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21.5.2021

University of Eastern Finland, Faculty of Science and Forestry
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Environmental Science
Karjalainen, Katri J.A.: Effects of insect outbreak on nitrous oxide emissions from subarctic ecosystems
Thesis, 92 pages, 3 appendices (21 pages)
Thesis instructors: Research Director Christina Biasi, Postdoctoral Researcher Lona van Delden, Assistant Professor Kristiina Karhu, Research Scientist Saara Lind
May 2021

Keywords: nitrous oxide, subarctic, mountain birch, autumnal moth, climate change

Greenhouse gases (GHGs) such as carbon dioxide (CO_2) and methane (CH_4) and their effects on global warming have been of great interest in Arctic research in recent years. However, one greenhouse gas, nitrous oxide (N_2O), has often been overlooked despite it being a gas almost 300 times stronger than CO_2. Only recently, N_2O hotspots have been identified on bare permafrost peatlands from Arctic tundra. Since then, the search for new N_2O sources has begun in the Arctic. Primary candidates are areas impacted by insect outbreak, because damaged or lack of plants can increase – similarly as in the bare peat areas- the emissions of N_2O due to absence of competition for nitrogen (N). Additionally, priming effects on soil N turnover due to increased abundance of dead plant biomass could trigger N_2O. Thus, insect outbreak where plants have been attacked, could lead to released N_2O, but studies are lacking so far.

The aim of the master's thesis, "Effects of insect outbreak on nitrous oxide emissions from sub-arctic ecosystems", was to find out whether N_2O fluxes from dead mountain birch trees differed to fluxes from living mountain birch trees and treeless tundra areas. The dead trees have been affected by an autumnal moth (Epirrita autumnata) outbreak 12 and 55 years ago. The autumnal moth has caused extensive damage to mountain birch trees in the Pulmankijärvi area in Utsjoki, Finnish Lapland, where the study was conducted in July 2019.

There were three field sites approximately two kilometres apart and 16 trees on each site from which fluxes were measured. The fluxes were measured using the chamber method and diffusion gradient method. Nitrous oxide fluxes were measured together with CH_4 fluxes to allow comparison of these two trace gases relevant for the climate.

Overall, the flux results were low. With N_2O, measured by the chamber method, treeless tundra had significantly higher fluxes compared to living trees and dead 55 trees had significantly lower fluxes compared to treeless tundra. Additionally, there was a moderate, positive correlation between N_2O and water-filled pore space (WFPS). Results from using the diffusion gradient method show N_2O emissions on site 3. Methane fluxes showed small uptake.

Although, the N_2O flux results were low they still provide valuable information about the level of fluxes in the area. A possible explanation for the low fluxes is that it rained considerably less than usually in July in the region when conducting measurements, and soils were thus relatively dry. Soil moisture is a major driver of N_2O fluxes, with low soil moisture limiting the emissions. These results give cause to evaluate whether flux measurements should also be conducted outside the growing season when there is higher soil moisture. Year-round and long-term measurements in the region would provide a more comprehensive understanding of the flux levels and what can be expected in the future as climate warming continues.

Itä-Suomen yliopisto, Luonnontieteiden ja metsätieteiden tiedekunta
Ympäristö- ja biotieteiden laitos
Ympäristötiede
Karjalainen, Katri J.A.: Hyönteistuhon vaikutukset typpioksiduulin päästöihin subarktisessa ekosysteemissä
Opinnäytetutkielma, 92 sivua, 3 liitettä (21 sivua)
Tutkielman ohjaajat: Tutkimusjohtaja Christina Biasi, Postdoc-tutkija Lona van Delden, Apulaisprofessori Kristiina Karhu, Tutkija Saara Lind
Toukokuu 2021

Asiasanat: typpioksiduuli, subarktinen, tunturikoivu, tunturimittari, ilmastonmuutos

Kasvihuonekaasut kuten hiilidioksidi (CO_2) ja metaani (CH_4) ja niiden vaikutukset ilmaston lämpenemiseen ovat olleet suuren kiinnostuksen kohteena viime vuosina Arktisessa tutkimuksessa. Tästä huolimatta yksi kasvihuonekaasu, typpioksiduuli (N_2O), on usein jäänyt huomioimatta vaikka se on melkein 300 kertaa vahvempi kaasu kuin CO_2. Vasta viime aikoina on tunnistettu N_2O:n niin kutsuttuja kuumia pisteitä paljailla turvealueilla, jotka sijaitsevat ikiroudan alueella Arktisella tundralla. Sittemmin uusien N_2O päästölähteiden etsintä Arktisella alueella on alkanut. Ensisijaisia ehdokkaita ovat alueet, jotka ovat joutuneet hyönteistuhon kohteeksi, sillä vahingoittuneet tai puuttuvat kasvit voivat kasvattaa, aivan kuten paljaat turvealueet, N_2O päästöjä, kun kilpailua typestä ei ole. Lisäksi priming-ilmiön vaikutukset maaperän typen vaihtuvuuteen kasvaneen kuolleen kasvibiomassan vaikutuksesta voi triggeröidä N_20 päästöjä. Hyönteistuhot voivat siis johtaa N_2O päästöihin, mutta tutkimukset ovat vielä puuttellisia.

Pro gradu -tutkielman **”**Hyönteistuhon vaikutukset typpioksiduulin päästöihin subarktisessa ekosysteemissä**”** tavoitteena oli selvittää erosivatko kuolleiden tunturikoivujen N_2O vuot elävien puiden ja puuttomien alueiden vuosta. Kuolleet tunturikoivut olivat olleet tunturimittarin (Epirrita autumnata) tuhon kohteena 12 ja 55 vuotta sitten. Tunturimittari on aiheuttanut laajoja tuhoja tunturikoivuille Pulmankijärven alueella Utsjoella, Lapissa, jossa tämä tutkimus tehtiin heinäkuussa 2019. Tutkimusalueita oli kolme noin kahden kilometrin säteellä toisistaan ja jokaisella tutkimusalueella oli 16 puuta, joista mitattiin vuot.

Vuot mitattiin käyttämällä kammiomittausmenetelmää sekä diffuusiogradienttimenetelmällä. Typpioksiduuli vuot mitattiin yhdessä CH_4 voiden kanssa, jotta näiden ilmaston kannalta merkittävän kaasun vertailu oli mahdollista.

Yleisesti ottaen vuot olivat matalia. Kammiomenetelmällä mitatut N_2O vuot erosivat toisistaan siten, että puuttomalla tundralla oli merkittävästi korkeammat vuot verrattuna eläviin puihin. Kuolleet 55 puissa taas oli merkittävästi matalammat vuot verrattuna puuttomaan tundraan. Lisäksi, N_2O ja vesitäytetty huokostilavuus (WFPS) korreloivat kohtuullisesti keskenään. Diffuusiogradienttimenetelmällä saatujen tulosten perusteella tutkimusalueella 3. esiintyy N_2O päästöjä. Metaanin sidontaa esiintyi pienissä määrin mittausalueilla.

Vaikka N_2O vuo tulokset ovat matalat, silti ne tarjoavat arvokasta tietoa voiden tasosta kyseisellä alueella. Mahdollinen selitys matalille voille on se, että mittausten aikana alueella satoi huomattavasti vähemmän kuin yleensä ja maaperä oli verrattain kuivaa. Maan kosteus on yksi tärkeimpiä ajureita N_2O voille ja matala kosteus on päästöjä rajoittava tekijä. Tulokset antavat aihetta pohtia tulisiko kasvihuonekaasuvuo mittauksia tehdä myös kasvukauden ulkopuolella, kun maan kosteus on suurempaa. Ympärivuotiset sekä pitkän ajan mittaukset alueella antaisivat kattavamman käsityksen voiden tasosta ja mitä voidaan odottaa tapahtuvan tulevaisuudessa ilmaston lämmetessä.

Foreword

First, I wish to thank my main supervisor Christina Biasi for providing me with an interesting master's thesis topic and the opportunity to conduct field work in Lapland. I also wish to thank her for her valuable expertise, guidance, and support.

I want to express my sincere gratitude to my other main supervisor, Lona van Delden. Her continuous support, guidance, pep talks, and belief that I will finish my thesis are the reasons I managed to complete and write it. Thank you, Lona.

I also want to thank my co-supervisors Kristiina Karhu and Saara Lind. I wish to thank Kristiina for her guidance and knowledge on the topic and I wish to thank Saara for her help with practicalities such as the use of the gas chromatograph and conducting statistical analysis. This thesis was done as part of the NOCA project at the Biogeochemistry Research Group at University of Eastern Finland.

I want to thank Kristiina "Mosse" Myller for her invaluable help with the field work. Without her I think I would still be there. Thank you Mosse also for your witty sense of humour and playing the game "is it a reindeer or post box" on our multiple drives to and from the field site.

To all my friends, thank you. Thank you for listening to me complain, whinge, stress, and rant on and on about my thesis, and thank you for answering my countless questions. Thank you for kicking me in the butt when it was needed and most of all thank you for believing in me and believing that I will finish my thesis and graduate. You know who you are.

Lastly, the biggest thanks to my family. To my parents, mum and dad, who have been my biggest support and always believed in me. To my big sister, Pia, who from all the way from Australia never once has missed the chance to ask, "is it ready yet?". And now, finally, I can answer her and say yes, it is! Mum, Dad, Pia, I love you and could not have done this without you.

Abbreviations

C = carbon

CH_4 = methane

CO_2 = carbon dioxide

EC = electric conductivity

GHG = greenhouse gas

GWP = global warming potential

IPCC = Intergovernmental Panel on Climate Change

KCl = potassium chloride

N = nitrogen

N_r = reactive nitrogen

N_2O = nitrous oxide

NH_4^+ = ammonium

N min = mineral nitrogen

NO_3^- = nitrate

O_2 = dioxygen

PPBV = parts per billion volume

SM = soil moisture

SOM = soil organic matter

WFPS = water-filled pore space

Contents

1 Introduction

The ongoing climate crisis which continuously increases global temperatures is the biggest threat to humanity. The amount of greenhouse gases (GHGs) in the atmosphere continues to increase due to anthropogenic activities. The Arctic in particular is warming at a rate twice as fast as the rest of the globe (WMO, 2020; IPCC, 2019). Rising soil temperatures and the thawing of permafrost accelerate soil organic matter (SOM) decomposition resulting in net emissions of carbon dioxide (CO_2) and methane (CH_4) to the atmosphere (Schuur et. al, 2015). It is known that permafrost soils contain large deposits of carbon (C) in Arctic and sub-Arctic regions (Schuur et. al, 2015). However, it is a less widely known fact that Arctic soils also contain vast reservoirs of nitrogen (N). According to an estimate done by Hugelius et al. (2020) northern peatlands cover 3.7 ± 0.5 million km^2 and store 415 ± 150 Pg carbon (C) and 10 ± 7 Pg nitrogen (N), of which almost half are affected by permafrost. Currently, the main GHG research focuses on carbon dioxide (CO_2) and methane (CH_4) (Treat et al., 2018) but increasing data on nitrous oxide (N_2O) dynamics indicate they are significant contributors as well (Voigt et al., 2020, review). This contribution can possibly shift peatlands from a C and N sink to a source as climate change continues (Frolking et al., 2011, Hugelius et al., 2020, Biskaborn et al., 2019).

With the warming Arctic climate, the large N reservoirs will slowly thaw and take part in decomposition. As part of the natural N cycle, nitrous oxide (N_2O) is produced mainly via nitrification and denitrification in the soil (Butterbach-Bahl et al., 2013) and then released to the atmosphere. Nitrous oxide is a strong greenhouse gas, almost 300 times stronger than CO_2; 298 CO_2 equivalents (Myhre et al., 2013) and participates in the destruction of stratospheric ozone (Ravinshakara et al., 2009). The emissions of N_2O have been increasing for about three decades (IPCC, 2018), mostly due to accelerated use of fertilizers (Syakila & Kroeze, 2011) and due to global warming. Due to the importance of N_2O for climate warming and atmospheric chemistry, quantifying and understanding factors driving the emissions is important so that they can be accounted for in national and global GHG budgets in the future. There is, however, still not enough information on N_2O emissions from the Arctic and subarctic ecosystems, and particularly knowledge on the drivers of the emissions is largely lacking.

It has been suggested that lack of vegetation is a key for producing N_2O in permafrost soils. Bare surfaces are abundant in the Arctic since frost actions and freeze-thaw cycles often erase and destroy vegetation (Voigt et al., 2020, review). A possible reason for increased N_2O emissions in the absence of plants is the soil microbe activity (Timilsina et al., 2020), nitrification and denitrification processes, and N turnover (Machacova et al., 2019). Insect outbreaks also negatively impact plant growth often causing plant death in large areas; however, no studies have yet investigated the effects on N_2O in high latitude soils. It is expected that insect outbreaks will increase in the future under warmer conditions in the Arctic because of longer and warmer growing periods (Bale et al., 2002). Particularly the outbreak of the autumnal moth which is widely distributed throughout the Holarctic region can be devastating for Arctic plants (Kankaanhuhta/Metla, 2005).

The autumnal moth (Epirrita autumnata) is a geometrid moth. The moth populations have shown cyclic, high amplitude fluctuations in density in the northern and mountainous parts of Fennoscandia. These have resulted in devastating outbreaks for 1-3 successive years. The main host for larvae in these areas is the mountain birch (Betula pubescens czerepanovii Orlova, Hämet-Ahti, 1963) and large areas of the subarctic mountain birch zone have been damaged or killed (Kankaanhuhta/Metla, 2005). The larvae eat the mountain birch leaves until only the stem is left. If the tree is healthy, it can survive this, but the survival depends also on the weather factors during growing season and whether other pests attack the tree (Metla, 2005). This widespread tree dying due to moth outbreaks might have significant impact on nutrient cycling by changing the nutrient uptake and litter input of the dead trees. Consequently, N_2O emissions might increase, however, this has not been studied yet. The main of this thesis was thus to investigate effects of moth outbreaks on N_2O emissions and soil nutrient content in subarctic ecosystems.

2 Literature review

2.1 Climate Change in Arctic and Subarctic Regions

The Intergovernmental Panel on Climate Change (IPCC) estimated in their Special Report on Global Warming 1.5°C in 2018 that the approximately 1.0°C of global warming above pre-industrial levels have been caused by human activities. In this report the IPCC uses the period 1850 – 1900 as a reference representing pre-industrial temperature (FAQ 1.2, IPCC, 2018). The IPCC also estimates that a temperature increase of 1.5°C will likely be reached between 2030 and 2052 if global warming continues to increase at the current rate. Human induced climate change includes rising temperatures, which also changes precipitation patterns, and causes extreme weather events to occur more frequently (Bonfils et al., 2020). Many regions are experiencing warming at a higher rate than the global annual average, including warming two to three times higher in the Arctic and Antarctic respectively (IPCC, 2018).

Surface air temperature in the Arctic has most likely increased by more than double compared to the global average over the last two decades. The loss of both sea ice and snow cover feedbacks contribute to the increased warming (IPCC, 2019). According to the Special Report on the Ocean and Cryosphere in a Changing Climate (SROCC) by the IPCC in 2019, polar regions are rapidly losing ice and their oceans are changing. This polar transition can have global and various consequences and effects. Permafrost temperatures have increased to record high levels since the 1980s to present day. Temperatures in continuous-zone permafrost increased by 0.39 ± 0.15°C and 0.37 ± 0.10°C in the Arctic and Antarctic during 2007 to 2016 (IPCC, 2019). Of the Earth's terrestrial surface area, approximately 17% is covered by permafrost regions and include subarctic, alpine, Arctic, and Antarctic ecosystems (Voigt et al., 2020, review). Permafrost is also warming globally (Biskaborn et al., 2019) and temperatures in the upper layers of permafrost have risen by 0.5-2.0°C within the last two decades (Romanovsky et al., 2010).

