3^-]$ or $[NO_2^-]$	Increasing oxidant
O_2	Increasing O_2
Carbon	Decreasing C availability
pH	Decreasing pH
$[H_2S]$	Increasing sulphide
Temperature	Decreasing temperature
Enzyme status	Low N_2O reductase activity

For N_2O to be produced, the availability of oxidant (N-oxide) has to exceed the availability of reductant (organic carbon) resulting in higher proportion of N_2O to N_2 (Table 1). Moreover, decreasing temperature and lower N_2O reductase activity also results in the same phenomenon (Table 1). The ratio of N_2O/NO_3^- has been observed to increase with lowering of oxygen partial pressure and increasing acidity (Goreau *et al.,* 1980; Martikainen, 1985).

Although denitrification is traditionally believed to be inhibited by O_2, there is substantial evidence for co-respiration or co-metabolism of O_2 and NO_3^- by different genera of cultured bacteria within the *Alpha-*, *Beta-* and *Gammaproteobacteria* under both alternating oxic/anoxic phases and fully aerated conditions (Robertson and Kuenen, 1984; Lloyd, *et al.,*

1987; Bonin and Gilewicz, 1991; Baumann *et al.,* 1996). In bacterial cultures, N_2O and N_2 are produced when O_2 concentrations range from near anoxic to c.90% air saturation (Robertson and Kuenen, 1984; Körner and Zumft, 1989). N_2O produced during nitrification and denitrification contains some differences. N_2O produced during nitrification is more depleted in ^{15}N and ^{18}O relative to substrates while N_2O produced during denitrification is less depleted (Butterbach-Bahl *et al.,* 2013).

Nitrogen oxides can be reduced by bacteria, archaea and fungi groups (Philippot *et al.,* 2007; Hayatsu *et al.,* 2008). These micro-organisms can be classified as complete denitrifiers and non-denitrifying N_2O reducers. Complete denitrifiers are facultative aerobes which are ecophysiologically homogenous with the potential to switch from oxidative respiration to denitrification when soil conditions are anoxic (Sanford *et al.,* 2012). Non-denitrifying N_2O reducers are denitrifiers with genes for N_2O reduction only with atypical NosZ gene which encodes for N_2O reductase (N_2OR) and are more diverse and tolerate a wider range of conditions such as anoxic, microaerophilic, oxic, psychrophilic, piezophilic, thermophilic, and halophilic (Sanford *et al.,* 2012). All denitrifiers in natural environments are assumed to be capable of complete denitrification resulting in N_2 as the final product (Firestone and Davidson, 1989). Bacteria and archaea containing the N_2OR also have the potential to convert N_2O to N_2 (Jones *et al.,* 2011).

2.3 Atmospheric N_2O fluxes from boreal soils

Microbial activity and abiotic environmental factors are important prerequisites for the efficiency of the N cycle in most terrestrial ecosystems, especially in N-limited ecosystems. N_2O fluxes from boreal forests are generally far less than those of tropical ecosystems and have perceived capabilities to have a much greater potential in these regions to act as a sink for N_2O (Kroeze *et al.,* 2007). In a study conducted in eastern Finland on afforested organic agricultural soils and organic agricultural soils in active use, the measured N_2O flux values were in the range of 100-2500μg N_2O-N m^{-2} h^{-1} with intermittent uptakes at sites with high water table level (Maljanen *et al.,* 2012). This is probably due to the fact that the boreal forests are in regions of low temperatures and produce little N_2O with emission rates less than 0.10 Tg N per year (Zhuang *et al.,* 2012). However, in boreal soils from southern Russia and Canada N_2O emissions have been found to be relatively high at rates above 0.20 kg N_2O-N ha^{-1} yr^{-1} and this is primarily due to high soil organic matter content and moist climate (Zhuang *et al.,* 2012).

Some studies have also shown N_2O uptake in soils in the boreal region. Net N_2O uptakes of up to $-77\mu g$ N_2O-N m^{-2} h^{-1} has been measured from drained and abandoned, drained and afforested and active peat extractions in Finland. This uptake has been observed when soil pH and mean soil C:N ratio ranged between 3.9-5.9 and 17.6-24.2 respectively; with higher N_2O uptake in soils with highly saturated water tables (Maljanen *et al.,* 2012). This is because N-limited and high moisture ecosystems such as boreal mires and pristine boreal peatlands support N_2O uptake (Chapuis-Lardy *et al.,* 2007). It is however important to note that there are huge uncertainties involving boreal N_2O fluxes due to high spatial and temporal variability (Ambus *et al.,* 2006; Kellmann and Kavanaugh, 2008).

The highest spatial variability in soil N_2O fluxes is always found in areas known as hot spots (Savage *et al.,* 2014). "Hot spots" are areas that display disproportionately high reaction rates of biogeochemical activity relative to the surrounding area (McClain *et al.,* 2003). Hot spots are often chemically limited because one reactant is unstable in the dominant biogeochemical environment, or because the reaction only proceeds under particular/specific conditions (for example, anoxia) (McClain *et al.,* 2003). Biogeochemical activity can be further categorized as "hot" in the temporal dimension also known as "hot moments" whereby there are periods of intense biogeochemical reactions for a short period of time relative to longer intervening time periods. Hot moments can be delineated at time scales ranging from instants to millennia (McClain *et al.,* 2003). For example, McClain *et al.,* (2003) reported that denitrification hot spots within unsaturated soil profiles become active during hot moments in unsaturated zones where hydrological flow paths are intermittent with strong seasonal variations.

Denitrification hot spots have been identified across spatial scales range from soil profiles to larger basins. In a soil profile of the scale 1-10m, denitrification hot spots have been observed to occur around patches of labile organic matter, for example, plant detritus or manure (Christensen *et al.,* 1990; Petersen *et al.,* 1996); at the anaerobic center of large soil aggregates (Seech and Beauchamp 1988; Højberg *et al.,* 1994); or in earthworm casts (Svensson *et al.,* 1986; Parkin and Berry 1994). These hot spots occur because of the movement of nitrate-rich water into an organic-reducing substrate (Parkin, 1987; Johnston *et al.,* 2001). Consequently denitrification hot spots require oxic-anoxic interfaces meeting a continual water flow, whereby oxic conditions are needed for nitrification to occur producing nitrate and anoxic conditions needed for denitrification and water acting as a transport medium (McClain *et al.,* 2003).

2.4 Factors affecting N_2O sinks

There are several physio-chemical factors affecting the potential for soils to serve as N_2O sinks. These include soil oxygen, soil pH, soil temperature, nutrient availability, soil moisture content and soil type.

2.4.1 Effects of soil oxygen on N_2O fluxes

Soil oxygen is important in controlling N turnover through the processes of nitrification and/or denitrification (Li *et al.,* 2000 and Schurgers *et al.,* 2006). Aerobic soils are generally sources of N_2O but small uptake rates have sometimes been observed in dry soils (Duxbury and Moiser, 1993) and wet grass pastures (Ryden 1981, 1983). In anaerobic soils, a higher possibility for complete denitrification from N_2O to N_2 occurs. Therefore anaerobic soils are generally assumed to be the main potential sinks for N_2O (Erich *et al.,* 1984). Despite this assumption, there has been no large, constant N_2O uptake observed in soils. For example, in flooded rice fields N_2O uptake has been largely dependent on the time of cropping seasons (Minani and Fukushi, 1984 and Parashar *et al.,* 1991). The observed small N_2O uptake in flooded rice fields can be attributed to slow rates of dissolution and transport of atmospheric N_2O in wet soils and hence prevent these sites from being a significant regulator of N_2O emissions (Mosier *et al.,* 1998).

In most surface soils, particularly in fertilized soils containing sufficient organic carbon and nitrate, the presence of oxygen most commonly limits denitrification (Firestone and Davidson, 1989). However, denitrification is extremely variable in space and time making it difficult to accurately ascertain rates of denitrification and N_2O exchange in the field (Lapitan *et al.,* 1999; Hofstra and Bouwman, 2005). N_2O was found to be produced and consumed in both aerobic and anaerobic conditions when oxygen concentrations in soils were primarily affected by soil moisture content (Martikainen *et al.,* 1993; Aerts and Ludwig, 1997). In oxygen-limited conditions, N_2O becomes the sole electron acceptor for denitrifying microbes thus altering N_2O fluxes in soils (Butterbach-Bahl *et al.,* 2013).

2.4.2 Effects of soil pH on N_2O fluxes

Under field conditions, denitrification rates have been found to be lower in acidic soils compared to neutral or slightly alkaline soils (Kroeze *et al.,* 2007). Simek and Cooper (2002) have described the amount of N_2O relative to N_2 formed during denitrification as pH dependent, mostly decreasing when pH increases. Hence, it can be deduced that the highest rates of N_2O reduction occurs in neutral to alkaline soils. This could be explained by N_2O reductase being hindered by a low pH (Richardson *et al.,* 2009).