Arctic ecosystems are strongly temperature limited, which means that warming may be capable of substantially offsetting arctic ecosystem functioning. This can affect the ecosystem's sink or source behaviour regarding the major GHGs: CO_2, CH_4, and N_2O (Voigt et al., 2017). Recent analysis done by Hugelius et al. (2020), determined the total peat C and N stock in the Northern Hemisphere. They estimated the total C stock being 415 ± 150 Pg and total peat N stock being 10 ± 7 Pg (mean ± RMSE). They also estimated that of this C stock 185 ± 70 Pg and of the N stock 7 ± 4 Pg are stored in permafrost peatlands which are substantial amounts of the total stock (Hugelius et al., 2020). Over the course of thousands of years, these C and N stocks have accumulated (Schuur et al., 2013) in the form of frozen and seasonally defrosted peat, soil, and litter (Koven et al., 2011). However, it is these very factors which have protected and maintained C and N in soil that are now changing due to climate warming (Schuur et al., 2013) and could now become available for decomposition, resulting in the release of CO_2 and CH_4 but also N as N_2O to the atmosphere (Voigt et al., 2017). Permafrost thawing as the result of climate warming leads tothe long-term immobile belowground C stocks being exposed to microbial decomposition and remobilization, resulting in the release of CO_2 and CH_4 to the atmosphere (Hayes et al., 2014). The extent of the permafrost-C feedback is inadequately constrained (McGuire et al., 2018) and not currently included in IPCC projections, which likely underestimates the Arctic's climate feedback (Koven et al., 2011; Schaefer et al., 2014).

Recently climate simulations have predicted an additional warming of 0.2 °C, caused by the loss of C from permafrost, by the end of this century (Burke et al., 2017; Schaefer et al., 2014). The loss of N in the form of N_2O is even less constrained, with only one number published by simple back on the envelope calculations. Accordingly, permafrost regions emit 007-0.63 Tg (1 teragram = 1 mega tonne) N_2O-N during the growing season (100 days) (Voigt et al., 2020, review).

2.2 Greenhouse gases

2.2.1 N_2O

Nitrous oxide is a long-lived trace gas in the atmosphere, and it has an average mixing ratio of 322.5 parts per billion volume (ppbv) (year 2009, WMO, 2010). Nitrous oxide concentrations in the atmosphere have risen by 19% since pre-industrial times (WMO, 2010). Nitrous oxide is a potent greenhouse gas, and it has a global warming potential (GWP) 298 times stronger than that of CO_2 for a 100-year timescale and it is responsible for 6.24 per cent of total global radiative forcing (WMO, 2010). Additionally, it is the single most important depleting substance of stratospheric ozone (Ravinshakara et al., 2009). Rising N_2O concentrations in the atmosphere over the last decades have led to an increased interest in understanding N_2O production pathways in order to come up with strategies to decrease the concentrations of N_2O (Kool et al., 2010). However, evaluating N_2O fluxes has been one of the most challenging topics in environmental biogeochemistry over the last 10 years (Groffmann et al., 2000).

2.2.2 N_2O Drivers

To better quantify N_2O soil emissions, it is essential to understand the N cycle from ecosystem and regional scales all the way up to global scales. Therefore, it is essential to understand the key drivers involved in the formation, consumption, and emission of N_2O. The challenge and aim are to integrate these together (Butterbach-Bahl et al., 2013). Groffmann et al. (2000) stated that "soil-atmosphere N_2O flux is one of the most difficult to quantify component of the terrestrial N cycle".

There are several factors which influence the N_2O gas exchange between soil and atmosphere, such as N input, precipitation, temperature, land use, and soil properties (pH, texture, C/N ratio) (Schaufler et al., 2010). In soils, sediments, and water bodies microbial production processes are the dominant sources of N_2O (Butterbach-Bahl et al., 2013). Emissions from agriculture, due to N fertilizer and manure management, and emissions from natural soils account for 56 – 70% of global N_2O sources. In both managed and natural soils, microbial nitrification and denitrification contribute approximately to 70% of global N_2O emissions (Syakila & Kroeze, 2011). In the boreal

region, early spring and winter with snow cover are important periods for the annual N_2O budget in addition to the growing season (Maljanen et al., 2003).

The availability of reactive nitrogen (N_r) is the major driver of N_2O soil emissions, making the use of fertilizer one major factor controlling N_2O fluxes from soils (Syakila & Kroeze, 2011). Nevertheless, increased N_2O soil fluxes are not only restricted to direct emission sites where N fertilizers are applied, but due to erosion, leaching, and volatilization, N_r is flowing from direct emission sites to downwind and downstream ecosystems (Butterbach-Bahl et al., 2013). This can result in natural N enrichments in ecosystems, creating new indirect N_2O emission hotspots (Galloway et al., 2003, Erisman et al., 2007).

Next to N_r availability, a major driver of N_2O emissions is soil moisture because it regulates the availability of oxygen to soil microbes. Nitrous oxide emissions are at their optimum level at 70 – 80% water-filled pore space (WFPS) range, depending on soil type (Davidson et al., 2000). At higher levels of soil moisture, the main end product of denitrification is N_2 (Butterbach-Bahl et al., 2013). The reason why soil water content is so important is due to its controlling of the transport of oxygen into soil and also controlling the transport of NO, N_2O, and N_2 out of soil. Nitric oxide, N_2O, and N_2 emissions are dependent on the balance of production, consumption, and diffusive transport of the gases in question. The oxidative process of nitrification dominates in dry, well-aerated soil, and NO being the more oxidized gas is the most common nitrogen oxide emitted (Davidson et al., 2000). Gas diffusivity is high in dry soils which leads to much of NO being able to diffuse out of the soil before it is used (Bollmann & Conrad, 1998). Gas diffusivity is lower, and aeration is also poorer in wet soils. Most of the NO is reduced before it leaves the soil, which results in N_2O, the more reduced oxide, being the dominant end product. In even more water-saturated and mostly anaerobic soil, much of N_2O is further reduced to N_2 by denitrifiers before it leaves the soil (Davidson et al., 2000). It seems that upland soils are rarely able to reach moisture conditions that are outside the optimum N_2O emissions range (Butterbach-Bahl et al., 2013).

Despite soil moisture having a predominant effect on N_2O emissions, it should be noted that denitrification is especially sensitive to increasing temperatures (Butterbach-Bahl et al., 2013). The Q_{10} of denitrification, meaning the "stimulation of denitrification following an increase in temperature by 10 °C", surpasses the Q_{10} of soil CO_2 emissions (Schaufler et al., 2010). This can be explained by the tight coupling between microbial C and N cycle (Butterbach-Bahl et al., 2013). Therefore, N_2O emissions are not solely directly affected by temperature effects on enzymatic processes involved in N_2O production (Butterbach-Bahl et al., 2013).

In addition, increased soil respiration induced by temperature, leads to a reduction in soil oxygen concentrations and an increase in soil anaerobiosis, which is a precursor and major driver (Butterbach-Bahl et al., 2013). In the N cycle there are several temperature sensitive microbial processes which pour reactive N compounds through its various oxidation states, such as N-mineralization and nitrification, which provide the substrate needed for denitrification. This has an accumulating effect on temperature increase on soil N_2O fluxes. What this means in the context of environmental change globally is that a positive feedback effect of warming on GHG emissions can be anticipated to be greater for N_2O than CO_2 (Butterbach-Bahl et al., 2013). However, limitations on substrate and moisture of microbial N cycling processes under climate change conditions may reduce the stimulating effect of temperature (Butterbach-Bahl & Dannenmann, 2011). Nevertheless, aapplication of these findings into global climate change models can significantly change predictions of the severity of future climate change projections and atmospheric composition (Butterbach-Bahl et al., 2013).

Global change drivers such as temperature and moisture and their impact on ecosystem processes are well studied when functioning alone or at most, with one interacting variable. There is understanding about how both drivers interact mechanistically but where we lack understanding is predicting how emissions can change when a third or fourth driver comes along (Butterbach-Bahl et al., 2013).

This is because of the nonlinearity of the processes involved and the effects of combined drivers can be synergistic or antagonistic rather than simply additive. This makes understanding the

underlying mechanisms much more complex (Larsen et al., 2011). Despite this, although effects of dampening with scale and treatment complexity can be a part of fundamental system behaviour so far, we do not understand the threshold effects and tipping points. These need to be considered when predicting global change effects (Butterbach-Bahl et al., 2013).

Furthermore, N_2O emission rates can be affected by the seasonal or spatial dynamics of soil moisture or temperature (Butterbach-Bahl et al., 2013). Temporary waterlogging, seasonal passing from drought to rewetting as well as transient zones between upland and wetland soils present ideal conditions for the transition from microbial oxygen to NO_3 respiration, and therefore, can create hot moments and hot spots for N_2O emissions (Groffmann et al., 2009).

Field N_2O emissions experience temporal variation and up to 95% of this variation can be explained by changes in soil moisture and soil temperature (Kitzler et al., 2006), the main drivers of denitrification (Butterbach-Bahl et al., 2013). The remaining unexplained emissions are related to drivers of oxygen supply such as available energy and substrate concentration and plant nitrate uptake drivers such as SOM quality, soil texture, pH, microbial respiration, predation, and heavy metal pollution or organic chemicals (Chapin et al., 2002).

Several interactions of soil, climate, and vegetation, influence N_2O emissions which can influence the N effect. This means that the N_2O-to-N_2 ratio can differ between ecosystems and in sandy soils, N saturation can possibly promote NO_3 instead of N_2O emissions. These confusing effects need to be solved so that a better understanding of the true mechanisms behind the impacts of N input can be achieved (Butterbach-Bahl et al., 2013). In any case, N content and availability of N_r are key drivers for N_2O emissions in both managed and natural soils. Though generally nutrient limited, N_r can occasionally be high in Arctic ecosystems because of natural and/or climate change related perturbations, such as distributed vegetation cover, soil warming, and permafrost thaw (Voigt et al., 2020, review).

2.2.3 Processes Responsible for N_2O Production and Consumption in Soils

There are also other abiotic processes which produce N_2O (Butterbach-Bahl et al., 2013). According to Butterbach-Bahl et al. (2013) "the current understanding of underlying processes, pathways, and controls of N_2O formation is still primarily based on studies with pure cultures of micro-organisms and soils under controlled conditions". However, to gain a comprehensive understanding of N_2O fluxes at a variety of spatiotemporal levels, an understanding of N cycling and loss rates of N_2O for the duration of essential microbial N transformation processes is required (Butterbach-Bahl et al., 2013). Hotspots and hot moments cause challenges that are problematic because the process of denitrification is carried out by microorganisms but is of interest at various larger scales such as streams and wetlands, crop fields, mixed landscapes, and regional watersheds (e.g., Gulf of Mexico, Chesapeake Bay, Baltic Sea), and the entire globe (Groffmann et al., 2009). The interest in denitrification at large scales stems from its effects on soil fertility, water quality, and air chemistry (Butterbach-Bahl et al., 2013).

Autotrophic nitrification and heterotrophic denitrification have traditionally been considered as the major processes forming N_2O (Kool et al., 2010). Nitrification is the "oxidation of ammonium (NH_4^+) to nitrate (NO_3^-) and nitrite (NO_2^-)" (Groffmann et al., 2000). Denitrification is "the anaerobic reduction of nitrogen oxides nitrate (NO_3^-) and nitrite (NO_2^-) to nitrogenous gases nitric oxide (NO), nitrous oxide (N_2O) and dinitrogen (N_2)" (Groffman et al., 2009). Nitrous oxide gas emissions occur during the intermediate steps in nitrification and denitrification processes in variable amounts, dependant on a wide range of soil conditions (Groffmann et al., 2000).

2.2.4 Hotspots and Hot Moments

Field measurements of N_2O from soils to atmosphere across various terrestrial ecosystems combined with laboratory incubation studies in controlled conditions provide an extensive set of measured emission fluxes. Due to these measurements, it is possible to provide empirical emission estimates over spatiotemporal scales (Butterbach-Bahl et al., 2013). However, upscaling N_2O budgets to national and regional scales remains an unresolved issue and current national estimates experience high uncertainty (Butterbach-Bahl et al., 2013). The main reason behind the high uncertainty is the very dynamic and variable character of N_2O emissions from soils, which are caused by various interacting controls (Butterbach-Bahl & Dannenmann, 2011).

Therefore, N_2O emissions from soil are characterized by “hotspots” and “hot moments” meaning they have enormous spatiotemporal variability (Butterbach-Bahl et al., 2013). Groffmann et al. (2009) describe hotspots as small areas and hot moments as brief periods. According to Groffmann et al. (2009) hotspots develop in the terrestrial environment “from the interaction of patches of organic matter with physical factors that control oxygen diffusion and thus anaerobiosis, and the transport and residence time of denitrification reactants”. This means that various soil and plant factors such as rooting patterns, soil structure at small (0.1 – 10 m) scales, hydrologic flow paths, topography, and geology at larger (>1km) scales, need to be taken into account to comprehend the spatial distribution of hotspots (Groffmann et al., 2009). Hot moments on the other hand “are driven by events that cause a convergence of reactants, e.g., drying-rewetting and freezing-thawing events” (Groffmann et al., 2009).

These events have become significant through studies which show their importance to fluxes through denitrification intermediates such as NO and N_2O (Groffmann et al., 2009). Hotspots and hot moments are caused by temporal and spatial phenomena and human alteration for agricultural and urban/suburban land use strongly affects these phenomena (Groffmann et al., 2009). Soil N_2O fluxes have notorious spatiotemporal variability due to being dependant of microbial N_2O production and environmental control consumption processes such as temperature, redox potential, and substrate availability.

Nevertheless, it is essential to understand the spatiotemporal variability of N_2O fluxes to better limit the extent N_2O soil-atmosphere exchange and to create measurement programmes which are statistically valid and able to determine fluxes from plot to regional scales (Butterbach-Bahl et al., 2013). In Arctic ecosystems, the spatiotemporal variability of N_2O emissions is poorly assessed.

2.2.5 Freeze-Thaw Cycles

Of special interest for N_2O are temperatures around 0 °C. The interest stems from many soil microbes being still active and freeze-thaw processes leading to pulse N_2O emissions which significantly contribute to the annual N_2O budget (Groffmann et al., 2009). A possible driver for this is the release of stored C during thawing. It is these transition effects that are the key in understanding the environmental controls of N_2O release (Butterbach-Bahl et al., 2013). Studies have shown that annual budgets of NO and N_2O fluxes from different ecosystem soils are often dominated by defined periods, for example, <5-20 days, which have extremely high emissions (Groffmann et al., 2009). The extremely high N_2O emission periods are usually at the end of winter, when the soil starts thawing, in temperate and boreal regions. In subtropical and tropical regions on the other hand, pulse NO and N_2O emissions have been observed after wetting of soil after prolonged dry periods (Groffmann et al., 2009).

Denitrification has been shown to be the predominant original source of N_2O production during freeze-thaw cycles (Morkved et al., 2006) and hence, making the N_2O pulses dependant on the nature and extent of anaerobic conditions in the thawing soil (Groffmann et al., 2006).
For substantial N_2O emissions to occur during freeze-thaw, the soil needs to be close to water saturation and/or microbial respiratory activity needs to be greater than O_2 diffusion into soil (Groffmann et al., 2009). Freeze-thaw cycles can reoccur daily and create diurnal patterns in N_2O emissions depending on environmental conditions (Groffmann et al., 2009). Repeated freeze-thaw cycles decline the magnitude of N_2O emissions because of the gradual utilisation of the accumulated substrate (Skogland et al., 1988; Prieme & Christensen, 2001; Ludwig et al., 2006).

The pulse N_2O emissions which occur during freeze-thaw cycles from agricultural and/or forest ecosystems have been demonstrated to be important or even dominate overall annual N_2O emissions (Christensen & Tedje, 1990; Papen & Butterbahc-Bahl, 1999; Teepe et al., 2000; Groffmann et al., 2006b). Boreal forests, tundra, and steppes are subject to long periods of frost on a regular basis (Sakai & Larcher, 1987). The physical structure of soil and solute distribution and also the activity of plants and microorganisms are greatly impacted by recurring soil freezing and thawing. When the soil freezes, water and nutrients are redistributed (Groffmann et al., 2009). When the soil is subjected to freeze-thaw events, also the surface structure is impacted and often, patterned ground features develop. Parts of these features often lack vegetation. It is the complex interactions between "climate, permafrost, vegetation, soils, and hydrology" that produce these patterned ground features in question (Walker et al., 2008, p.1).

Repo et al. (2009) measured N_2O fluxes from peat circles which are round patches of peat without vascular plants and have diameters of 4 – 25 m and areas between 10 to 500 m^2. They discovered that the N_2O fluxes from the peat circles were exceptionally high compared to other vegetated surface types. The measured cumulative N_2O emissions during the snow-free period (138 days) were 1.2 ± 0.3 g N_2O m^{-2} which was significantly higher than fluxes from other surfaces (Repo et al., 2009). Peat circles contain high amounts of NO_3^- because plants are absent and thus, there is also lack of competition for mineral N between plants and microbes. This results in the NO_3^- produced being already available for denitrifiers which are the most productive producers of N_2O in the soil (Repo et al., 2009). It is known that a C:N ratio higher than ~25 in boreal peat soils make N_2O emission negligible but a ratio below this increase emissions rapidly (Klemedtsson et al., 2005).

2.2.6 N_2O Fluxes from Arctic and Subarctic Ecosystems –A Research Gap

In general, boreal upland forest soil N_2O emissions are small (Klemedtsson et al., 1997; Simpson et al., 1997; Brumme et al., 2005) yet global warming and intensified soil management practises could increase N_2O microbial production processes (nitrification, denitrification; Davidson, 1991) which is of concern (Maljanen et al., 2006). Nitrous oxide emissions from Finnish forest soils are not well known and are commonly thought to be small. However, according to Maljanen et al. (2006) their results in fact show that fertile forest soils may emit considerable quantities of N_2O. This indicates that total N_2O emissions from Finnish forests are most likely underestimated (Maljanen et al., 2006).

Tundra ecosystems contain large reservoirs of SOM and play thus a key role in the global C balance. Increased emissions of CO_2 and CH_4 from tundra soils can affect global climate and as a result, global warming (Schuur et al., 2013). The large SOM reservoir also contains large amounts of organic N (Post et al., 1985). However, the availability of mineral N in tundra is considered to be low because of mineralization of organic matter in cold climates is slow (Nadelhoffer et al., 1991) and low N deposition (Dentener, 2006). Traditionally, it has been thought that there is shortage of mineral N which is one of the central reasons for low N_2O emissions from tundra soils (Christensen et al., 1999; Ludwig et al., 2006; Siciliano et al., 2009). However, newer studies show that both N turnover and N_2O emissions can be significant in Arctic ecosystems (Voigt et al., 2020, review).

Taken together, N_2O can be released in certain habitats and under certain conditions in the permafrost region, particularly where reactive N availability exceeds the immediate needs of organisms and the system becomes N saturated. Thus, the general paradigm that all permafrost soils are N limited and N_2O is negligible is not true (Voigt et al., 2020, review). One system, which has not yet been studied, are tundra ecosystems impacted by insect outbreak. Through the attack, plant and disturbance soil nutrient regimes might be elevated, stimulating emissions of N_2O. Thus, to produce the first inventory based circumpolar N_2O budget, the N_2O measurements need to capture all possible hotspots.

In addition, near-zero fluxes need to be reported so that N_2O emissions are not overestimated because of biased site selection of high-emitting sites (Voigt et al., 2020, review).

Various studies have been conducted on N_2O fluxes and the underlying processes behind them. However, a comparison of over 200 studies conducted on CO_2 and more than 100 studies regarding CH_4 exchange in the Arctic, only about 40 published studies have examined in situ N_2O fluxes globally across permafrost regions (Voigt et al., 2020, review). From these 40 studies, only about half were from polar regions, mainly from the Tibetan plateau. Additionally, N_2O flux measurements in permafrost regions are scarce and measurements during non-growing season are lacking. Yet, N_2O flux measurements and studies from ecosystems in the subarctic region are even more rare. This results in the extent of N_2O fluxes across the vast permafrost regions being uncertain (Voigt et al., 2020, review) and therefore, there is a need for more studies.

Previous studies which have measured N_2O fluxes have conducted measurements on peatlands, boreal soils, and other ecosystems. For example, Repo et al. (2009), Marushchak et al. (2011, 2013) have conducted studies in Seida, which is in the discontinuous permafrost zone in northeast European Russia (Repo et al., 2009). The peat plateau complex in Seida has peat deposits which are several metres thick and many small thermokarst lakes (Marushchak et al., 2011). In this study, measurements were also conducted in Utsjoki, Finnish Lapland on three palsa mires located in the discontinuous permafrost zone (Marushchak et al., 2011). Voigt et al. (2017) also conducted an open-top chamber experiment 2.5 km from the Seida study site established by Marushchak et al. (2011) (Voigt et al., 2017). Treat et al. (2018) have also conducted studies on the Seida site but these have focused on CO_2 and CH_4 and have not included N_2O. Additionally, Elberling et al. (2010) have conducted studies in northeast Greenland and Abbot et al. (2015) in Alaska, USA. However, all these studies have been conducted in discontinuous permafrost regions whereas my study was conducted in the subarctic region which does not experience permafrost. While conducting research for my study it became clear that there are very few studies published on N_2O fluxes from subarctic regions.

Schaufler et al. (2010) studied all three (CO_2, CH_4, N_2O) GHGs using "soil cores collected from the NitroEurope Level-3 'Super Sites', which are distributed all over Europe" (Schaufler et al., 2010). Thirteen different sites were included in the level 3 'super sites'. These sites represented four different ecosystem types: forest, grassland, arable land, and wetland. (Skiba et al., 2009). There were two sites from Finland; a forest site in Hyytiälä (61°51´N 24°17´S) and wetland site in Lapland called Lompolojänkkä (68°00´N 24°13´S) (Skiba et al., 2009). The results from this study are not directly comparable with my own study but it will provide valuable information on what the level of N_2O fluxes have been in other ecosystems and parts of Finland. The results from the study were that there were significant differences in N_2O fluxes between sites. Highest emissions were measured from grassland sites (Easter Bush, UK and Bugac, Hungary) and lowest from Finnish soils (Hyytiälä and Lompolojänkkä). The highest fluxes were 514.4 ±133.5 N_2O-N/μg N m^{-2} $hour^{-1}$ (Easter Bush) and 211.9± 63.0 N_2O/N μg N m^{-2} $hour^{-1}$ (Bugac) (Schaufler et al., 2010). Lowest fluxes were 3.1±0.4 N2O-N/N μg N m^{-2} $hour^{-1}$ (Hyytiälä) and 3.2±0.3 N_2O-N/N μg N m^{-2} $hour^{-1}$ (Lompolojännkä). In this study, highest N_2O emissions were measured at 80% WFPS and they also found a significant relationship between N_2O emissions and soil moisture. However, no significant correlation was found between N_2O and soil temperatures over all soil moisture states and there were no significant correlations between N_2O fluxes and C/N, pH, N fertilization or N deposition (Schaufler et al., 2010).

Maljanen et al. (2003) studied N_2O fluxes from a drained organic soil in Eastern Finland for two years. They measured fluxes from April 1996 to April 1998 using the static chamber technique (Maljanen et al., 2003). The study site was an old shore and organic sediment of a pond which had been drained in 1957 and planted with birch. In 1997 only grass was grown on the main field and barely was cultivated on two separate plots. During both years, up to 3-5 experimental plots were kept bare by regular tilling every second week.

Maljanen et al. (2003) discovered that all of the different soils were sources of N_2O. Highest Emissions measured in 1996 after spring thaw in late April. The measured fluxes were 12.6, 14.2, and 2.0 mg N_2O-N m^{-2} d^{-1} from barely, bare and forest soils respectively (Maljanen et al., 2003). Up to 10.5 mg N_2O-N m^{-2} d^{-1} high emissions were measured during a warm period in

August 1996 from the barely soil. Also, N_2O emissions after spring thaw were higher in May 1997 than in 1996. Emissions measured in early summer from grassland, bare, and forest soils were 2-5 times higher than emissions later during summer 1997. Maljanen et al. (2003) also discovered that mean N_2O fluxes were always lower from forest soils than from cultivated soils.
In this study they found that the water table level is an important factor which determines N_2O production during snow-free periods. They also found that 55 % of variation in weekly mean N_2O fluxes was explained by water table, CO_2 release, and soil temperature at 5cm depth together (Maljanen et al., 2003). Nitrous oxide emissions were similarly related with WFPS. Cultivated soil N_2O fluxes were highest with WFPS between 80 and 90%, whereas forest soil N_2O fluxes were low with WFPS being 40-70%. Furthermore, the mean N_2O fluxes were 10 times higher at WFPS of 70-80% than at WFPS of 40-70% (Maljanen et al., 2003).

Generally, N_2O fluxes decreased near autumn yet increased again in winter during air temperature below 0 °C and the soil had snow cover. Highest emissions, up to 10 mg N_2O-N m^{-2} d^{-1}, in winter were measured when air temperature was close to zero and depth of snow cover was 30 cm (Maljanen et al., 2003). During spring thaw maximum emissions occurred, as has been reported earlier for boreal soils (Goodroad and Keeney, 1984a; Christensen and Tiedje, 1990). In contradiction to some studies conducted in the temperate region, the lowest N_2O emissions occurred in the autumn (Maljanen et al., 2003).

When air temperature dropped below 0 °C, N_2O emissions increased again (Maljanen et al., 2003) as has been reported for some boreal organic soils (Huttunen et al., 2002) and mineral soils (Teepe et al., 2001). It is not understood what the mechanism behind this increase is (Maljanen et al., 2003). However, several authors have reported enhanced N_2O emissions following freezing of surface soils (Christensen and Tiedje, 1990; Flessa et al., 1998; Papen and Butterbach-Bahl, 1999; Teepe et al., 2000). Most of the studies show that high N_2O emissions are associated with freeze-thaw cycles which result in C being available for denitrification (Maljanen et al., 2003) In cultivated soils, winter fluxes accounted for up to 60% and in forest soils near 36% of the annual N_2O flux (Maljanen et al., 2003).

Nitrous oxide emissions over 1 mg m^{-2} d^{-1} were measured during winter when snow depth was between 20 to 60 cm. Thus, even without freeze-thaw cycles, high N_2O emissions occurred in the present soil (Maljanen et al., 2003). This demonstrates the importance of snow acting as insulation which has been reported by Papen and Butterbach-Bahl (1999). Another important factor controlling winter fluxes is the timing of snowpack development (Brooks et al., 1997). In the boreal region, winter fluxes are a significant part of annual emissions. This needs to be considered in any annual gas balance calculations (Maljanen et al., 2003).

2.2.7 CH_4 Production and Consumption from Soils, with Focus on Arctic and Subarctic Ecosystems

In the atmosphere, CH_4 is the most plentiful reduced compound, and it has an important role in Earth's C cycle. Methane is a strong GHG; compared to CO_2 it is second in its importance related to climate change (Myhre et al., 2013). Carbon released as CH_4 and CO_2 matters as (Schuur et al., 2013; Treat et al., 2015; Schädel et al., 2016) "CH_4 is a 28-34 times stronger GHG than CO_2 on a 100-year time horizon, based on the GWP approach" (IPCC, 2013).

Production of CH_4 in the environment is controlled by factors which the climate influences. Increased production of CH_4 will result in warming the Earth which then leads on to CH_4 being produced at a faster rate causing a positive climate feedback (Dean et al., 2018). The Earth's C cycle contains continuous transformations of C between organic and inorganic pools in the atmosphere, geo- and hydrosphere, and terrestrial biosphere. Carbon dioxide in the atmosphere is the fully oxidized form of C and it is fixed by the marine and terrestrial biosphere. When organic matter is decomposed, C in the biomass of the organic matter can be, depending on environmental conditions, converted into CH_4 (Dean et al., 2018).

Methane fluxes are highly temperature prone (Bellisario et al., 1999). However, CH_4 production is bound to anaerobic conditions (Voigt et al., 2017). Consequently, waterlogged soils emit large amounts of CH_4, as the depth of the water table frequently overrules the effect of temperature (Liblik et al., 1997). Recently, it has been discovered that CH_4 uptake that happens in dry, arctic tundra soils can be of immense significance for the arctic regional CH_4 balance (Jorgensen et al., 2015). The amount of CH_4 that enters the atmosphere is dependent on three factors: "the rate of production, the rate of transport from production to atmosphere, and the rate of consumption along the production pathway" (Dean et al., 2018, p. 207). These three factors result in CH_4 emission fluctuations which affect CH_4 concentrations in the atmosphere over glacial-interglacial cycles. Methane emission fluctuations can have impacts on current and future climate warming by forming positive feedbacks (Dean et al., 2018).

Methane is emitted from various natural and anthropogenic sources. It is estimated that natural sources since 1980 have contributed to global anthropogenic emissions between 33 – 54% and anthropogenic sources have contributed between 46 – 67% (Kirschke et al., 2013). Wetlands are a dominant natural source of CH_4, but also freshwater systems are a significant contributor (Dean et al., 2018). Other natural sources of CH_4 are geological sources, coastal sediments and oceans, methane hydrates, and fauna. Main anthropogenic sources include agriculture, biomass burning, waste, and fossil fuels, which include both infrastructural fossil CH_4 leakage and methanogenic processes (Dean et al., 2018). Recently, it has been suggested that fossil fuel sources of CH_4 are a much larger part of the total anthropogenic CH_4 budget, even up to 60% greater than estimated previously (Schwietzke et al., 2016).

As an example, as permafrost thaws CH_4 will be released, and these regions can possibly be affected by changes in temperature. These temperature changes do not only affect the microbial activity and the deepening of the thawed soil active layer (Bardgett et al., 2008) but additionally it can alter precipitation patterns and change hydrologic flow paths (Rawlins et al., 2010). Thus, changing environmental conditions can result in altered microbial communities and as a result, CH_4 emissions too (Dean et al., 2018).

2.2.8 CO2

In the atmosphere CO_2 is the most important long-lived GHG associated with human activities. In 2017, the annual global average level of CO2 was 405.5 ppm (WMO, 2018). In 2019, the annual global average level of CO2 was approximately 410.5 ppm which is accounts for a 148% increase compared to pre-industrial levels (WMO, 2020). Anthropogenic CO_2 emissions are caused by burning of fossil fuels, deforestation, and other land use changes (WMO, 2020).

Arctic CO_2 exchange is affected by increased temperatures which accelerate microbial processes and increases availability of nutrients (Chapin et al., 1995). This results in CO_2 being released to the atmosphere and higher heterotrophic respiration rates (Dorrepaal et al., 2009). However, studies suggest that elevated temperature with longer growing seasons and increased nutrient availability alter the composition of plant species (Chapin et al., 1995) and thus promote the growth of plants (Rustad et al., 2001; Hobbie et al., 2002). This results in increased CO_2 uptake by plants (Voigt et al., 2017) and can either partly or fully compensate for increased losses of CO_2 from soils (Schuur et al., 2013). This is not, however, always the case though (Lund et al., 2012) and therefore the importance of vegetation regulating arctic CO_2 emissions is highlighted (Voigt et al., 2017).

2.3 Priming

Many studies that investigate the transformation of substances added to soil have noticed a side effect: an increased release of soil-derived C as CO_2 or N as NH_4^+ or as NO_3^- compared to the mineralization in the soil without any additions. This results from the interactions between the transformation of the added substances and natural soil cycles of both elements. These non-additive interactions which result in an extra release of soil-derived C or N have been summarized by the term “priming effect” (Kuzyakov et al., 2000). Priming is extremely difficult to measure in field conditions (Meyer et al., 2021, unpublished) but priming has been studied via incubation experiments (Karhu et al., 2016).