Moreover, soil pH is a major factor in the process of nitrification either directly or through its effects on soil cation exchange capacity (Robertson, 1989). Optimum pH values for nitrification are noted to range from 6.5 to 8 (Simek and Copper, 2002). When nitrate production increases through nitrification in soils at neutral pH, it could lead to a limited reduction of atmospheric N_2O (Kroeze *et al.,* 2007). According to Maljanen *et al.,* (2012), sites with slightly higher mean soil pH (4.9) produced lower N_2O than soils with slightly lower mean soil pH (4.4)

2.4.3 Effect of soil temperature on N_2O fluxes in natural ecosystems

Soil temperature is a significant factor affecting denitrification which is one of the major processes affecting N_2O fluxes. Typical temperature coefficient (Q_{10}) values for denitrification range from 5 to 16 (Ryden, 1983) and the ratio of N_2O to N_2 produced decreases with increasing temperature (Firestone and Davidson, 1989). Sommerfeld *et al.,* (1993) observed that N_2O emissions were high during the warm growing season in a temperate climate, and this was attributed to high microbial activity. According to Butterbach-Bahl (2013), many microbial processes in the nitrogen cycle are temperature-sensitive. Hence when temperature increases, soil respiration increases leading to decreased soil oxygen concentrations and increased soil anaerobiosis. Therefore, it might be possible that the potential for N_2O uptake through denitrification increases at higher temperatures. However, there have also been some contradictory results showing high N_2O emissions at low soil temperatures during the winter and also during freezing and thawing events (Papen and Butterbach-Bahl, 1999; Maljanen *et al.,* 2007).

2.4.4 Effect of nutrient availability on N_2O fluxes

N-limitation in soils has been shown to cause intermittent N_2O uptake (Rosenkranz *et al.,* 2006; Vanitchung *et al.,* 2011). In an acacia reforestation site with *Acacia mangium* trees, emission of N_2O was observed possibly due to the N-fixation activity of the trees which provided extra nitrogen to the soil (Vanitchung *et al.,* 2011). Higher N_2O emissions have also be observed in nitrogen fertilized cropping systems and also in tree-based systems where litter fall provide additional nutrient availability (Palm *et al.,* 2002).

Atmospheric nitrogen deposition has been found to positively correlate with N_2O emissions (Butterbach-Bahl *et al.,* 1998 and Zechmeister-Boltenstern *et al.,* 2002). Even minor increases in deposition rates over extended durations may lead to changes in the nitrogen cycle of sensitive ecosystems and affects N_2O sink potentials in soils (Bobbink *et al.,* 1998; Bouwman *et al.,* 2002). This is largely because nitrogen enrichment of ecosystems partly

suppresses nitrogen limitation resulting in a reduced N_2O sink strength. According to Kroeze *et al.,* (2007) even when sink activity is seasonal, this reduction of sinks of N_2O may be important considering the extensive areas of nitrogen-affected ecosystems which may in turn lead to increasing atmospheric N_2O concentrations. Therefore, the total availability of nitrogen can also be a major driver of N_2O soil emissions (Butterbach-Bahl *et al.,* 2013).

High nitrate availability has been usually associated with high N_2O emissions (Maljanen *et al.,* 2012). In temperate regions, substrate accumulation in small water films in soils has also been known to result in high N_2O emissions (Papen and Butterbach-Bahl, 1999; Teepe *et al.,* 2000). Organic soils rich in C substrates have been found to be a significant source of N_2O emissions in boreal regions (Alm *et al.,* 2007). Blicher-Mathiesen and Hoffmann (1999) have noted that high nitrate concentrations commonly inhibit N_2O reductase activity which elucidates the strong correlation between nitrate availability and N_2O build-up observed in soils (Skiba *et al.,* 1998). This supports the observation of net uptake of N_2O in grassland and forest soils where nitrate concentrations are typically low (e.g. $<1\text{-}2$ µg NO_3^- B/g soil; Butterbach-Bahl *et al.,* 1997). Nitrate concentrations are predicted to be lowest in environments with limited use of N-fertilizers and/or high plant uptake of nitrogen and also where nitrification does not occur (e.g. lack of oxygen and ammonia) (Kroeze *et al.,* 2007). Hence, this results in limited amount of electron acceptors in these environments and N_2 will be predominantly evolved. Moreover, in soils with an absence of oxygen, adequate organic carbon is present to support N_2O reduction (International Plant Nutrition Institute, 2016).

2.4.5 Effects of soil moisture content

One critical factor that affects boreal N_2O fluxes is soil moisture content. This can be assessed differently e.g. as precipitation (Werner *et al.,* 2007), water-filled pore space (WFPS) (Davidson *et al.,* 2000) or water table level (WTL) (Maljanen *et al.,* 2012). At above 60% WFPS denitrification will occur because there is no absolute anaerobic situation and N_2O can be produced as a by-product but when WFPS percentages are higher, the anaerobic situation is more pronounced and the production of N_2O will decrease (Davidson *et al.,* 2000).

Studies in boreal ecosystems showed that raising the water level close to the soil surface is likely to reduce N_2O emissions with N_2O emissions possibly mitigated when WTL is lower than 70cm (Maljanen *et al.,* 2012). Thus, it can be deduced that soil moisture content is closely coupled with soil microbial activity which consequently affects nitrification and denitrification determining N_2O fluxes. High soil moisture content results in anoxic conditions which amplify N_2O production through denitrification (Vanitchung *et al.,* 2011).

Soil moisture content also exerts considerable influence on activities of soil microbes, delivery of electron donors (NH_4^+, DOC) and electron acceptors (O_2, NO_3^-) and in the diffusion of N trace gases from soils (Firestone and Davidson, 1989). It also regulates the availability of oxygen to soil microbes; and high water level in the soil enables high microbial N turnover rates ensuring that there are substrates available for soil microbes (Goldberg *et al.,* 2010; Butterbach-Bahl *et al.,* 2013).

In addition, WFPS might be linked with nitrate concentrations in affecting N_2O emissions. Limited nitrate conditions and higher WFPS stimulate denitrification enabling denitrifiers to utilize N_2O as an electron acceptor and inhibiting nitrification (Vanitchung *et al.,* 2011). Moreover, increased N substrates and easily degradable C availability have also been found to increase microbial N_2O emissions (Papen and Butterbach-Bahl, 1999).

2.4.6 Effect of soil type on N_2O fluxes

2.4.6.1 Soil Carbon-to-Nitrogen ratio (C:N ratio)

Soil carbon-to-nitrogen ratio (C:N ratio) determines the decomposability of soil organic matter which in turn has a critical impact on soil nitrogen availability. Relationships between soil C:N might play a key role in nitrous oxide emissions. Soil residues with lower C:N ratios have been observed to have a higher decomposition rate hence providing more dissolved organic carbon (DOC) and therefore increasing N_2O emissions (Huang *et al.,* 2004). Soil C:N ratio affects the mineralization of plant residues and consequently N_2O emissions (Aulakh *et al.,* 1991 and Németh *et al.,* 1996). Organic amendments to a well-aerated soil which decrease C:N ratios have been shown to increase N_2O emissions (Bremmer and Blackmer, 1981). Substrates with C:N ratios <20 have a higher decomposition rate resulting in ammonium release via mineralization increasing N_2O emissions. Substrates with ratios of 25-75 also decompose quickly but N mineralization is inhibited by increased microbial immobilization and protein complexation by polyphenols when the cells lyse. In a modelling study in Scandinavia and the Baltic States, soils with elevated C stocks had considerably higher N_2O emissions than ambient soils; with values of >0.75 kg N ha^{-1} being calculated (Kesik *et al.,* 2005). High N_2O emissions were also predicted via modelling for soils with high amounts of organic carbon content in the forest floor in Southwest Finland and in the Northern parts of Sweden (1.0 to 1.8 kg N ha^{-1} yr^{-1}) (Kesik *et al.,* 2005).

2.4.6.2 N_2O emissions from Organic vs mineral soil types

N_2O emissions from mineral soils in boreal forests are in the range of 0.1 and 0.3 kg N ha-1 yr^{-1} (Brumme *et al.,* 2005), while forest soils, rich in organic matter, emitted N_2O in the range of 1.0 to 10.0 kg N ha-1 yr^{-1} (Maljanen *et al.,* 2001, 2003; von Arnold *et al.,* 2005). N_2O emissions from peat soils (organic soil) that have been used for agriculture prior to forestation emitted the most N_2O (Maljanen *et al.,* 2003). Therefore, it can be concluded that large N_2O emissions occur as a result of drainage and cultivation of organic soils due to enhanced mineralization of old, N-rich organic matter (Guthrie and Duxbury, 1978; Martikainen *et al.,* 1996; Velthof *et al.,* 1996). Nutrient poor organic forests were noted to emit negligible amounts of N_2O (Regina *et al.,* 1996). However, drained organic soils with no fertilizer additions were noted to show much higher emissions of N_2O of up to 100 kg N- N_2O ha-1 yr^{-1} than mineral soils (Regina *et al.,* 1996).

2.4.6.3 Natural vs altered ecosystems

Human activities like agriculture affect the nitrogen cycle by increasing N_2O emissions. The production of synthetic nitrogen fertilizers and cultivation of N_2-fixing plants play a key role in steadily increasing nitrogen into the biosphere thus altering the nitrogen cycle (Vitousek *et al.,* 1997). Nitrogen fertilizers that contain ammonium and nitrate have been known to elevate the emissions of N_2O immediately after addition (Eichner, 1990, Chang *et al.,* 1998). Drainage along with other agricultural practices such as ploughing, liming and fertilization contribute to higher pH values in soil and stimulate decomposition of N-rich organic matter (Maljanen *et al.,* 2012) and nitrogen mineralization (Freeman *et al.,* 1996).

It is known that emissions of N_2O from pristine peatlands are negligible but in drained peatlands there is an increase in N mineralization leading to greater emissions of N_2O due to nitrification and denitrification (Regina *et al.,* 2004). In Nordic countries, agricultural activities conducted in organic soils resulted in N_2O emissions on average four times higher than those from mineral soils, indicating that N_2O derived from soil organic carbon decomposition dominates overall fluxes (Mu *et al.,* 2014). Moreover, in organic soils the variability of soil C/N ratio may be one of the dominant factors determining N_2O emissions in organic soils (Mu *et al.,* 2014).