Depending on whether studies focused on N or C, there are different definitions (Kuzyakov et al., 2000). Studies focused on N support the following definition: “the priming effect is extra soil N which is taken up by plants after addition of mineral N fertilizer, compared with non-N treated plants” (Jenkinson et al., 1985; Leon et al., 1995). This means N uptake by plants therefore is production oriented. Jenkinson et al. (1985) suggested another definition for “added nitrogen interaction”, although inexact but frequently used: “priming is every effect on N already in the soil by adding N to the soil”. The priming effect can also be defined as organic compounds stimulating the soil microbial community to decompose more SOM (Bingeman et al., 1953).

Although many mechanisms have been proposed for priming, soil nutrient availability and microbial nutrient demand often strongly influences the responses (Dijkstra et al. 2013, Carrillo et al. 2014, Chen et al. 2014, Meier et al. 2017). The nutrient mining interpretation for priming is based on the idea that labile OM is used as an energy source which supports microbial activity, with microorganisms co-metabolizing SOM to release and obtain N from soil (Craine et al. 2007, Meier et al. 2017). This means that in N-poor Arctic and subarctic soils “microbial responses to inputs of labile OM may be driven by microbial demand for N” (Hicks et al., 2020). Hartley et al. (2010) in their study of subarctic mountain birch forest and tundra soils found that labile OM did have a priming effect on the decomposition of soil C.

They also discovered that this priming response was reduced when labile OM was added together with inorganic N (Hartley et al., 2010). This suggests that microbial demand for N caused priming (Hartley et al., 2010). So, because of shrub expansion in the Arctic it is possible that increased litter inputs combined with high C/N ratio could complicate N limitation to microorganisms which increase the susceptibility of N-poor soils to priming (Hicks et al., 2020).

Decomposition happens when microorganisms break down OM into its basic inorganic parts (Hicks et al., 2020). The mineralization rates of C and N are often assumed to be coupled but recently studies have discovered a strong decoupling of C and N mineralization after labile C has been added and microorganisms have specifically targeted the N-rich components of SOM (Murphy et al. 2015; Rousk et al. 2016; Ehtesham and Bengtson 2017). A study of boreal forest soils conducted by Wild et al. (2017) found that adding labile C increased the demand for microbial N, but it did not result in microbial N-mining from SOM. Instead, the microorganisms immobilized available N (Wild et al., 2017). Therefore, further studies are required so the N-control of SOM mineralization in response to labile OM inputs in high-latitude soils can be assessed (Hicks et al., 2020). It has been suggested that positive priming effects are especially important in N-limited ecosystems (Dijkstra et al., 2013) but studies on priming effects in boreal ecosystems are scarce (Linden et al., 2014; Linkosalmi et al., 2015). Additionally, it has been established that NH_4^+ causes larger priming effects than NO_3^- (Rennie and Rennie, 1973; Kowalenko and Cameron, 1978; Steele et al., 1980; Stout, 1995)

Stimulating SOM decomposition linked with microbial N-mining could explain why there is less soil C stored in subarctic forest soils compare to subarctic tundra, despite the forest having higher plant productivity (Hartley et al. 2012, Parker et al. 2015). On the contrary, N mineralization accelerating because of warmer temperatures (Salazar et al. 2020) could reduce microbial demand for N. This leads to microbial N-mining being reduced as a response to increased labile OM inputs in the rhizosphere (Hicks et al., 2020). Tree death from pest outbreak and possibly shrubification later could influence priming by increasing microbial demand for N. Based on the literature I hypothesise that pest outbreaks will increase priming, and will trigger thus N turnover, possibly leading to increased N_2O emissions, at least in the short term.

2.4 Disturbances including Insect Herbivory in Arctic Ecosystems

Outbreaks of pest insects cause defoliation and tree mortality (Jepsen et al., 2009). Insect outbreaks affect about 36.5 million hectares of the global forest area annually (Kautz et al., 2017) and are globally one of the most important disturbance factors (Jepsen et al., 2009).

The Food and Agriculture Organization (FAO, 2005) of the United Nations defines forest disturbances as "the environmental fluctuations and destructive events that disturb forest health and/or structure and/or change the resources or the physical environment at any spatial or temporal scale". These disturbances can be caused by fire, diseases, insect pests, and severe weather and they are important influences on forest ecosystems (van Lierop et al., 2015).

During normal circumstances, in healthy forests, disturbances caused by diseases and pest insects are an integral part of the forest ecosystem (Dajoz, 2000). Nonetheless, disturbances of catastrophic scales can have undesired effects on forest ecosystems and can affect environmental functions, which affect biodiversity and livelihoods and impacts on climate change (Schowalter, 2012).

Temperature is the dominant abiotic factor which directly affects herbivorous insects. The amount and range of forest insect pests is predicted to rise due to global warming (Bale et al., 2002). There seems to be a positive correlation between warmest summer month temperature and level of herbivore damage, especially in high latitude, cold-limited ecosystems (Kozlov et al., 2008, 2015). Large-scale insect outbreaks which have occurred in forests across the Northern hemisphere have been linked to warming climate, for example outbreaks of geometrid moth in Northern Fennoscandia (Jepsen et al., 2008). It is likely that factors such as competition, natural enemies, host phenology, climatic conditions, forest age structure, and resource distribution play a part (Berryman 1996; Ruohomäki et al. 1997; Ruohomäki et al. 2000; Niemelä et al. 2001; Selås et al. 2001).

Silfver et al. (2020) discovered during their two-year study that warming and herbivore reduction, on its own and together, increased mineral N availability in the soil by almost 8-fold by the end of the second full growing season. They also discovered that reduced herbivory had a stronger effect on N mineralization than warming and warming increased N availability only under natural herbivory (Silfver et al., 2020). Effects on N_2O were never studied so far.

Climate acts directly on an insect; either by determining the growth and development rate or as a mortality factor (Bale et al., 2002). There are also other various effects of climate change on insect herbivores which can be direct or indirect. Direct effects are such as impacts on physiology and behaviour and indirect effects are when insects respond to climate-induced changes mediated through other factors, mainly the host plant (Bale et al., 2002). The direct temperature effects are expected to be greater and more important than any other factor (Bale et al., 2002). Also, direct effects of increasing temperatures may well be greater in polar regions compared to temperate or tropical zones, reflecting the more serious environmental conditions, and the projection of considerably greater temperature rises in the areas in question (Hodkinson et al., 1998).

2.4.1 Autumnal Moth Epirrita Autmnata

The geometrid moth species autumnal moth (Epirrita autumnata) and winter moth (Operophtera brumata) are widespread in Fennoscandia (Jepsen et al., 2008). Their names reflect a difference in the timing of emergence of adult moths in autumn (Jepsen et al., 2009). The autumnal moth is a light grey coloured butterfly, and it has a dark crossline on its front wings. The adult moth's wingspan is 30 – 40 mm (Metla, 2005). The moths swarm from end of August until October, then the females lay their eggs on the branches of birch trees and the moths winter in the egg stage. The caterpillars hatch in spring in synchronisation with birch leaf flush (Kaitaniemi et al. 1997a; Kaitaniemi and Ruohomäki 1999). The caterpillars are green and can be 22 – 26 mm in size (Metla, 2005). They are highly polyphagous, meaning they feed on many types of foods (Ruohomäki et al., 2000). The caterpillars have been documented on more than 15 species of deciduous shrubs, dwarf-shrubs, and trees (Seppänen, 1970). The length of the caterpillar stage is highly variable; depending on temperature and forage quality it can last anything between just over two weeks up to almost two months (Ruohomäki et al., 2000). The caterpillars eat birch leaves until they pupate (Metla, 2005). They pupate before mid-June within a thin cocoon in the litter and this stage lasts until autumn (Ruohomäki et al., 2000). The moth's life cycle has adapted to latitudinal changes in summer length by adjusting the duration of the pupal stage. Finland being 1200 km in length from south to north, the length of the pupal stage differs from more than three months in the south to approximately 1.5 months in the north (Haukioja et al., 1988). The length of the pupal stage is partly genetically determined but additionally also influenced by environmental signals, at least by temperature (Harrison 1920; Peterson and Nilssen 1996; Tammaru et al. 1999).

The synchronisation of caterpillar and leafing phenology is important for the caterpillars' development since they can only maintain fast growth on young leaves (Haukioja et al. 1978; Ayres and MacLean 1987; Tammaru 1998; Kause et al. 1999b). The growth rate then affects many aspects of the moth's life: it is decisive in determining the caterpillar stage, pupal mass (Kause et al., 1999), and fecundity (Tammaru, 1998). Fecundity is determined by Bradshaw and McMahon (2008) as "the physiological maximum potential reproductive output of an individual

(usually female) over its lifetime". The fecundity of the moth is directly dependent on resources (Haukioja & Neuvonen, 1985a) which have accumulated during the caterpillar stage (Ruohomäki et al., 2000). According to Ruohomäki et al. (2000) "each milligram of additional mass is equivalent to ≈ 2.6 more eggs". The autumnal moth has a high reproductive potential which allows rapid population increases (Haukioja et al., 1988a). Thus, natural factors permit autumnal moth populations to initiate outbreaks. Nonetheless, these natural factors are not sufficient to explain the outbreaks (Tammaru & Haukioja, 1996).

The autumnal moth displays cyclic population outbreaks at approximately 10-year intervals, which cause widespread defoliation and occasionally, mortality of mountain birch forests in northern Fennoscandia (Kallio & Lehtonen, 1973). Northern Fennoscandia is situated in the arctic/alpine-boreal transition zone, including northern parts of Finland, Norway, and Sweden (Jepsen et al., 2009). A pronounced increase in mean annual temperatures has occurred in the entire region during the past 15 years (Jepsen et al., 2008). The increase is most noticeable in the northern and continental eastern parts. It is only in these coldest regions where winter temperatures potentially lethal to the overwintering eggs of autumnal moth are experienced (approx. -35°C, Macphee 1967; Tenow & Nilssen 1990). At the same time, the frequency of extreme winter cold occurring has decreased noticeably (Jepsen et al., 2008). The natural forest in Fennoscandia is dominated by mountain and pubescent birch (Betula pubescens czerepanovii Orlova; Betula pubescens Ehrh) (Hämet-Ahti, 1963) at the northern and alpine tree limit, and in the west. In the east, it is boreal mixed and coniferous forest. Birch is the main host tree to the autumnal moth in the region (Jepsen et al., 2008). It is in these northern-boreal birch forests where both, winter and autumnal moth, are the most important cause of disturbance (Jepsen et al., 2009).

The distribution range is defined as the "geographical area where the species has been found to occur" (Jepsen et al., 2008). The distribution range of the autumnal moth according to Tenow (1972) includes the entire northern Fennoscandia whereas the winter moth has been found in all lowland districts in Norway, in all of Sweden and in most of Finland, except in the most eastern districts. The distribution range of both moth species is larger than the region which experiences

regular outbreaks and consequently forest damage. This is defined as the outbreak range (Tenow 1972, Neuvonen et al., 1999). The distribution range is assumed to be determined by climate but the factors or combination of factors which permit population outbreaks in only some parts of the species' range is not understood (Jepsen et al., 2008).

Various factors such as competition, natural enemies, resource distribution, climatic conditions, host phenology, and forest age structure are likely to have an effect (Berryman 1996; Ruohomäki et al. 1997; Ruohomäki et al. 2000; Niemelä et al. 2001; Selås et al. 2001). The potential effect of warming climate has been of interest recently. How will global warming affect the outbreak dynamics and distribution of the outbreak range of these insects. It is an issue which has not yet been resolved and causes much debate (Bylund 1999; Neuvonen et al. 1999; Neuvonen, Bylund & Tømmervik 2005). The insect outbreak and insect death could boost N availability due to increased N input by insect feces, and leaf-litter in the topsoil which results in increased decomposition and easily available C (Sistla et al. 2013; Pausch and Kuzyakov 2018).

2.4.2 Moth Damage in Finland and its Effects

Out of different insect attacks in Finnish Lapland, the mass occurrences of the autumnal moth are known best; reports cover more than a century (Kallio & Lehtonen, 1973). There are reports of autumnal moth outbreaks occurring in 1927, 1955, 1965, 2004, and 2005 (Kallio & Lehtonen, 1973; Jepsen et al., 2009). Additionally, there are signs of even older damages. People of the Talvadas village and other villages in the Teno valley have talked about big birch damages which occurred in the first decade of the 20th century (Kallio & Lehtonen, 1973). Damage caused by autumnal moth may possibly have far reaching effects on the whole ecosystem (Lehtonen & Yli-Rekola, 1979). In low productivity mountain birch forests, the impacts of severe moth defoliation is especially damaging, and it may take decades before full regrowth of lost foliage occurs (Jepsen et al., 2013).

Utsjoki, the northernmost administrative district in Finland, is about 5000 km2 in size and situated south of the latitude 70° (Kallio & Lehtonen, 1973). In 1965 – 1966 it experienced an abrupt change in landscape of the subarctic birch zone ecosystem. The caterpillars of the autumnal moth defoliated an area of approximately 1350 km^2 in the Utsjoki area (Kallio & Lehtonen, 1973), and about 5000 km^2 in all Finnish Lapland (Lehtonen & Yli-Rekola, 1979). Jepsen et al. (2009) estimated that the outbreaks of 2004 ad 2005 defoliated 10 – 15% of birch forest in northern Fennoscandia. 10 600 km^2 of the forest was affected by severe defoliation during one or more years during those outbreaks (Jepsen et al., 2009).

The consequences of the 1965 – 1966 outbreak were severe, likely due to the preceding cold summers (Kallio & Lehtonen, 1973); the damage took place during a period when the average summer temperature was the lowest (Lehtonen & Heikkinen, 2009). Kallio & Lehtonen have argued that due to the lack of energy reserves the birch trees' defence and recovery capacity was rather weak, and over wide areas the main trunks were more or less permanently defoliated. The birch forest ecosystem experiences notable changes in the environmental conditions quite quickly after moth damage (Lehtonen & Yli-Rekola, 1979). Kallio & Lehtonen predicted in their paper in 1973 that the defoliated area will largely turn into treeless tundra. The tree layer vanishes, so illumination at the field and ground layers increases. Additionally, the amount of

nutrients in the soil increases notably because the production of debris is abundant after tree death, and the trees are no longer taking nutrients from the soil. In normally N-poor areas, the increase in N may be particularly prominent (Lehtonen & Yli-Rekola, 1979). This could cause N_2O emissions to rise, as studied here.

University of Turku's Kevo Subarctic Research Station, which is situated in Utsjoki, undertook a study to classify, and map the moth damage and to study its biology (Kallio & Lehtonen, 1973). They started their investigation of the damaged area in 1969, four years after the outbreak occurred (Kallio & Lehtonen, 1973). Excursions were made in the whole 5000 km2 area of Utsjoki in order to map the damage. Kallio & Lehtonen (1973) found centres of damage in the following areas: around the rivers Utsjoki-Kevojoki, Pulmankijoki in north-east Utsjoki, and around the river Kaamasjoki, the last being a less coherent area than the first two. There are also smaller areas as well and the mapping also included Inari, but it was studied less thoroughly (Kallio & Lehtonen, 1973). A damaged birch tree can recover to some extent; twigs and parts of the basal stem are able to form adventive shoots (Kallio & Lehtonen, 1973). In their mapping of damaged areas, Kallio & Lehtonen (1973) found that about half of the damaged areas had more than 90% of green destroyed. Typical areas of total damage are in the western part of Kevo Nature Park, also a high degree of damage is seen in some central parts of Pulmankijoki area and in Utsjoki Ailigas. However, the damage has not been as severe in areas east of the Utsjoki valley. Unfortunately, there are no continuous areas of high recovery (Kallio & Lehtonen, 1973). My study was conducted close to Pulmankijärvi.