Several studies have noted that clayey and highly fertile soils result in higher N_2O emission (Matson and Vitousek, 1987; Verchot *et al.,* 1999). Forest soils are more inclined to provide acidic conditions which may select microorganisms to different processes according to their tolerance of pH ranges (Muñoz *et al.,* 2010). Acidic soil conditions of coniferous forests in

Western Europe contain heterotrophic bacteria and fungi promoting nitrification with the bacteria *Arthrobacter sp.* seemingly most highly adapted to initiate heterotrophic nitrification (Brierley and Wood, 2001).

In the forests of South-Central Chile, dominated by volcanic soils the N-cycle is extremely efficient resulting in low productions of N gases to the atmosphere due mainly to the physicochemical characteristics of these soils (Chorover, 2002; Godoy *et al.,* 2003). In pristine soils, N conservation can be attributed to consumption by microorganisms and vascular plants by net primary production (Muñoz *et al.,* 2010). There is a scarce production of nitrate in these soils resulting in nitrification requiring an extra-consumption of energy at the ecosystem level (Huygens *et al.,* 2008). Hence, this results in an increased amounts of N immobilized by microbial action and adsorption of inorganic forms of N onto clay colloid surfaces (Bengtsson and Bergwall, 2000; Huygens *et al.,* 2008). It has also been noted that land use change from native forest to forest plantations and grassland remarkably increased N mineralization and nitrification in soils of New Zealand (Parfitt *et al.,* 2003).

2.4.7 Hole in the pipe model

Firestone and Davidson (1989) have proposed a model known as "hole-in-the-pipe" (HIP) which explains how microbiological and ecological factors affected soil emission of NO and N_2O fluxes. Several studies have indicated that nitrogen fertilization encouraged production of one or both gases (Williams *et al.,* 1992). On the contrary, in unfertilized soils net nitrogen mineralization and net nitrification have been found to positively correlate with N_2O emissions (Robertson and Tiedje, 1984, Matson and Vitousek, 1987). In the HIP model, the total production of NO and N_2O is assumed to be directly linked to the availability of nitrogen in the soil. The HIP model also attempts to link soil water content to the ratios of N_2O:NO emissions as a function of soil water content (Davidson *et al.,* 2000).

The HIP model is expressed by leaky pipes representing two major processes nitrification and denitrification. The rate of flow of nitrogen through these pipes is comparable to the rates of nitrification and denitrification and shows nitrogen cycling through the ecosystem (Davidson *et al.,* 2000). "Holes" in the pipe represent trace gases of NO and N_2O leaking out of the pipe and the sizes of these holes correspond primarily to soil water content (Davidson *et al.,* 2000).

3. Materials and Methods

3.1 Study site

The first study site was located in eastern Finland in Heinävesi (62º26'N, 28º 38'E) which is an upland forest of Myrtillus type (MT) (Hotanen *et al.,* 2008). The main tree species located at the site are spruce (*Picea abies*), pine (*Pinus sylvestris*) and birch (*Betula pendula* and *Betula pubescens*) with a stand age of 80 years and the dominant soil type in this area can be characterized by a humus layer and mineral soil.

The second study site was located in eastern Finland in Salmisuo, Ilomantsi (62°47′N, 30°56′E) in a low-sedge *Sphagnum papillosum* pine fen. The Salmisuo peatland complex is an eccentric bog with some minerotrophic strips. Plant species indicate average water tables and nutritional statuses of the mire types and microsites. Flarks are the wettest microsites, with an average water table depth from 0 to −5 cm while lawns have an average water level depth 5–20 cm below the peat surface. Hummocks rise above their surroundings and have an average water table over 20 cm below the peat surface. The vegetation in Salmisuo enabled all three distinctive forms - hummocks, lawns and flarks, to be recognized in the low-sedge S. *papillosum* pine fen.

The proportion of different microsites were determined by evaluating their coverage in 20 vegetation squares (1×1 m, along a transect with 5-m intervals). The most typical hummock species was *Sphagnum fuscum* (Schimp.) Klinggr., with S. *angustifolium* (Russow) C. Jens. and *Eriophorum vaginatum* L. also found in the lower parts of the hummocks. The lawn moss layer was dominated by S. *angustifolium* and S. *balticum* (Russow) C. Jens., accompanied by some S. *magellanicum* Brid. and S. *papillosum* Lindb. The field layer in the lawns consisted mainly of E. *vaginatum* with *Andromeda polifolia* L., *Vaccinium oxycoccos* L. and Carex *pauciflora Light*. The major mosses in the flarks were S. *majus* (Russow) C. Jens., S. *balticum* and S. *angustifolium*, and the only vascular plants on flarks were *Scheuchzeria palustris* L. and E. *vaginatum*. Between the mineral soil and the low-sedge S. *papillosum* pine fen there was a narrow tall-sedge fen lag consisting of a lawn vegetation in which the major moss species was S. *angustifolium* and the field layer was dominated by C. *rostrata* Stokes. In this study, upper peat layers of 0-10cm and lower peat layers of 10-20cm were sampled.

Organic and mineral soil at the Heinävesi site (Fig. 2) and profiles of 0-10cm depth and 10-20cm depth at the Salmisuo site were collected on the 2nd of June 2014 and the 8th of July

2014 respectively. Soils were collected and sealed in ziplock bags and refrigerated in cold room of temperatures up to 4°C until physical and chemical analysis of soils were conducted. Soils were then homogenized manually by picking out roots and debris. Soil pH was measured from soil-water suspension (1:2-3 v/v) by using a pH meter (WTW pH-Electrode SenTix® 81, Germany) while electrical conductivity was measured using an EC meter (WTW TetraCon® 325, Germany). Soil organic matter was also analyzed for the respective soil layers utilizing the weight loss on ignition method (Reeuwijik, 2002). Samples were first oven-dried at 105°C in a muffle furnace and thereafter the samples were burnt at 450°C (Reeuwijik, 2002). The weight loss that occurred at this temperature was then correlated to the soil moisture content.

Figure 2: Organic and mineral layers of forest soil at Heinävesi site

3.2 Experimental set-up

3.2.1 Effect of oxygen on N_2O, NH_4^+ and NO_3^- concentrations

The experiment was carried out in flask-microcosms (Figure 3) in oxic and anoxic conditions. Sacrificial sampling was carried out during the experiment. To ensure that there was no N_2O consuming activity already present, pre-incubation was done for 5 days whereby bottles were degassed and flushed with 100% helium. This degassing and flushing was done twice to ensure that there was complete removal of accumulated N_2O during the 5 days. After the pre-incubation period, N_2O consumption was activated in the respective soil layers (mineral/organic; upper 0-10cm/lower 10-20cm) of the Heinävesi and Salmisuo soils with

addition of 15-N N_2O (98% atm, 1500 ppb) to the headspace of the bottles. The respective soil layers of each soil site were then subjected to oxic and anoxic conditions. Oxic conditions were achieved by adding 21% volume of O_2 to the headspace of the bottles while anoxic conditions did not have any additions of O_2. Sampling of nitrous oxide gas, nitrate and ammonium was then done at various time points for each site (0, 3, 12, 48, 72, 168 hours for Heinävesi and 0, 3, 24, 48, 72, 168 hours for Salmisuo). There were five replicates for each treatment (anoxic/oxic conditions) and incubation experiments were conducted at room temperatures of 22°C.

Figure 3: Flask-microcosm experiment (Peat samples)

N_2O concentrations were then determined by Gas Chromatography analysis (Hewlett Packard 5890 Series II, U.S.A). Ammonium-N (NH_4^+-N) and nitrate-N (NO_3^-N) were extracted from integrated soil samples with 0.5M K_2SO_4 (soil:K_2SO_4 1:2 v/v, 175 rev min-1, 1 hour) at various time points during the incubation period (168h). The extracts were filtered (Blauband 589[3] BlueRibbon filter paper) overnight and stored at -20°C until analyzed for NH_4^+ and NO_3^-. The concentration of NH_4^+ and NO_3^- in the extracts were measured by UV-Visible spectrophotometry (Philips PU 87501 UV/VIS) using microtiter plate format and following the protocol of Fawcett and Scott (1960) for NH_4^+ (630 nm) and Griess method for NO_3^- in aqueous solution (544 nm) (Miranda *et al.,*2001).

3.2.2 Effect of electron donors on mineral forest soil and lower 10-20cm peat soil on N_2O concentrations in oxic conditions

We also wanted to understand the effects of electron donors on the N_2O fluxes in mineral layer of Heinävesi and 10-20cm layer of Salmisuo sites. Five electron donors (succinate, butyrate, formate, propionate, lactate) were added to soils (Heinävesi: mineral; Salmisuo: 10-20cm depth). There were 3 replicates of each treatment and 3 controls for each site. Soils were added to bottles and pre-incubated in anoxic conditions for five days at 14°C and degassed and flushed with 100% Helium. N_2O consumption was activated by adding 15-N N_2O (98% atm, 1500 ppb) to the headspace of the bottles. Oxic conditions were then ensured in the bottles by adding 21% volume of O_2 to the headspace of bottles. This was then followed by the addition of 1mM of each electron donor to the respective bottles. Sampling of nitrous oxide was done at various time points (0, 3, 5, 24, 48, 72, and 168 hours) for both soils.

3.3 Statistical Analysis

Statistical analysis was performed with the IBM SPSS Statistics 21. The Shapiro-Wilk test of normality of variables was first carried out on all data to check for normality of data. As the data did not conform to normality, non-parametric tests (Kruskal-Wallis and Mann-Whitney U) were used to compare the data sets between sites over time.

4. Results

4.1 Soil properties of the study sites

Heinävesi forest soils have a considerably lower NH_4^+ content than Salmisuo peatland soils with the lowest NH_4^+ content observed in the mineral layer of Heinävesi soils and the highest NH_4^+ content in the 10-20cm layer of the Salmisuo soil (Table 2). The same trend was observed for the NO_3^- concentrations, with the Heinävesi soils having a notably lower NO_3^- content than the Salmisuo soils. The lowest NO_3^- content was observed for the mineral layer of the Heinävesi soil but the highest NO_3^- content was observed in the upper (0-10cm) layer of the Salmisuo soil. For the Salmisuo soil the upper layer (0-10cm) had nearly twice as much NO_3^- than the lower layer (10-20 cm) (Table 2).

Table 2: Background Soil properties of the study sites

Site	Soil Type	µg N-NH4/g_{dw}	µg N-NO3/g_{dw}	pH	Temperature	EC (µ S/Cm)	Soil Moisture (%)
Heinävesi	Mineral	2.78 ± 0.90	0.023 ± 0.003	4.89	21.1	15	8.31
Heinävesi	Organic	5.30 ± 0.57	0.033 ± 0.008	4.68	21.1	14	31.71
Salmisuo	0-10	26.78 ± 0.26	0.87 ± 0.53	4.25	22.3	20	88.59
Salmisuo	10-20	33.62 ± 1.61	0.46 ± 0.13	4.76	22.3	18	90.76

Both soils had very similar pH range and were largely acidic. The upper layer (0-10cm) of the Salmisuo soil was the most acidic, followed by the organic layer of the Heinävesi soil, the lower layer of Salmisuo soil (10-20cm) and the mineral layer of Heinävesi respectively (Table 2). In general, the Salmisuo peat soil had higher soil moisture content than the Heinävesi upland forest soils. Both peat layers (Salmisuo soil) have similar soil moisture contents but the organic upland forest soil (Heinävesi soil) had higher soil moisture content than the mineral layer (Table 2).

4.2 Effects of Anoxic conditions on N_2O fluxes in forest soils (Heinävesi)

When forest soils were exposed to anoxic conditions, N_2O concentrations decreased in both mineral and organic soil layers (Figure 4a). A pronounce decrease in N_2O concentrations in both soil layers was observed between 3 and 48 hours of incubation with the organic soil showing greater mean net consumption of N_2O (decreased in concentration) than the mineral soil throughout the experiment (Figure 4a, Table 3). The effect of anoxia on this decrease in N_2O was statistically significant during this time frame (Table 4). Anoxic conditions also significantly resulted in the near total consumption of the N_2O in the organic layer by the end of the experiment (Figure 4a). The forest mineral layer however, was only able to reduce half of the N_2O concentrations by the end of the experiment compared to initial N_2O fluxes (Figure 4a).

In addition, a statistically significant increase in ammonium concentrations in the organic layer of the forest soil was measured throughout the experiment under anoxic conditions (Figure 4b, Table 5). For the mineral layer, the effect of anoxia was not significant since the concentrations of ammonium remained constant and negligible throughout the experiment (Figure 4b, Table 5). On the other hand, nitrate concentrations in both soil layers (organic and mineral) showed a slightly decreasing trend throughout the experiment (Figure 4c), but it was not statistically significant (Table 6).
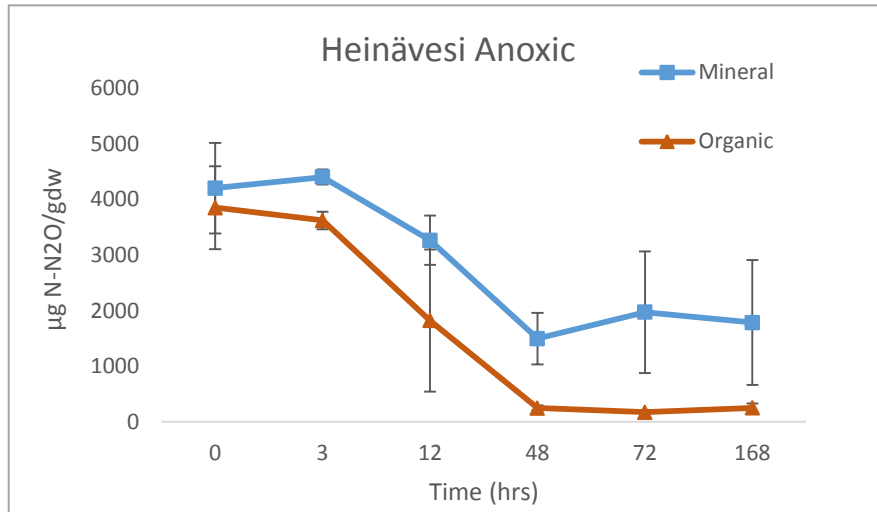
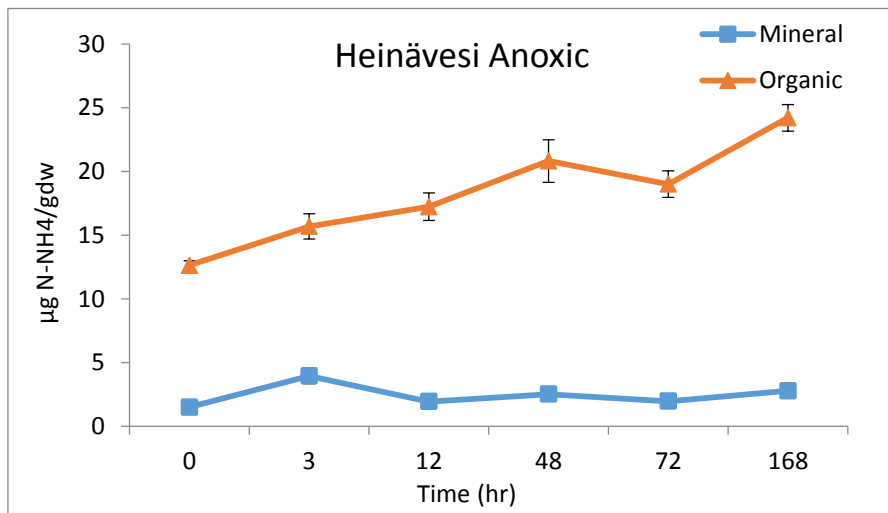Figure 4a: Effect of anoxic conditions on N_2O fluxes in Heinävesi soils

Figure 4b: Effect of anoxic conditions on NH_4^+ concentrations in Heinävesi soils
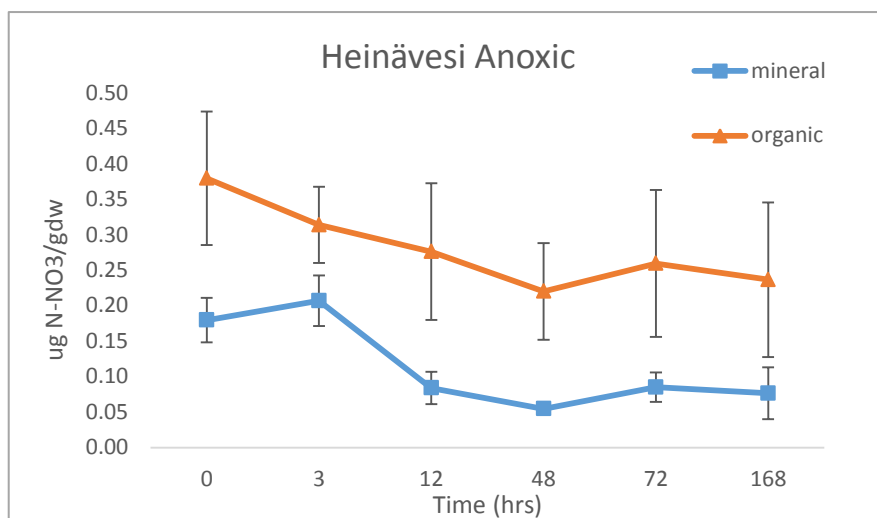

Figure 4c: Effect of anoxic conditions on NO_3^- concentrations in Heinävesi soils

Table 3: Net N_2O consumption/production potential in Heinävesi and Salmisuo soils
in anoxic/oxic treatment

Site	Soil Type	Anoxic Treatment	Oxic Treatment
Heinävesi	Mineral	-91.28	1.10
Heinävesi	Organic	-322.14	493.53
Salmisuo	0-10cm	-187.38	-40.61
Salmisuo	10-20cm	-319.06	-63.15

Table 4: Effect of oxygen on nitrous oxide (N_2O-N) concentrations (* indicating $p < 0.05$)

Site	Soil	Time	Significance
Heinavesi	Mineral	0	0.221
		3	0.016 *
		12	0.009 *
		48	0.009 *
		72	0.047 *
		168	0.117
	Organic	0	0.329
		3	0.009 *
		12	0.016 *
		48	0.009 *
		72	0.009 *
		168	0.009 *
Salmisuo	0-10cm	0	0.117
		3	0.016 *
		24	0.009 *
		48	0.009 *
		72	0.009 *
		168	0.009 *
	10-20cm	0	0.047 *
		3	0.009 *
		24	0.009 *
		48	0.009 *
		72	0.009 *
		168	0.009 *

Table 5: Effect of oxygen on ammonium (NH_4^+-N) concentrations (* indicating $p < 0.05$)

Site	Soil	Time	Significance
Heinavesi	Mineral	0	0.94
		3	0.465
		12	0.068
		48	0.602
		72	0.475
		168	0.076
	Organic	0	0.034 *
		3	0.028 *
		12	0.009 *
		48	0.009 *
		72	0.009 *
		168	0.009 *
Salmisuo	0-10cm	0	0.465
		3	0.602
		24	0.221
		48	0.602
		72	0.028
		168	0.034
	10-20cm	0	0.117
		3	0.699
		24	0.602
		48	0.624
		72	0.289
		168	0.053