Lehtonen (1987) studied the recovery, shoot formation, of the defoliated trees in the damaged areas. Lehtonen studied eight different experimental and control areas. The experimental and control areas are permanent study sites, and the fences' purpose is to eliminate reindeer grazing and to indicate its effects on recovery (Lehtonen, 1987). The field work was conducted in years 1973, 1979, and 1982. In the experimental areas, the degree of damage was estimated for the first time in 1970 and in control areas in 1973 (Lehtonen, 1987). According to Lehtonen (1987) the recovery of trees in experimental and control areas did not differ significantly in any of the years 1973, 1979, and 1982. However, through correlation calculations Lehtonen (1987)

discovered that the recovery process had begun soon after the damage and trees in well recovering areas maintained their vitality. Nonetheless, trees in the studied areas have been able to improve their state during 1973 – 1982 only in a few cases. In 10 out of 16 studied cases the total number of basal shoots had decreased and the number of totally shootless, meaning dead, trees has increased in almost all cases. Therefore, it can be stated that the recovery has been ineffective (Lehtonen, 1987).

When mountain birches are in question, the understanding of plant tolerance to insect herbivory is lacking. For many years, the interest has been focused on the altered chemical features of defoliated trees (Huttunen et al., 2012). The changes have related to induced plant resistance via herbivore attacks, mainly by autumnal moth, with most studies investigating the metabolic changes in the above-ground plant structures. Certainly, there are serious consequences to mountain birch physiology and metabolism caused by leaf damage (see e.g., Ruohomäki et al. 1997; Kaitaniemi et al. 1998; Lempa et al. 2004; Ruuhola et al. 2008). Reports concerning growth and survival of northern trees following above-ground damage have focused on carbon assimilation (Prudhomme 1982) or shoot elongation (e.g., Kaitaniemi et al. 1999). However, in plant-herbivore interactions the role of roots is often overlooked, even though their function as storage reserves and recovery promoters is indisputable (Huttunen et al., 2012). Therefore, continuous studies in the damage areas are needed (Lehtonen 1987).

2.5 Measurement Methods

To this day, the closed chamber technique is the most widely used measuring technique for quantifying N_2O fluxes. It is inexpensive and simple to use and allows for studying treatment effects as well as to carrying out specific process studies. Yet, it has some shortcomings due to effects on environmental conditions: plant damage, soil compaction, temperature effects and disturbance of diffusion gradients for example. Other faults include limited soil surface coverage (usually less than $1m^2$) resulting in the spatial heterogeneity often being insufficiently addressed, inserting of collars into soil and cutting of roots or regarding the temporal coverage of measurements (Butterbach-Bahl et al., 2013). Because of restricted manpower, the latter is often limited to weekly-to-monthly measurement intervals. This results in flux estimates during peak emission periods, following fertilizer application or during spring-thaw periods for example, being associated with high uncertainty (Butterbach-Bahl et al., 2013). Nevertheless, the closed chamber technique is widely used in N_2O studies globally and provides reliable results. It also provides a good means to connect flux with soil process studies, since both parameters can be studied simultaneously from the same plot.

Using automated chamber systems responds to the problem of temporal coverage in flux measurements but the problem of spatial representativeness is not so easily solved. Spatial variability occurs in both agricultural and natural systems (Butterbach-Bahl et al., 2002) and drivers are often small-scale changes in soil properties (texture, gas diffusivity, soil organic C, or water availability), nutrient availability, or plant cover (Butterbach-Bahl et al., 2013).

The N cycle closes by complete denitrification meaning the reduction all the way to N_2, which returns N_r to the stable pool in the atmosphere. Measuring the denitrification flux of N_2 is very difficult because of its high atmospheric background. Nitric oxide and N_2O fluxes have been measured more than N_2 fluxes but they do not provide comprehensive information on denitrification (Groffmann et al., 2006a). Even though the amount of data on actual nitrification and denitrification rates in soils is increasing, there is still little known about the production and consumption of N_2O as well as N_2 emissions at field to landscape scales (Butterbach-Bahl et al., 2013).

This lack of knowledge is mainly because of methodological problems of measuring N_2 production by denitrification and to unravelling N_2O production processes at field scale (Butterbach-Bahl et al., 2011). These methodological problems combined with the lack of knowledge at the process level is still hindering the assessment of disturbances and their effects on N cycling at regional to global scales (Butterbach-Bahl et al., 2013).

Detailed understanding of factors – and processes regulating N_2O dynamics in permafrost regions are still lacking and needs to be urgently improved. For example, only rare and inconclusive information is available about the microbial processes which produce N_2O in cold-climate ecosystems (Gil et al., 2017). During freeze-thaw cycles denitrification has been shown to be the predominant original source of N_2O production (Mørkved et al., 2006). More information on microbial pathways would be important to stimulate fluxes and responses of N_2O emissions from permafrost soils to climate induced changes.

Knowledge about the processes and fluxes of N_2O and Nr have advanced greatly in recent years. Nevertheless, the understanding of soil N cycling processes and the importance of microbial diversity, for example, regarding the extent and spatiotemporal dynamics of N_2O fluxes from soils, is yet incomplete (Butterbach-Bahl et al., 2013).

3 Study aim

The main aim of this study is to identify if tree death caused by insect outbreak has a significant effect on N_2O emissions and whether this is changing over time (decades).

To reach this aim, this study focuses on three objectives:

1. To measure and quantify N_2O fluxes from three different mountain birch sites in Finnish Lapland that have been affected by autumnal moth outbreak in the past.
2. To identify possible environmental drivers of these N_2O fluxes such as temperature, soil moisture, and nutrient availability.
3. To identify possible dynamic over time after the tree death by comparing localised tree statuses such as living, dead, and treeless tundra.

I hypothesise that there will be significant N_2O fluxes as the study site is in a subarctic region and as moth damage has dramatic, long-lasting effects on plant growth. I further speculate that living trees will have the lowest N_2O dynamics due to lower availability of nutrients because of competition between plants and microbes for N and treeless tundra will have the highest emissions because of more nutrients available. Priming of N turnover due to increased litter input as the result of dead wood of trees, could intensify this effect.

4 Materials and Methods

4.1 Study site

The field experiment was conducted between 1st and 18th July 2019. The research site Utsjoki (69°9'N, 27°9'E, 130-156 m a.s.l) was located 12 km south of the Nuorgam village and approximately 5 km west of lake Pulmankijärvi, subarctic Finnish Lapland. The average annual temperature was -1.6°C and mean annual precipitation was 448 mm during the period 1981-2010, measured in Utsjoki, i.e., 35 km west of the study site (Finnish Meteorological Institute, 2020). In this area the defoliation caused by the autumnal moth is more than 90% according to Kallio & Lehtonen (1973).

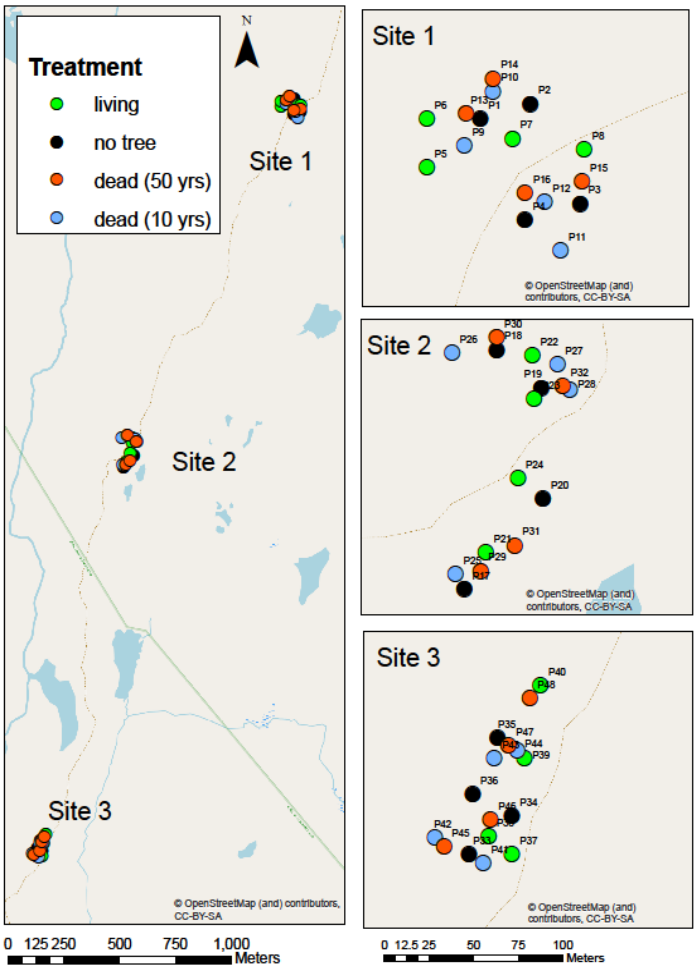

Photo 1. Map of study site including plots, with overview of all three sites and sites 1 -3 more specifically. Map by Nele Meyer.

There were three field sites, approximately two kilometres apart and 1 ha in size, which were damaged by the insect outbreak. The sites were selected based on three factors: the site had to contain all tree types (treatments), the sites had similar altitudes to one another, and the sites were not on a slope but as flat as possible (personal communication with Nele Meyer).

There were 16 plots on each site: 48 plots in total. Plots were numbered in numerical order; plots 1 to 16 were on site 1, plots 17 to 32 were on site 2, and plots 33 to 48 were on site 3. There were four different tree types: treeless tundra, living, dead 12 years, and dead 55 years and four replicates of each tree. Treeless tundra means the area was naturally treeless. Dead 12 refers to the severe autumnal moth outbreak which affected the trees between 2006 and 2008 and dead 55 refers to the moth outbreak which occurred between 1960 and 1965 (Meyer et al., 2021, unpublished). The trees were selected based on the distance to other trees and representativeness of the treatment. There was at least two metres between selected trees and the dead trees could not have any leaves on them (personal communication with Nele Meyer). Below, Tables 1. and 2. show precipitation and temperature data for years 2017 to 2020 from the area.

Table 1. Monthly precipitation in millimetres from May to September for years 2017 to 2020 in Utsjoki Nuorgam. Data from Finnish Meteorological Institute.

	2017	**2018**	**2019**	**2020**
May	14.9	18.1	55.2	29.1
June	59.8	54.3	31.5	39.0
July	130.3	93.4	29.6	94.3
August	99.0	89.4	46.4	62.2
September	55.6	70.8	23.9	31.6

Table 2. Monthly temperatures in °C from May to September for years 2017 to 2020 in Utsjoki Nuorgam. Data from Finnish Meteorological Institute.

	2017	**2018**	**2019**	**2020**
May	2.0	6.8	3.8	4.0
June	7.6	9.0	8.1	10.2
July	13.0	16.3	11.5	13.1
August	10.4	11.2	10.4	10.7
September	6.8	7.3	6.9	7.3

4.2 Chamber Measurements

Chamber gas flux measurements were done following the method published by Maljanen et al. (2006) and were conducted only at site 1 and measurements were taken once a week over the three-week period. Measured gases were N_2O, CH_4, and CO_2. The chambers were placed over the underground vegetation which appeared similar between the tree types. Greenhouse gas fluxes from all ecosystem components excluding the trees were thus measured. Measurements were taken approximately 50 cm from the selected tree.

Chambers were made of steel ventilation pipe and a plastic bucket. The height of the chambers was 24 cm with an inside diameter of 31 cm. The lid was inserted into the other end of the pipe tightly. A hole was made into the basin for a rubber plug. Two plastic tubes were put through the plug; one for levelling the pressure and one to insert the 3-way valve syringe, which was used to take the gas sample. The syringes used had a volume of 30 ml. Also, a small fan was inserted inside the chamber for ventilation purposes. The fan was connected by wires to a battery while measurements were done to ensure a well-mixed headspace gas sample.

The chambers were inserted into the ground directly without collars. Chambers were inserted into the ground and measurements were taken 50 cm from the tree. The plots were selected on the first day of field work and thus, places for chambers were determined. The chambers were inserted into the ground after using a knife to cut the thick organic layer. After inserting the chambers, their height was measured in order to be able to determine the exact headspace volume. Samples were taken at time intervals 5, 20, 35, and 50 minutes. Syringes were flushed 2 -3 times by quickly pulling and pushing the syringe piston before beginning to take gas samples. At each time interval, 15 ml of sample was taken into the syringe and then transferred into pre-evacuated glass vials. The inside temperature of the chamber was measured at the end of each measurement.

Photo 2. Chamber measurement set-up with field template and thermometer with soil probe.

4.3 Soil Gas Concentration

Nitrous oxide, CH_4, and CO_2 fluxes were also determined by diffusion gradient method following Maljanen et al. (2003). The protocol (NOCA protocol, version 1.) was established in the Biogeochemistry Research Group at University of Eastern Finland and provided to me by my supervisors. For full protocol, see supplementary information (Appendix 3.). With this method, soil gas is withdrawn via a metal probe into a syringe and then transferred into a glass vial.

Soil gas samples were taken once on all three sites. Samples were taken from 3 out of 4 plots per treatment type and one sample per depth per treatment per plot. In total, there were 36 soil gas samples. Gas samples were taken from four different depths: 2, 5, 10, and 20 cm. The metal probe had the depths marked with thin lines and there was also a rubber disk around the probe, which could be set at the required depth. There were four plots at which a sample from 20 cm could not be taken due to the soil being too rocky (plots 17, 28, 35, and 36). A 3-way valve syringe (volume 30 ml) was connected to the soil gas probe and both valves were turned so that gas can flow from the probe to syringe. The syringe was flushed 2 – 3 times by quickly pulling and pushing the syringe piston before starting to take gas samples. The rubber disk on the probe was set to the first sampling depth of 2 cm and the probe was carefully pushed into the soil. The syringe piston was pulled slowly backwards, sampling only 1 – 2 ml of gas. The 3-way valve was opened to the atmosphere and the piston was pressed to remove the gas used to flush the probe.
The valve was turned back to its previous position and the syringe piston pulled backwards until about 15 ml of gas was taken. After this, the 3-way valve of the syringe was closed in order to disconnect it from the probe without leakage. An injection needle was attached to the syringe so that the gas sample could be transferred into a glass vial.

For dead 12 years trees there are results from both measurements only from the first two weeks because of losing the gas chromatograph sample list.

4.4 Soil Sampling

Soil sampling was done on all three sites according to standard protocols used at the Biogeochemistry Research Group at UEF (e.g., Voigt et al. 2017).

The location for the soil sampling pit was right next to the soil gas sampling spot. A spade was used to dig a hole, with depth of approximately 30 cm. The digging was done carefully as to trying to keep one side of the soil profile “clean” and intact, avoiding mixing the soil horizons. A photo was taken of each soil profile. Soil samples were taken from depths of 0 – 5 cm, 5 -10 cm, and 10 – 20 cm. Samples were taken by using a volumetric ring.

For the first layer, above ground vegetation was removed from the sampling area and the soil ring was pushed vertically into the soil. Then by turning the soil ring, within the soil, the soil inside the core was disconnected from the surrounding soil.

For depths 5 -10 and 10 – 20 cm, the soil ring was pushed horizontally into the soil wall, with the centre point being at sampling depth. For both depths, the soil ring was turned within the soil so that it was disconnected from the surrounding soils. After this, the soil ring was gently pulled out from the soil. Clearly different soil horizons were not mixed, but either separated into organic and mineral soil after the sample was taken or if the horizons were very distinct then separate samples of each were taken. The samples were placed into resealable plastic bags marked with depth, soil type, and plot number. The fresh weight of each sample was weighed at the end of the day at the Kevo Subarctic Research Institute laboratory.

4.5 Environmental Parameters

Auxiliary measurements such as air temperature, soil temperature at depths of 2, 5, 10, 15, and 20 cm, and soil moisture were measured at each plot every time both chamber and soil gas measurements were done.

Air temperature was measured by holding the thermometer at hip height pointing away from the body. The thermometer was held until the reading stabilised, then it was recorded. Soil temperatures from five different soil depths were measured too. Soil temperatures were measured close to the chambers and with soil gas measurements often from the exact same spot. The thermometer with soil probe was pushed into the ground at each depth and it could stabilize for a few minutes before recording the temperature.