Table 6: Effect of oxygen on nitrate (NO_3^--N) concentration (* indicating $p<0.05$)

Site	Soil	Time	Significance
Heinavesi	Mineral	0	0.086
		3	0.465
		12	0.142
		48	0.064
		72	0.297
		168	0.456
	Organic	0	0.329
		3	0.564
		12	0.076
		48	0.717
		72	0.784
		168	0.175
Salmisuo	0-10cm	0	0.251
		3	0.465
		24	0.245
		48	0.472
		72	0.327
		168	0.251
	10-20cm	0	0.754
		3	0.347
		24	0.602
		48	0.251
		72	0.347
		168	0.754

4.3 Effects of Oxic conditions on N_2O fluxes in forest soils (Heinävesi)

Under oxic conditions, the organic layer showed a gradual and steady increase in N_2O fluxes throughout the experiment with the presence of oxygen significantly increasing N_2O fluxes between the 3rd hour till the end of the experiment (Figure 5a, Table 4). In the mineral layer, N_2O concentrations oscillated around the average value of (5937.5 ± 677.0μg N-N2O/gdw) throughout the experiment with a statistically significant increase between the 3rd hour and 12th hour of incubation (Figure 5a, Table, 4). Organic soil showed a mean net production of N_2O while mineral soil had a negligible mean net production of N_2O (Table 3).

Ammonium concentrations in organic layer showed a generally declining trend. There were slight increases in ammonium concentrations in the organic layer between the 12th and 48th hour and also towards the end of the experiment (72 to 168 hours) (Figure 5b). Nitrate concentrations increased steadily throughout the experiment in organic layer but the effect of oxygen was not statistically significant (Figure 5b, Table 6). In mineral layer, there were no distinguishable changes in ammonium concentrations but slightly declining nitrate concentrations throughout the experiment (Figure 5b, 5c). There was also no statistically significant effect of oxygen on ammonium and nitrate concentrations in mineral layer during the experiment (Table 5, 6).
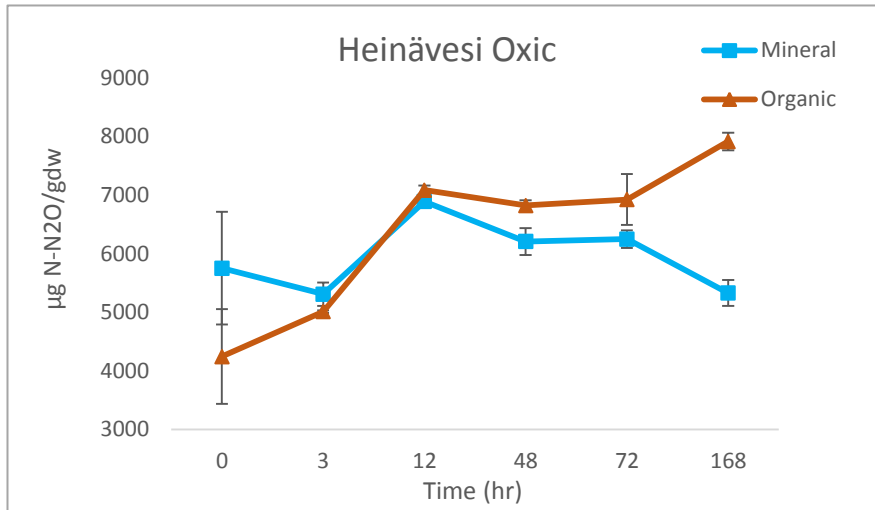
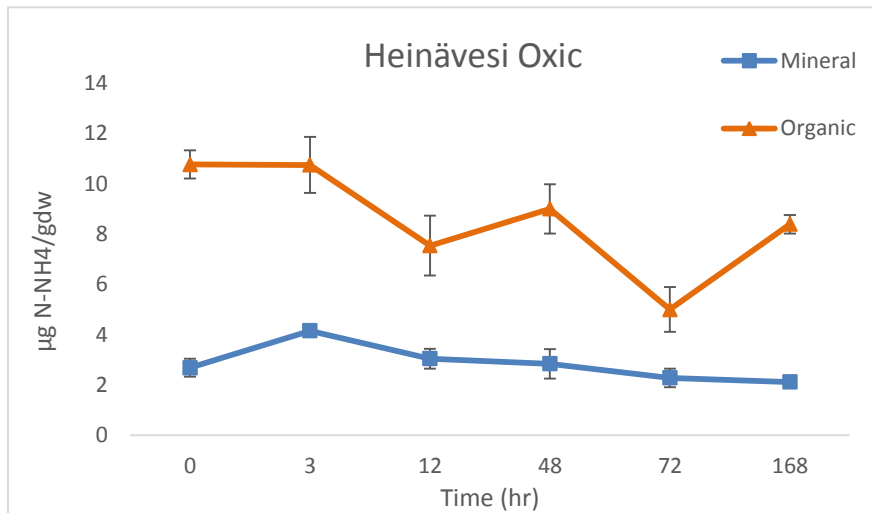Figure 5a: Effect of oxic conditions on N_2O fluxes in Heinävesi soils

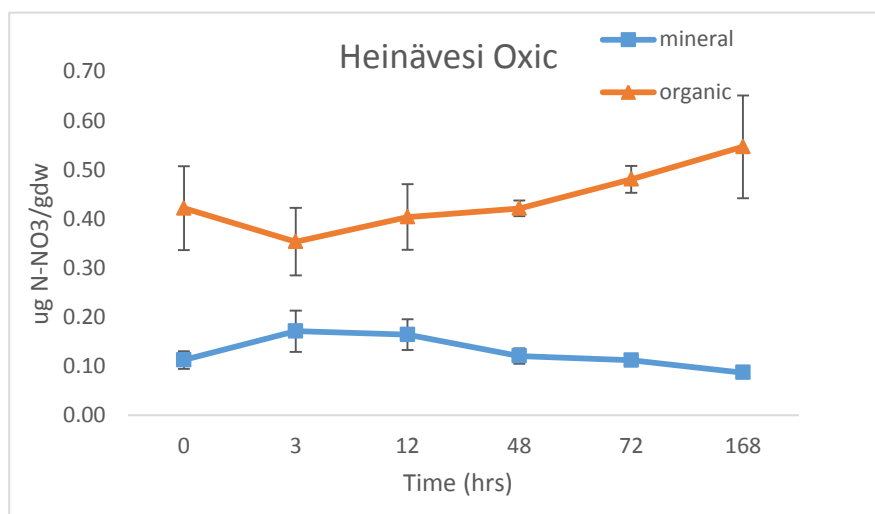Figure 5b: Effect of oxic conditions on NH_4^+ concentrations in Heinävesi soils

Figure 5c: Effect of oxic conditions on NO_3^- concentrations in Heinävesi soils

4.4 Effects of anoxic conditions on N_2O fluxes in peat (Salmisuo)

Peat soils exposed to anoxic conditions had declining N_2O concentrations throughout the experiment in both layers (Figure 6a). Similar to the forest soils in anoxic conditions (Figure 4a), there was a statistically significant decrease in N_2O fluxes in both peat layers between 3 and 72 hour of incubation (Table 4). The lower peat layer (10-20cm) exhibited greater net consumption of N_2O than the upper peat layer (0-10cm) (Figure 6a, Table 3). Additionally, anoxic conditions significantly resulted in the near total consumption of N_2O in the lower peat layer (10-20cm) by the end of the experiment (Figure 6a). In the upper peat layer (0-10cm) N_2O was not completely consumed but its concentration was roughly 7 times lower compared to the initial N_2O concentration (Figure 6a).

At anoxic conditions, ammonium concentrations in the upper peat layer (0-10cm) remained fairly steady but peaked at the 48th time point of incubation and then subsequently declined till the 72nd hour (Figure 6b). Nitrate concentrations in the upper peat layer (0-10cm) remained consistently low throughout the experiment (Figure 6c). In the lower peat layer (10-20cm), ammonium concentrations fluctuated between a minimum of 9.86µg $N-NH_4$/gdw and a maximum of 17.22µg $N-NH_4$/gdw throughout the experiment (Figure 6b). Nitrate concentrations in the bottom peat layer (10-20cm) also showed a rapid decline in the first 3 hours and then declined steadily to the end of the experiment (Figure 6c). The effect of anoxia on the concentrations of ammonium and nitrate were not statistically significant in either soil layer (Table 5, 6).
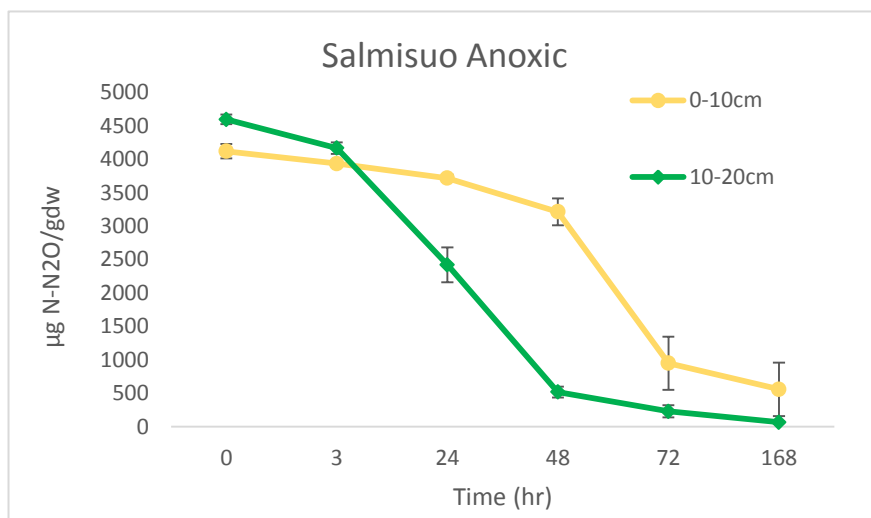
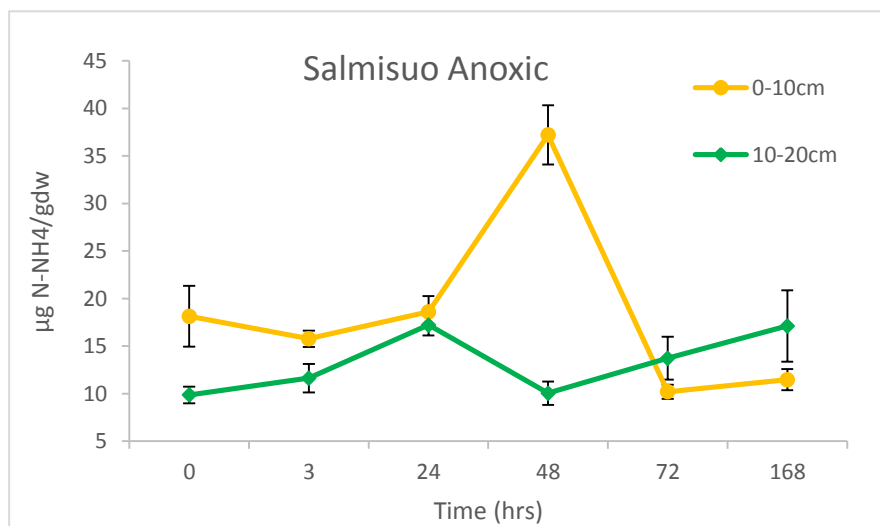Figure 6a: Effect of anoxic conditions on N_2O fluxes in Salmisuo peat

Figure 6b: Effect of anoxic conditions on NH_4^+ concentrations in Salmisuo peat

Figure 6c: Effect of anoxic conditions on NO_3^- concentrations in Salmisuo peat

4.5 Effects of Oxic conditions on N_2O concentrations in peat (Salmisuo)

In the upper peat layer (0-10cm) N_2O concentrations remained fairly steady in the first 72 hours of incubation while in the lower peat layer, (10-20cm) N_2O concentrations declined slowly between 0 and 72 hours. After the 72nd hour time point, N_2O concentrations in both peat layers declined abruptly till the end of the experiment (Figure 7a). The lower peat layer (10-20cm) averaged a higher consumption potential than the lower peat layer (0-10cm) (Table 3). The effect of oxic treatment on N_2O concentrations of both peat layers was statistically significant throughout the entire experiment with exception for the upper peat layer (0-10cm) at the beginning of the experiment (Table 4).

Ammonium concentrations in the lower peat layer (10-20cm) fluctuated between a minimum of 5.03μg $N-NH_4$/gdw and a maximum of 46.09μg $N-NH_4$/gdw throughout the experiment (Figure 7b) while nitrate concentrations increased for the first 24 hours and then decreased steadily to end of the incubation period (Figure 7c). In the upper peat layer (0-10cm), ammonium concentrations increased between 0 and 48 hours and then steadily decreased until the 72nd hour before remaining constant to the end of the experiment (Figure 7b). Nitrate concentrations in upper peat layer (0-10cm) remained fairly constant throughout the experiment (Figure 7c). The effect of oxygen on ammonium and nitrate concentrations was not statistically significant throughout the experiment for either peat layer (Table 5, 6).

Figure 7a: Effect of oxic conditions on N_2O fluxes in Salmisuo peat

Figure 7b: Effect of oxic conditions on NH_4^+ concentrations in Salmisuo peat
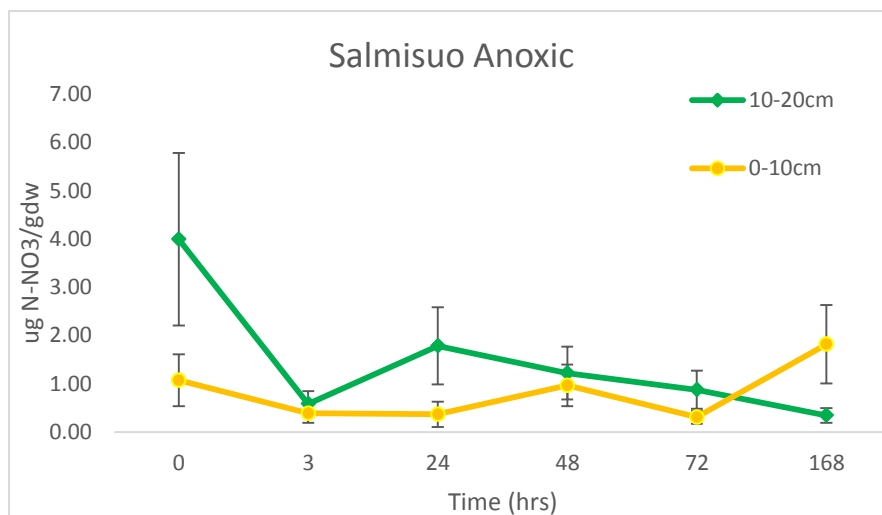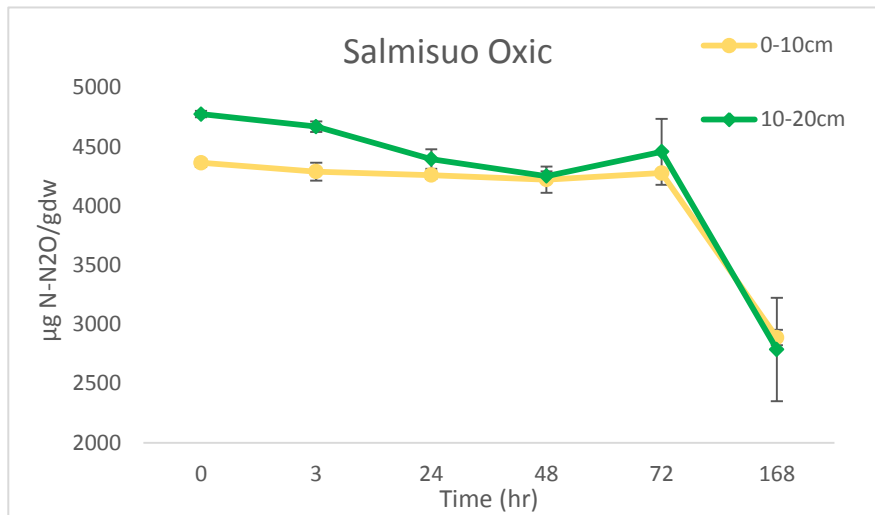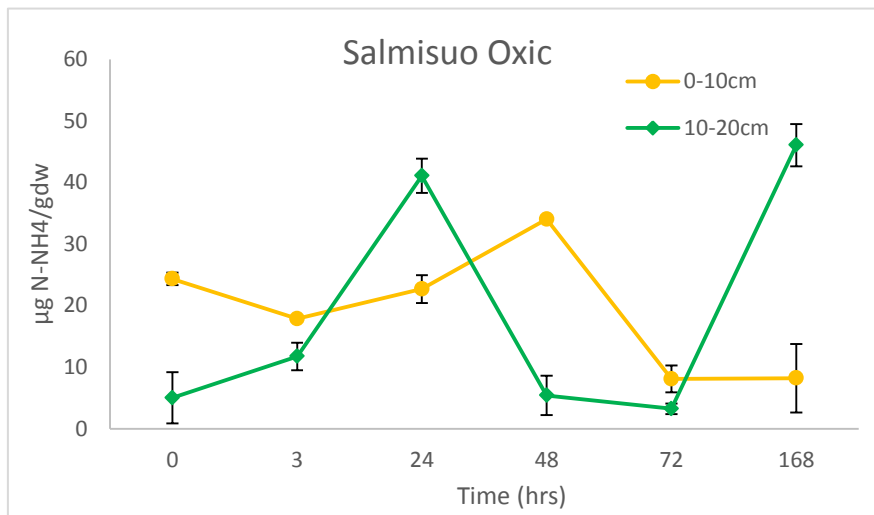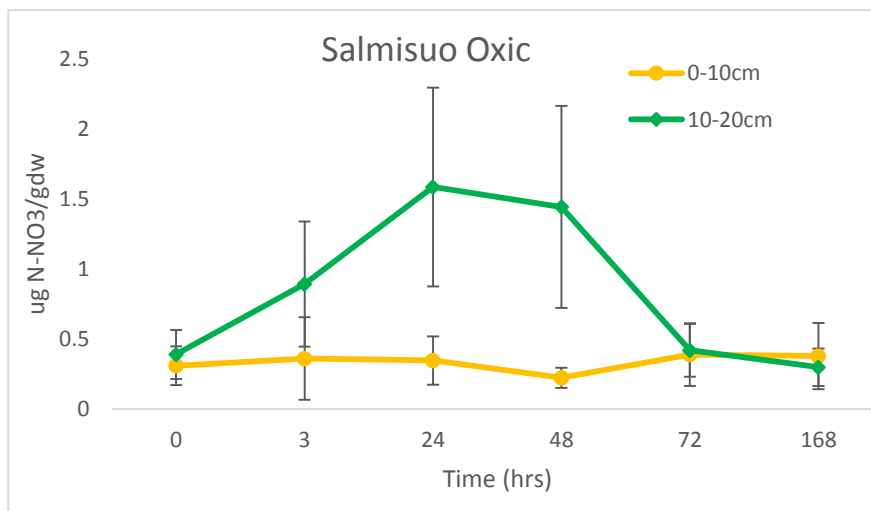