Soil moisture was measured at three different spots near the chamber and soil gas probe (Theta Probe). The moisture metre was pushed into the ground and the reading was recorded. Of the three readings, an average was calculated and used. From soil samples, pH and electric Conductivity (EC) (WTW, Xylem Analytics, Germany) were determined. The pH and EC were measured in water by using a pH and electric conductivity meter. Both readings could stabilize before recording them.

4.6 Laboratory Analysis

Gas samples were analysed by a gas chromatograph (Agilent, USA) and results were calculated using Excel. Nitrate (NO_3^-) and ammonium (NH_4^+) levels were determined according to Carter and Gregorich (2008) from soil extracts.

In short, soil samples were extracted by weighing 30 ml of soil into extraction flasks and adding 100 ml 1 M KCl (potassium chloride) into the flasks. Flasks were closed and placed on to rotary shaker for one hour. While flasks are shaking, filters are placed in a beaker filled with milli-Q water for 30 minutes. After this, they are placed into funnels with tweezers and 100 ml sample bottles are placed below each funnel. After shaking for one hour, the soil was poured into funnels and the funnels were covered with aluminium foil to prevent evaporation. The samples were filtered overnight.

A standard series was prepared by diluting from nitrate stock solution (100 nmol/l) and from ammonium stock solution (1000 mg NH_4+-N l^{-1}). For the nitrate standard series, the stock solution was diluted to concentrations of 500, 250, 125, 62.5, 31.25, 15.63, 7.81, and 3.91 µmol/l. For the ammonium standard series, the stock solution was diluted to the concentrations of 5.0, 2.5, 1.25, and 0.3125 mg/l.

For nitrate analysis, 100 µl of sample, standards, and blanks were pipetted into a 96-well microtiter plate. 100 µl of Griess reagent and 20 µl VCl3 were added. The samples were incubated at 37°C for 90 minutes. After incubation, the absorbance of purple dye was measured at 540 nm (1420 VICTOR3™ plate reader, PerkinElmer Inc., USA). Then, for ammonium analysis, 50 µl of each sample and standard were pipetted into wells on the microtiter plate. Under a fume hood, 50 µl of sodium-phenate, 75 µl of 0.01 % sodium-nitroprusside, and 75 µl of 0.02 M sodium-hypochlorite were added into each well. The blue colour was allowed to develop for 30 minutes at room temperature before measuring the absorbance. The absorbance was measured at 650 nm (1420 VICTOR3™ plate reader, PerkinElmer Inc., USA).

4.7 Flux Calculations and Statistical Analysis

Gas fluxes were calculated from analysed gas concentrations using Microsoft Excel. Gas concentrations were subject to quality control which meant setting a specific R^2 value as a limit. For N_2O, the R^2 value was set at 0.8 and nothing under that value was accepted into calculations. For CO_2 and CH_4 the R^2 values were 0.98 and 0.95.

Statistical analysis was conducted by SPSS Statistics 25 -programme (IBM, USA). Statistical analysis was done with the results from gas and soil samples. Statistical analysis was done to test for normal distribution, correlations, and significant differences. Normal distribution was determined by interpreting the Shapiro-Wilk significance ($p > 0.05$) because of the data set being small; 48 samples for each variable. N_2O ($p = 0.348$) and CO_2 ($p = 0.158$) fluxes followed normal distribution, but CH_4 fluxes were low overall as was the significance ($p = 0.047$) and thus, the fluxes were not normally distributed. NO_3^- was normally distributed ($p = 0.113$) but NH_4^+ and Nmin were not ($p = 0.000$). Soil moisture and WFPS also followed normal distribution, having p -values of 0.244 and 0.270. The variables which did not follow normal distribution were transformed to log 10 scale.

Homogeneity of variance was tested, and the p-value had to be greater than 0.05 so that the assumption of homogeneity of variance was met. N_2O, CO_2, and CH_4 all had a p-value greater than 0.05. However, NO_3^-, NH_4^+, and Nmin all had p-values lower than 0.05. The one-way ANOVA test was used to test for any statistically significant differences between groups. Due to all the data not being normally distributed and not meeting the assumption of homogeneity of variance, two post-hoc tests were selected and used to determine significant differences between treatments. The least significant difference (LSD) post-hoc test was used for N_2O, CO_2, and CH_4. The Games-Howell post-hoc test was used for NO_3^-, NH_4^+, and Nmin.

The data was also tested for correlations between variables. The Pearson correlation coefficient was used for normally distributed variables and for other variables, Spearman's rank-order correlation coefficient was used. For correlation matrixes, see Appendix 1. and 2.

5 Results

5.1 Flux Results

The fluxes of all three gases varied between treatments. Generally, CO_2 fluxes were highest, followed by CH_4 and finally N_2O. In Table31. the range of fluxes is presented per treatment for each greenhouse gas.

Table 3. Range of fluxes per treatment.

	CH_4_[mg/m2d]		**CO_2_[mg/m2d]**		**N_2O_[µg/m2d]**	
	MIN	**MAX**	**MIN**	**MAX**	**MIN**	**MAX**
Treeless tundra	-3.62	-0.23	2350	7873	-8.08	83.3
Living	-2.91	-0.27	2508	6047	-36.2	54.4
Dead12	-9.39	-0.32	3436	8005	6.68	36.8
Dead55	-3.90	-0.36	2941	8393	-13.9	29.6

5.1.1 N_2O

All calculated N_2O fluxes were below detection limit (100 µg m^{-2} d^{-1}) and therefore have limited validity. However, as some ANOVA analysis indicated significant differences, I will discuss treatment differences with reservations. The fluxes ranged from -36.2 to 83.3 µg N_2O m^{-2} d^{-1} (Table 3.), and mean N_2O flux measured during the three-week campaign for treeless tundra, living, dead 12, and dead 55 was 40.1, 13.5, 22.1, and 17.1 µg N_2O m^{-2} d^{-1}.

Treeless tundra was significantly higher compared to living tree (p = 0.043) and dead 55 was significantly lower compared to treeless tundra (p = 0.049). There was a moderate, positive correlation between N_2O and WFPS % (0.451). There was a weak negative (-0.384) correlation between N_2O and CO_2. Overall, there was a temporal decrease in fluxes within the treeless tundra and living tree treatments. In dead 55 tree type there was a temporal increase over time.

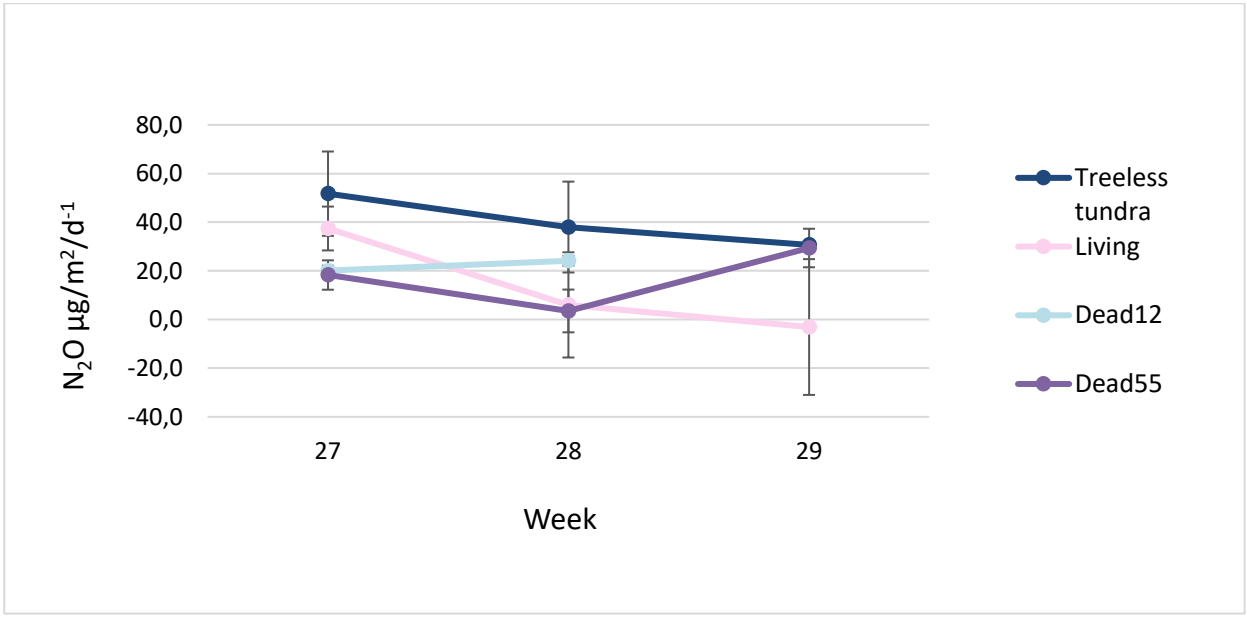

Figure 1. Graph representing N_2O fluxes from treeless tundra, living, dead12, and dead55 trees as µg/m2/d over three-week measuring period from a tundra ecosystem impacted by insect outbreak. Treeless tundra means naturally treeless area, living trees are trees that are growing and viable, dead 12 trees were affected by an insect outbreak during 2006-2008, and dead 55 trees were affected by an insect outbreak during 1960-1965.

5.1.2 CH_4

All calculated CH_4 fluxes were also below detection limit. The fluxes ranged from -9.40 to -0.23 mg CH_4 m^{-2} d^{-1} (Table 3.), and mean CH_4 flux measured during the three-week campaign for treeless tundra, living, dead 12, and dead 55 was -2.06, -1.04, -1.64, and -1.62 mg CH_4 m^{-2} d^{-1}. There were no significant differences between tree types nor any correlations between CH_4 and other variables.

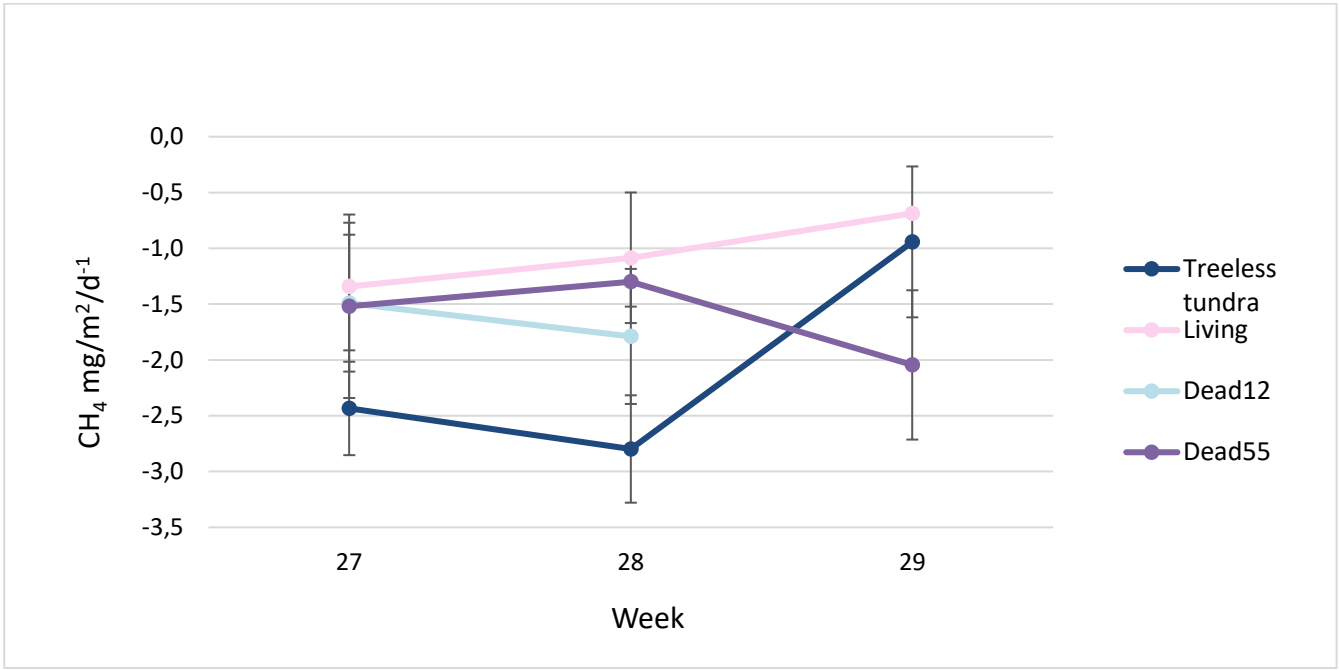

Figure 2. Graph representing CH_4 fluxes from treeless tundra, living, dead12, and dead55 trees as µg/m2/d over three-week measuring period from a tundra ecosystem impacted by insect outbreak. Treeless tundra means naturally treeless area, living trees are trees that are growing and viable, dead 12 trees were affected by an insect outbreak during 2006-2008, and dead 55 trees were affected by an insect outbreak during 1960-1965.

5.1.3 CO_2

Calculated CO_2 fluxes ranged from 2350 to 8390 mg CO_2 m^{-2} d^{-1} (Table 3.), and mean CO_2 flux measured during the three-week campaign for treeless tundra, living tree, dead 12, and dead 55 was 5078, 4669, 4766, and 6010 mg CO_2 m^{-2} d^{-1}. Living tree was significantly lower compared to dead 55 treatment (p = 0.031). Treeless tundra and dead 12 treatment were in the mid-range with respect to CO_2 fluxes. There was a weak positive correlation between CO_2 and NH_4^+ (0.344) and Nmin (0.339).

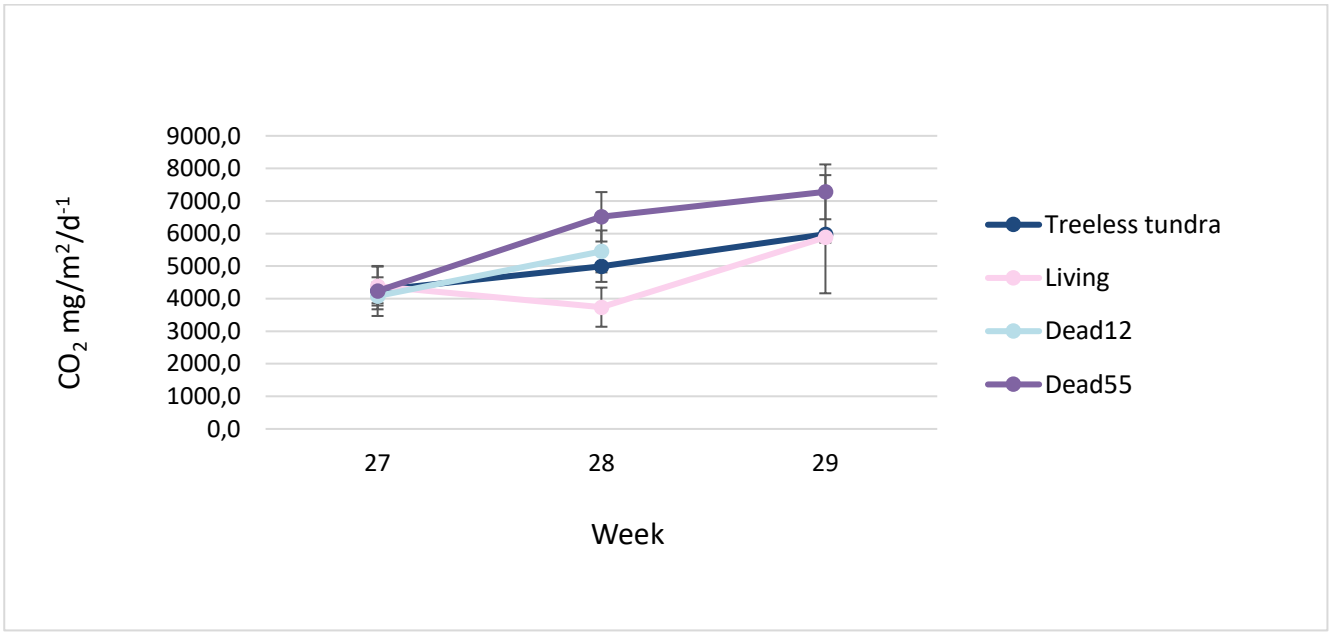

Figure 3. Graph representing CO_2 fluxes from treeless tundra, living, dead12, and dead55 trees as µg/m2/d over three-week measuring period from a tundra ecosystem impacted by insect outbreak. Treeless tundra means naturally treeless area, living trees are trees that are growing and viable, dead 12 trees were affected by an insect outbreak during 2006-2008, and dead 55 trees were affected by an insect outbreak during 1960-1965.