Figure 7c: Effect of oxic conditions on NO_3^+ concentrations in Salmisuo peat

4.6 Effects of different electron donors on N_2O fluxes from the mineral layer of Heinävesi forest and the lower peat layer (10-20cm) of Salmisuo

Different carbon sources resulted in different net N_2O flux rates in mineral (Heinävesi forest) and peat soils (Salmisuo peat soil) (Figures 8a and 8b). Both soils showed a decline in net N_2O flux rates in the incubations with propionate, indicating the preference for this carbon source during N_2O consumption processes. However, the peat soil (10-20cm layer) from Salmisuo soils exhibited a comparatively larger net N_2O consumption potential with propionate than the mineral forest soil from Heinävesi (see scale in the figures 8a and 8b). The incubations with butyrate and lactate exhibited net N_2O production potential in both soils but the peat soil (10-20cm layer) showed a larger net N_2O production potential. The addition of succinate resulted in a near negligible consumption of N_2O in mineral soils but a considerably greater net N_2O consumption potential in the peat soil (10-20cm layer). In the incubations with formate contrasting effects on net N_2O fluxes were observed with net N_2O production in mineral soil and net N_2O consumption in peat (10-20cm layer).
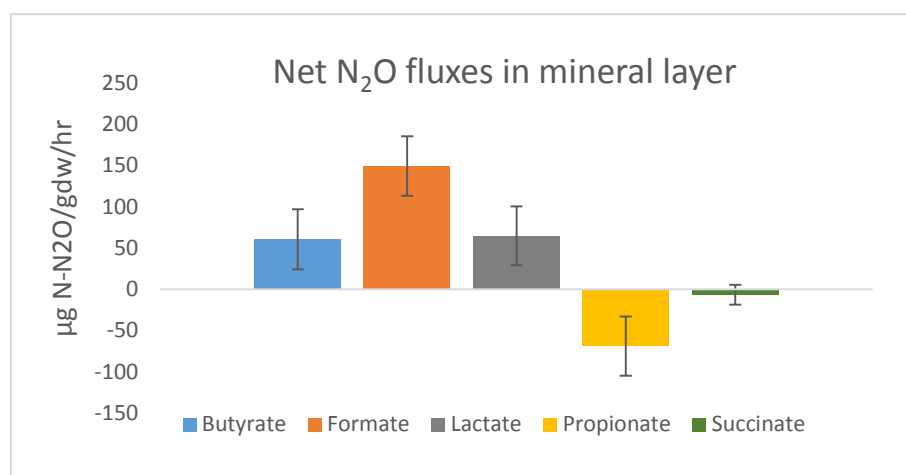

Figure 8a: Effect of different electron donors on net N_2O fluxes in mineral soil
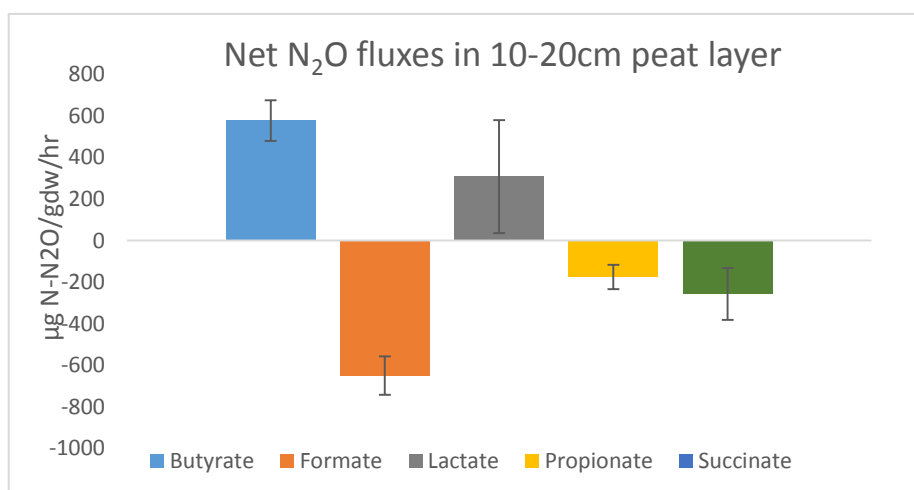

Figure 8b: Effect of different electron donors on net N_2O fluxes in 10-20cm peat

5. Discussion

5.1 Effects of anoxic conditions on N_2O fluxes in forest soils (Heinävesi)

Denitrification is a significant process regulating the nitrogen cycle. In prevailing anoxic conditions, like the conditions in our experiment, the dominant process noted to be able to consume N_2O is denitrification (Conrad, 2002). The results of the present study support these observations because there was a pronounce decline of N_2O concentrations within the first 48 hours of the soil incubation under anoxic conditions for both soil layers. Denitrification also produce N_2O as an intermediate (Butterbach-Bahl *et al.,* 2003; Voss *et al.,* 2013; Ward, 2013) and this probably explains the initial concentrations of N_2O in both soil layers. The differences observed in N_2O consumption rates between the soil layers could be attributed to the differences in physico-chemical properties of each soil layer.

In organic soils, anoxic conditions could have been intensified by the increasing ammonium concentrations during anaerobic decomposition of organic matter (Liikanen, 2003; Ward, 2013). The conditions for complete denitrification could then be built up in the organic soil, resulting in lower N_2O fluxes over time and led to nearly all the N_2O fluxes in the organic layer to be consumed by the end of the experiment. The decreasing nitrate concentrations over time observed in organic soils implies a lack of electron acceptors and supports the notion that N_2 was mainly produced instead of N_2O in these soils. Overall, the results suggested that the organic layer from the forest soil (Heinävesi soils) has a high potential as a sink for N_2O (Erich *et al.,* 1984).

In the mineral soils, there was also a net decrease in N_2O fluxes over time but the process of denitrification did not seem as acute as in the organic layer. This could be due to the soil properties of the mineral layer which contain relatively low ammonium and nitrate concentrations. Several studies have shown that nitrogen limited conditions support N_2O consumption (Flechard *et al.,* 2005; Rosenkranz *et al.,* 2006; Vanitchung *et al.,* 2011). Moreover relatively low nitrate concentrations, like the concentrations measured in the mineral layer from Heinävesi soils ($<1\mu g/gdw$ soil) throughout the experiment, have also been noted to support N_2O uptake because nitrate is a limiting factor affecting denitrification (Seitzinger, 1988, Butterbach-Bahl, 1997).

The net greater N_2O consumption potential observed in the organic soil layer compared to the mineral soil layer of Heinävesi forest soil has also been described previously for an N-limited Mediterranean pine forest study (Rosenkranz *et al.,* 2006). In that study, the net greater N_2O

consumption potential observed in the organic soil has been attributed to the higher soil moisture content of the organic compared to the mineral soil which intensifies the process of anaerobiosis (Kent *et al.,* 2002). This consequently increases the activity of complete denitrifiers containing NosZ gene resulting in a comparatively higher rate of N_2O consumption in these soils (Kang *et al.,* 2007; Wolf *et al.,* 2010).

5.2 Effects of oxic conditions on N_2O fluxes in forest soils (Heinävesi)

The process of coupled nitrification denitrification seems to be the dominant process for increasing N_2O fluxes (Butterbach-Bahl *et al.,* 2013) in organic soils whereby there is production of nitrate by nitrite oxidizers, which is immediately denitrified *in situ* by denitrifiers. Although denitrification is thought to be strictly anaerobic, it has been also observed to occur in well-aerated soils (Nielsen and MacDonald, 2013). Oxic treatment could have resulted in soil respiration which then could have led to anaerobic conditions in soil aggregates within the organic soil (Werner *et al.,* 2007). In aerobic soil aggregates, ammonium is oxidized to nitrate which then provides the substrate required for denitrifiers to reduce nitrate in the anaerobic soil aggregates (Kremen *et al.,* 2005). The occurrence of this process seemed to be supported by the generally declining trend in ammonium concentrations and consequent increase in nitrate concentrations and N_2O fluxes.

The process of aerobic denitrification is also known to play a pertinent role in environments not limited by oxygen (Patureau *et al.,* 2000) and can result in potential to produce N_2O (Lloyd, 1993). This process seems to be more prevalent in the mineral soils whereby nitrate seems to be consistent with steady concentrations of N_2O. Denitrifiers are facultative anaerobes and are also able to utilize oxygen when available (Hallin *et al.,* 2009) because aerobic respiration is more thermodynamically favourable. However, in aerobic conditions denitrification does not result in the final reduction of N_2O to N_2. N_2OR enzyme encoded by the NosZ gene in denitrifiers has the ability to reduce N_2O to N_2 (Sanford *et al.,* 2012) and when N_2OR enzyme is exposed to oxygen, the N_2OR enzyme becomes deactivated within a cell (Baggs *et al.,* 2009) resulting in N_2O not being reduced to N_2.

The net N_2O production potential was extremely high in organic soils compared to mineral soils which had nearly negligible net N_2O production. Organic soils could be richer in carbon substrates compared to the mineral soils which lead to comparatively higher net N_2O emissions in oxic conditions (Klemedtsson *et al.,* 2005; Alm *et al.,* 2007). Moreover, the comparatively higher availability of nitrogen substrates and possibly higher degradable

carbon availability in organic soils than mineral soils could have resulted in the higher net N_2O production in aerobic conditions (Papen and Butterbach-Bahl, 1999).