5.2 Ammonium and Nitrate levels

5.2.1 Mineral Nitrogen Content

Mineral N content was calculated per hectare by summing up the NH_4^+ and NO_3^- content from all samples taken over one profile and taking bulk density into account. The results are presented per site and per treatment (Fig. 4.). The average nutrient content for treeless tundra, living, dead 12, and dead 55 was 31.8, 42.5, 24.0, and 32.2 kg NO_3^- and NH_4^+/ha/profile depth.

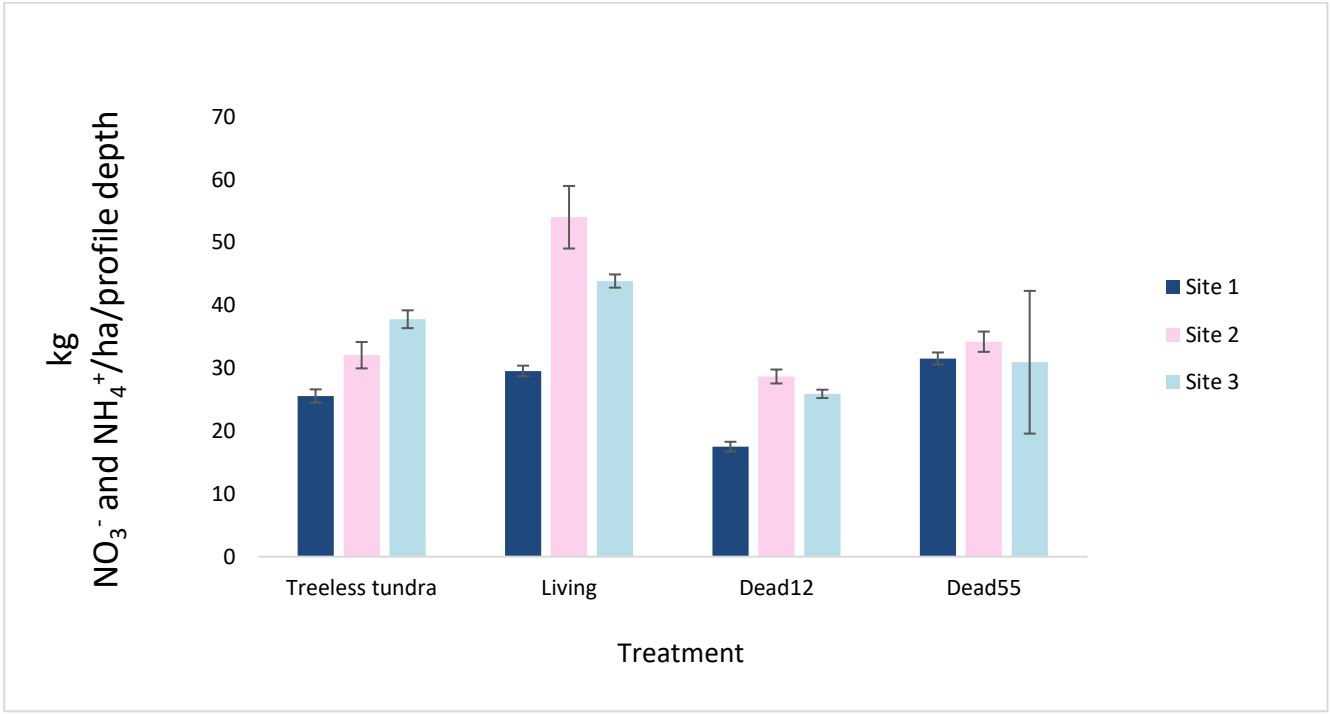

Figure 4. Mineral N content as kg NO_3^- and NH_4^+/ha/profile depth from all 3 sites impacted by insect outbreak in subarctic tundra. For explanation of treatments, see caption of figure 1.

5.2.2 Mineral nitrogen concentrations

Total mineral nitrogen (Nmin) was calculated on a per kilogram soil basis (Fig. 5.) and ranged from 0.00 to 48.5 mg Nmin/kgDW. When averaged across all three sites, the lowest amount was in dead 12 treatment and the highest in dead 55 treatment; this trend was also true when site 2 was excluded (Fig. 5.). With total mineral nitrogen there were statistically significant differences between treatments. Treeless tundra was significantly lower (p = 0.042) compared to living treatment. Dead 55 treatment was significantly higher (p = 0.000) compared to all other treatments; treeless tundra, living, and dead 12.

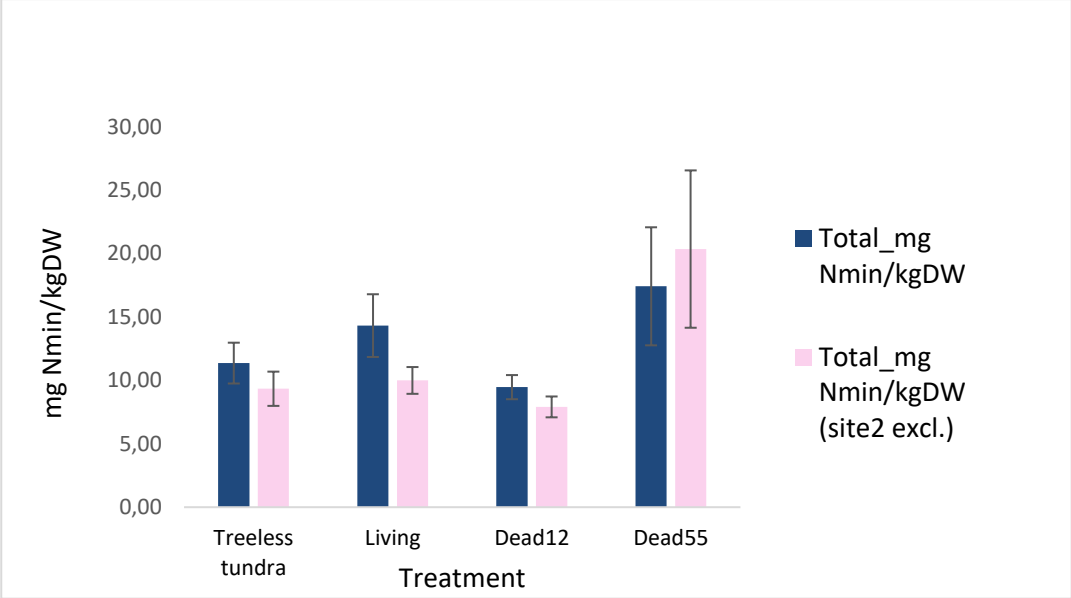

Figure 5. Total mineral nitrogen presented per treatment with standard deviation from all 3 sites impacted by insect outbreak in subarctic tundra. For explanation of treatments, see caption of figure 1. Results presented also with site 2 excluded.

Nitrate and NH_4^+ also had significant differences between treatments. With NO_3^-, treeless tundra was significantly lower (p = 0.002) compared to living treatment. Living treatment was significantly higher compared to both dead 12 and dead 55 treatments (p = 0.004, p = 0.000). With NH_4^+, dead 55 treatment was significantly higher (p = 0.00) compared to all other treatments on sites 1 and 3, but not on site 2. There was a positive correlation between NO_3^- and NH_4^+ (0.441). Both NH_4^+ and Nmin were negatively (-0.480, -0.430) correlated to N_2O.

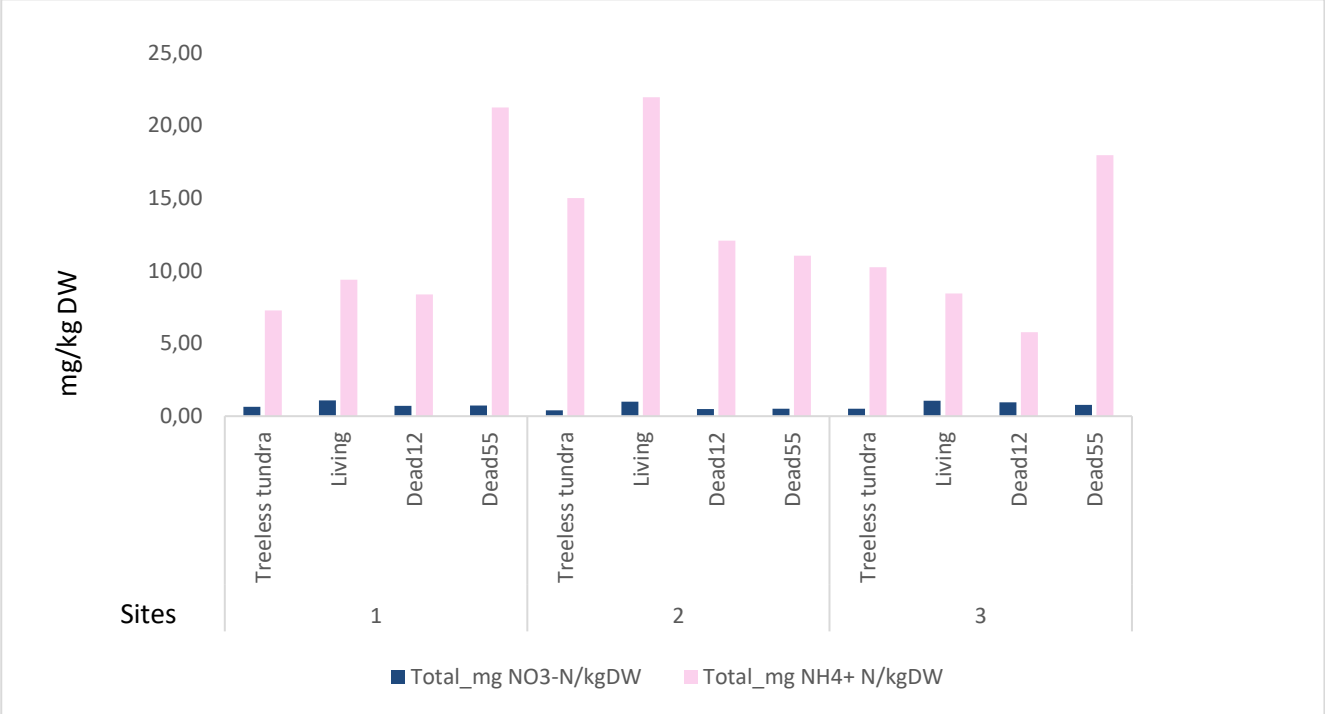

Figure 6. Total NH_4^+ and NO_3^- presented from all 3 sites impacted by insect outbreak in subarctic tundra. For explanation of treatments, see caption of figure 1.

Figures 7. and 8. show the amount of NO_3^- and NH_4^+ as a percentage of total mineral nitrogen. The amounts are shown in organic and mineral soil separately. From both figures (7. & 8.) it is clear that NH_4^+ is dominant over NO_3^-. The amount of NO_3^- is slightly higher in organic soil, approximately between 4 – 8 %, but in mineral soil it is very low; only 2 – 5 %.

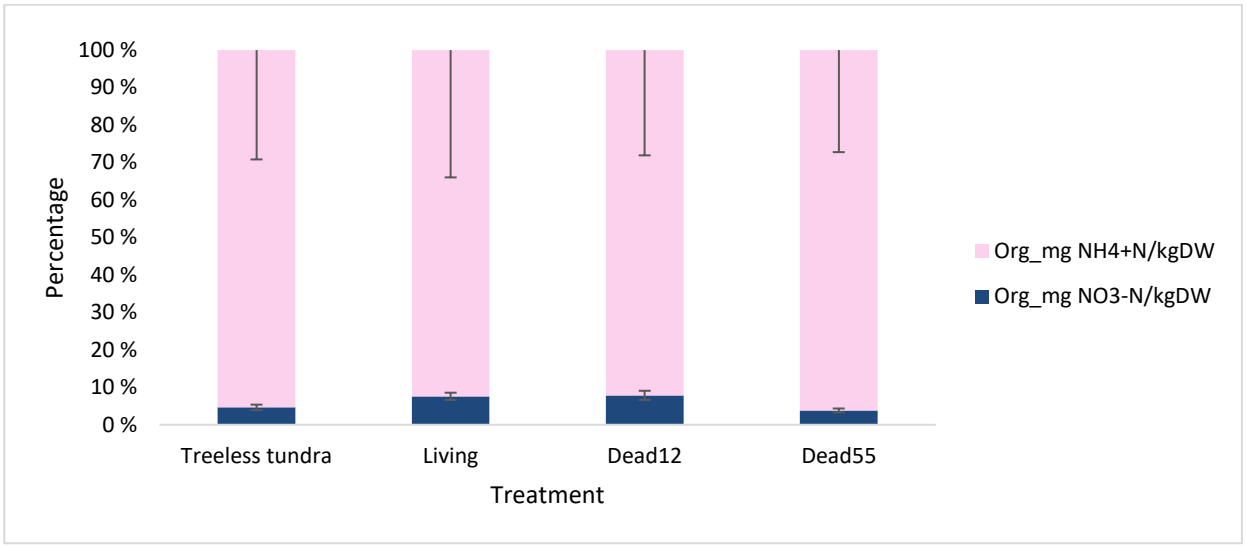

Figure 7. Percentage of NH_4^+ and NO_3^- in organic soil per treatment from all 3 sites impacted by insect outbreak in subarctic tundra. For explanation of treatments, see caption of figure 1.

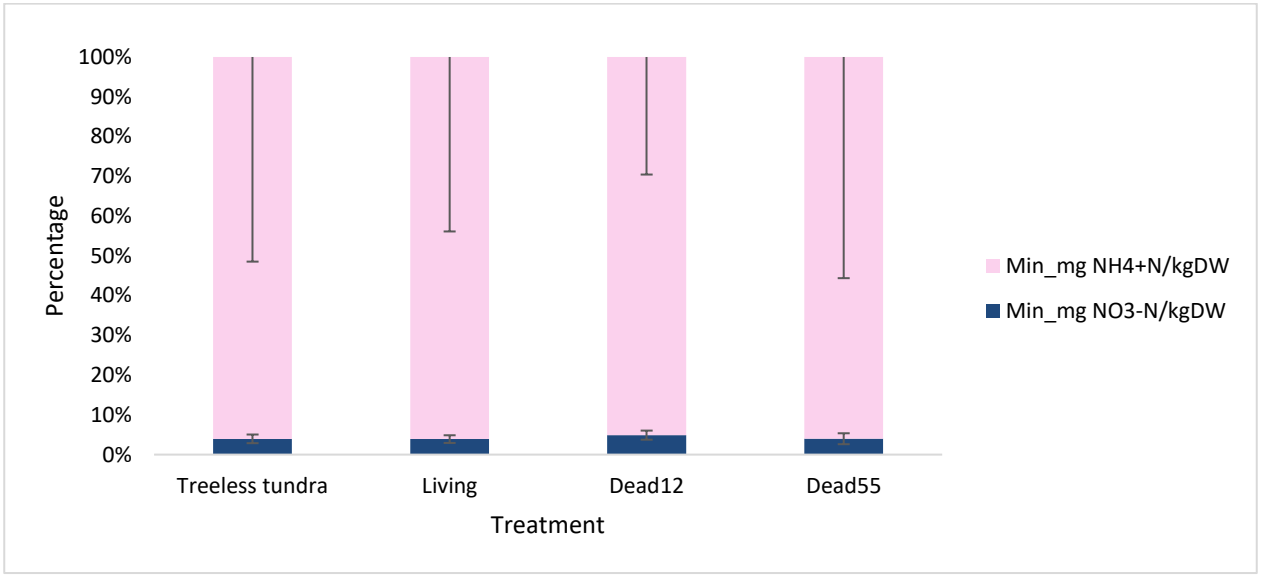

Figure 8. Percentage of NH_4^+ and NO_3^- in mineral soil per tree type from all 3 sites impacted by insect outbreak in subarctic tundra. For explanation of treatments, see caption of figure 1.