5.3 Effects of Anoxic conditions on N_2O fluxes in peat (Salmisuo)

According to earlier studies, wetlands have been regarded as sources of N_2O (Davidson, 1991). However, recent studies including the results from our study, have shown that acidic wetlands have also the potential to act as a sink for N_2O (Inubushi *et al.,* 2003; Hadi *et al.,* 2005; Chapuis-Lardy *et al.,* 2007; Goldberg *et al.,* 2010).

In anoxic conditions, N_2O seemed to be produced and consumed by microbial denitrification in both peat layers but at different rates. This suggestion is supported by the physico-chemical properties of the peat soil including the high soil moisture content in the peat layers, which favours rapid denitrification, and the extremely low nitrate concentrations throughout the experiment, indicative of low nitrification potentials in both peat layers. Low nitrate concentrations have also been observed in earlier studies and associated with low rates of N_2O emissions (Martin and Holding 1978; Rosswall and Granhall 1980; Verhoeven 1986; Rangeley & Knowles 1988). Moreover, peat layers are sources of organic carbon, and dissolved organic carbon in anaerobic conditions can support the reduction of N_2O because denitrifying bacteria obtain their energy from soluble carbon needed for denitrification (International Plant Nutrition Institute, 2016).

The lower (10-20cm) layer of peat resulted in a quicker net consumption of N_2O than the upper (0-10cm) layer in the first 48 hours. This suggests that the denitrification potential and the potential to consume N_2O were higher in the lower (10-20cm) peat layer. This could be due to the soil characteristics and resulting microbial community differences in the two distinct layers. Peat decomposition is primarily driven by microorganisms and subsequent soil microbial community composition plays a key role in ecosystem processes and nutrient cycling (Balser and Firestone, 2005; Bardget *et al.,* 2008; McGuire and Treseder, 2010). Differences in microbial communities have been noted to exist between peatland types (i.e. Bogs and Fens; Jaatinen *et al.,* 2007, Ausec *et al.,* 2009, Robroek *et al.,* 2014) and also among depths down the peat profiles (Morales *et al.,* 2006 and Jaatinen *et al.,* 2007). Microbial bacteria could be more predominant in the lower (10-20cm) peat layer than fungi and have been known to pre-dominate deeper more anoxic peat layers because they are able to utilize alternative electron acceptors (Killham and Prosser, 2007). This possible difference in

microbial community in the peat depths could have resulted in the different N_2O production/consumption rates.

The highly anoxic conditions of the peat soils combined with the anoxic treatment seemed to favour complete denitrification of N_2O to N_2 in the peat layers of Salmisuo soil (Klemedtsson *et al.,* 2005; Cuhel *et al.,* 2010; Braker and Conrad, 2011). Acid-tolerant denitrifiers in both peat layers likely reduced N_2O to N_2 (Palmer *et al.,* 2010, 2011) which resulted in a decrease in N_2O fluxes over time, but at different rates. Hence, it can be concluded that active denitrification-associated N_2O production and consumption occurs in acidic wetland (Goldberg *et al.,* 2008, 2010).

5.4 Effects of Oxic conditions on N_2O fluxes in peat (Salmisuo)

There were consistent N_2O fluxes emitted from both peat layers for the first 72 hours of the experiment. N_2O fluxes were generally higher in the lower (10-20cm) peat layer compared to the upper (0-10cm) peat layer. One reason could be that the oxic conditions could have resulted in ammonification and nitrification being dominant in the lower (10-20cm) peat layer resulting in N_2O fluxes due to inefficient denitrification (Williams and Wheatley, 1988; Regina *et al.,* 1996). This process seemed more dominant in the lower (10-20cm) peat layer compared to the upper (0-10cm) peat layer in the first 24 hours where both ammonium concentrations and nitrate concentrations were higher in the lower (10-20cm) peat layer than in the upper (0-10cm) peat layer. Throughout the experiment although ammonium concentrations fluctuated in the upper (0-10cm) peat layer, there was no discernable change in nitrate concentrations which were consistently low. The much higher ammonium concentrations in the upper (0-10cm) peat layer seem to have an inhibitory effect on the second step of nitrification whereby NO_2^- is oxidized to NO_3^- (Monaghan and Barraclough, 1992; Clough *et al.,* 2003; Ali *et al.,* 2013). Hence, this suggests that nitrification did not necessarily contribute to N_2O fluxes in the upper (0-10cm) peat layer and most N_2O fluxes in this layer probably occurred due to soil respiration.

The process of soil respiration by soil microbes in the peat layers could have contributed to CO_2 fluxes which in turn resulted in the consistent N_2O fluxes. In several studies, increased CO_2 fluxes have resulted in increased N_2O fluxes (Kammann *et al.,* 2008). Elevated CO_2 1.7 times higher than ambient CO_2 has been noted to increase N_2O by 2-3 times (Arnone and Bohlen, 1998; Baggs *et al.,* 2003a, b) compared to ambient CO_2 conditions. This effect of subsequent increase in N_2O due to elevated CO_2 has also been observed to occur in soils

(Kettunen *et al.,* 2006). When CO_2 fluxes are increased, there could have been an increase in carbon allocation in the peat layers which provide energy for denitrifiers resulting in N_2O fluxes. Denitrification can then be also simultaneously occurring in anoxic microsites of both peat layers leading to N_2O consumption.

After 72 hours, denitrification seemed to be occurring in both peat layers resulting in a decrease in N_2O fluxes. As oxygen is consumed by the soil microbes in respiration, nitrate is then used in the lower (10-20cm) peat layer as the next favourable electron acceptor resulting in the decrease in N_2O fluxes. In the upper (0-10cm) peat layer, the low nitrate concentrations result in nitrate becoming limiting and with denitrifiers using N_2O as electron acceptors reducing N_2O to N_2 (Vanitchung *et al.,* 2011). Differences in microbial communities did not seem to play a key role in the N_2O fluxes in oxic treatment compared to anoxic treatment.

5.5 Effect of different electron donors on net N_2O consumption/potential in oxic conditions in mineral forest soil and peat

In our study, we also wanted to understand how the addition of different electron donors affected the net nitrous oxide consumption potential in the mineral forest soil and the lower (10-20cm) peat in oxic conditions. The mineral soil produced negligible N_2O in upland boreal forest while the 10-20cm had the most consumption potential amongst the 4 soil layers studied. Electron donors are pertinent because it has been shown that different electron donors stimulate distinct microorganisms (Ginige *et al.,* 2004; Osaka *et al.,* 2008; Baytshtok *et al.,* 2009). Therefore, it can be assumed that differences in microbial community structure and their tolerance/susceptibility to transient stressors could give rise to different emissions on different C-sources (Lu *et al.,* 2011). In soils, most denitrifying bacteria are heterotrophic and require carbon sources for cell growth and nitrate reduction (Lee and Rittmann, 2003). Carbon sources also have the ability to influence oxygen consumption rates in soils by directly increasing aerobic heterotrophic respiration or indirectly supporting the anaerobic production of reductants such as Fe(II) which then reacts with oxygen (Schlesinger, 2005).

The effect of electron donors in mineral forest soil and the lower (10-20cm) peat on net N_2O consumption potential in our study were variable. The microbial community at the lower (10-20cm) layer of peat seemed to have a preference for formate>succinate>propionate as electron donors in catalyzing N_2O reduction; with formate being most efficient in increasing net consumption of N_2O. The addition of these carbon sources seem to amplify the net reduction of N_2O 3-14 times higher compare to the oxic treatment with no addition of carbon

sources. This indicates that even a small addition of carbon sources have the ability to produce anaerobic conditions in soils that are partially or completely saturated (Schlesinger, 2005); enhancing complete denitrification and N_2O consumption. In the mineral soil, only propionate seemed to amplify N_2O consumption only by 1.5 times. This indicates that addition of propionate in the mineral soil layers could have resulted in anaerobic sites occurring within the soil aggregates which have been observed in upland forest soils (Højberg *et al.,* 1994, Schlesinger, 2005). However, in our study, the effect of supplemental electron donors on N_2O fluxes was unexpectedly not significant.

6. Conclusion

This study shows that denitrification plays a crucial role in regulating N_2O fluxes in boreal soils. The effect of oxygen on N_2O fluxes is different in boreal soil types and their layers. In anaerobic conditions, both forest and peat soils are able to consume N_2O although at different rates. In aerobic conditions, N_2O fluxes of forest and peat reacted differently with organic soils showing markedly increase in N_2O concentrations throughout the experiment and peat soils consuming N_2O after 3 days. Hence, these results indicate that N_2O fluxes in northern boreal soils are sensitive to aerobic/anaerobic conditions and are strongly affected by nitrate concentrations. The results from the addition of carbon sources under aerobic conditions to the mineral layer of the forest soil (negligible N_2O production) and to the lower peat layer (10-20cm) (high potential for N2O consumption), implied that certain carbon sources can preferentially reduce N_2O fluxes.

However, it is difficult to elucidate exactly the processes that are occurring in the boreal soils. Additional stable isotope analysis and microbial community analysis is pivotal in determining exactly which processes and which microbes contributed to the N_2O fluxes. Moreover, N_2O fluxes can be markedly different depending on seasonal changes particularly in freeze-thaw cycles. It is important to note that this study was conducted primarily in the summer of 2014 and N_2O fluxes are generally known to be higher in the summers than during other seasons. Further studies of N_2O fluxes throughout the year and with these additional assessments could significantly improve our understanding of N_2O fluxes in northern boreal ecosystems.

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