5.2.3 C:N Ratios

Figure 9. and 10. show the total soil C:N ratio from all sites per treatment individually for each site, and average C:N ratios for each treatment. The C:N ratios of the soils ranged from 26.9 to 34.1. As can be seen, the difference between the sites were larger than the differences between the treatments; there were no statistically significant differences between the treatments.

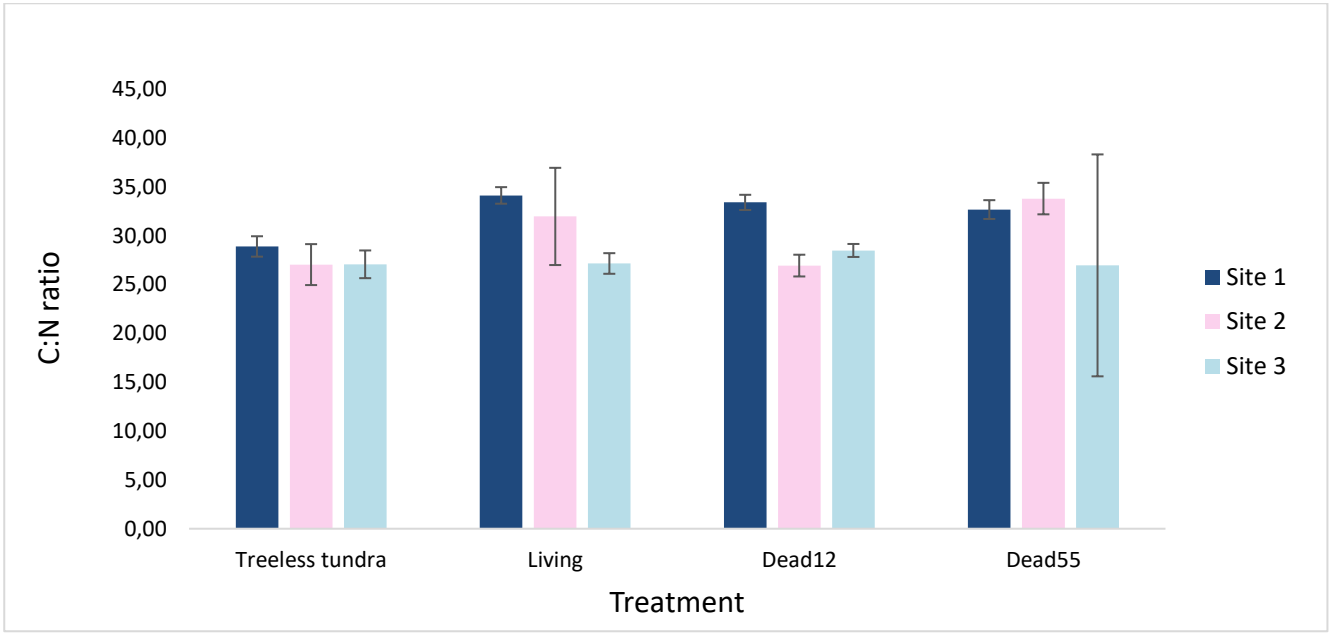

Figure 9. C:N ratio per site per treatment from all 3 sites impacted by insect outbreak in subarctic tundra. For explanation of treatments, see caption of figure 1.

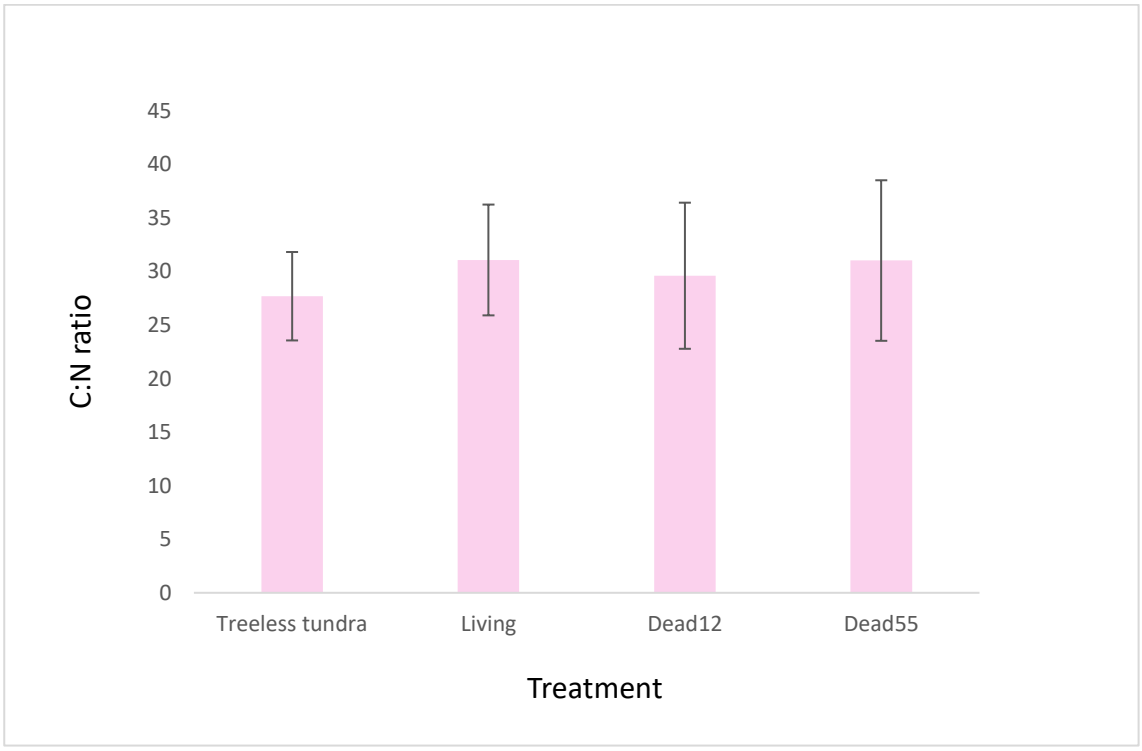

Figure 10. C:N ratio per treatment from all 3 sites impacted by insect outbreak in subarctic tundra. For explanation of treatments, see caption of figure 1.

5.3 Soil Gas Concentrations

5.3.1 N_2O and CH_4 concentrations

Nitrous oxide soil gas concentrations ranged from 0.32 to 0.73 ppm (Fig. 11.) for all depths and treatments. Treeless tundra had highest concentrations when measured from sites separately and also when all measured fluxes were averaged across all three sites (fig. 11. & 12.). There, the concentrations increased with depth. There were no significant differences between the treatments.

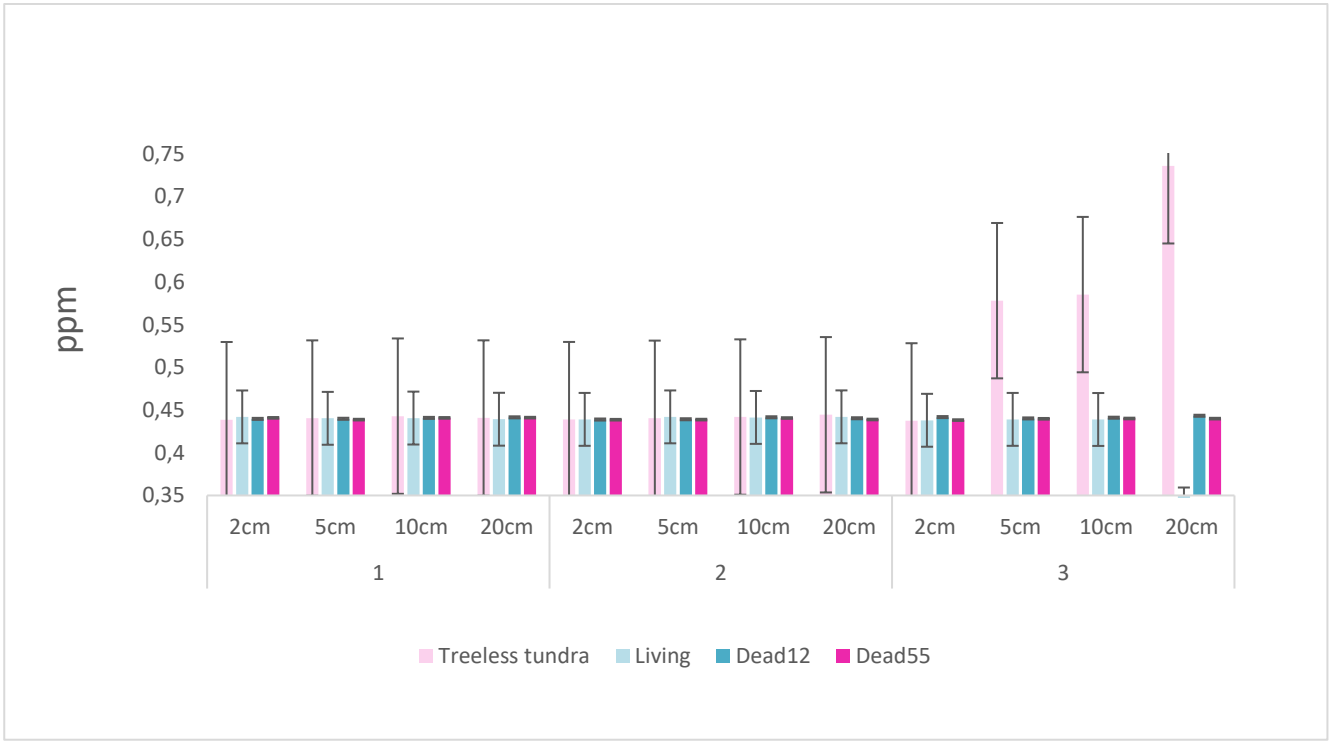

Figure 11. N_2O soil gas concentrations from soil depths of 2, 5, 10, and 20 cm from all 3 sites impacted by insect outbreak in subarctic tundra. For explanation of treatments, see caption of figure 1.

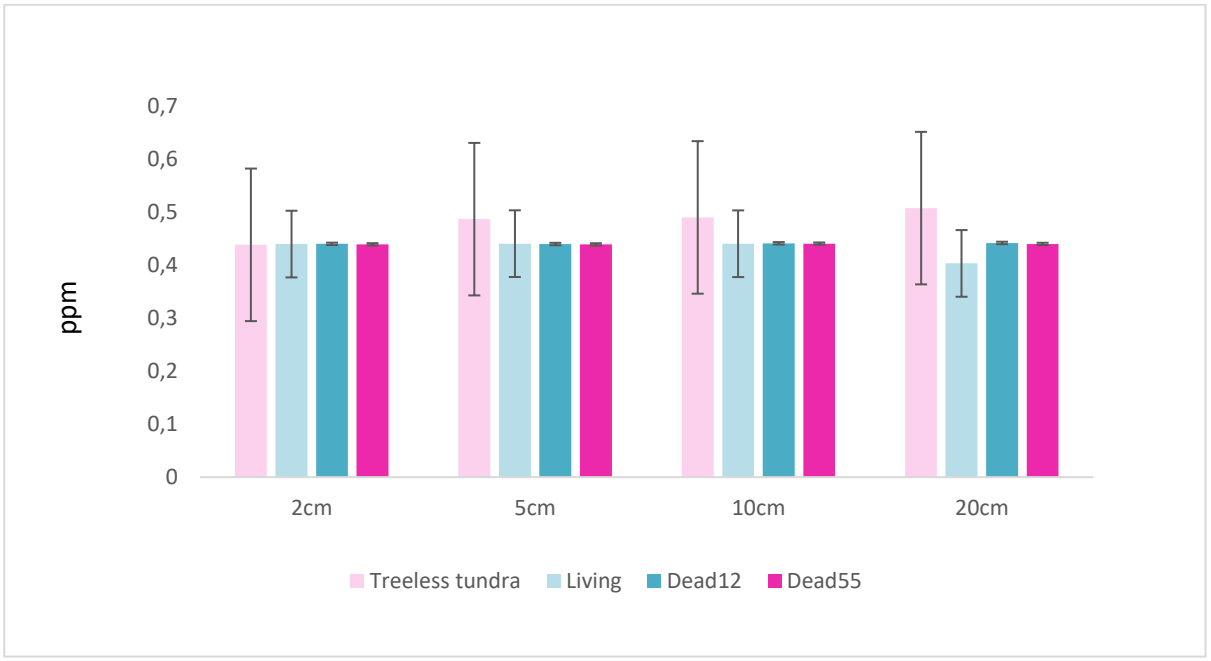

Figure 12. N_2O soil gas concentrations from soil depths of 2, 5, 10, and 20 cm averaged across all 3 sites impacted by insect outbreak in subarctic tundra. For explanation of treatments, see caption of figure 1.

Methane concentration decreased tendentially with depth, from ambient concentration in surface layers to 1.90 ppm at 20 cm depth (Fig. 13. & 14.). The concentration at 2 cm depth was significantly higher compared to the concentration at 5 cm depth ($p = 0.002$) and both 10 and 20 cm depths ($p = 0.000$). There were no significant differences between the treatments at any depth.

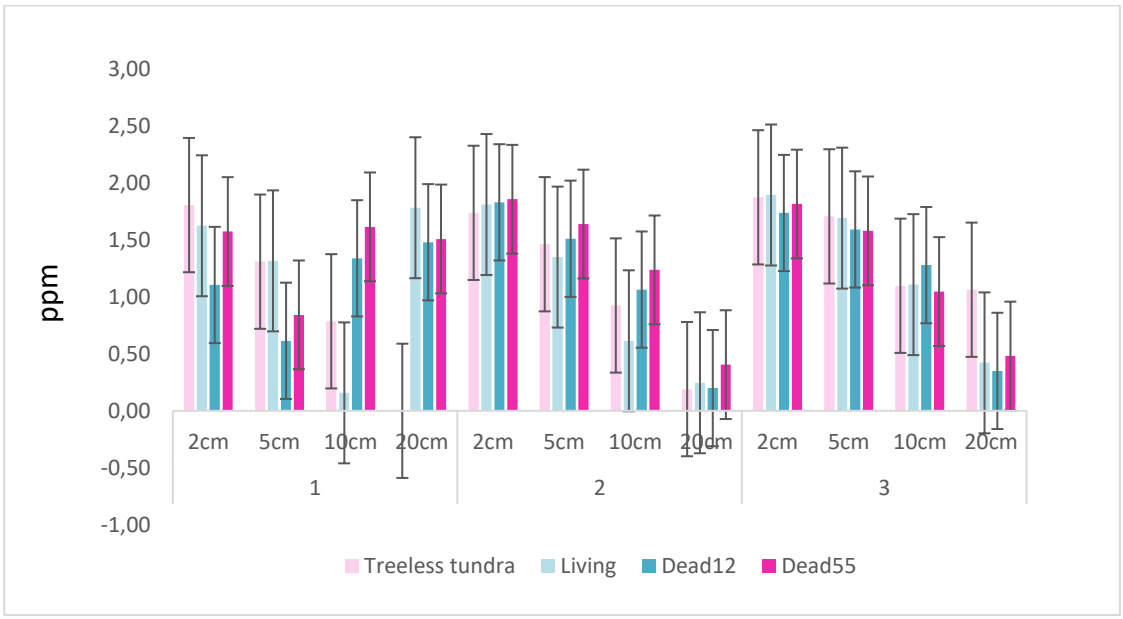

Figure 13. CH_4 soil gas concentrations from soil depths of 2, 5, 10, and 20 cm from all 3 sites impacted by insect outbreak in subarctic tundra. For explanation of treatments, see caption of figure 1.

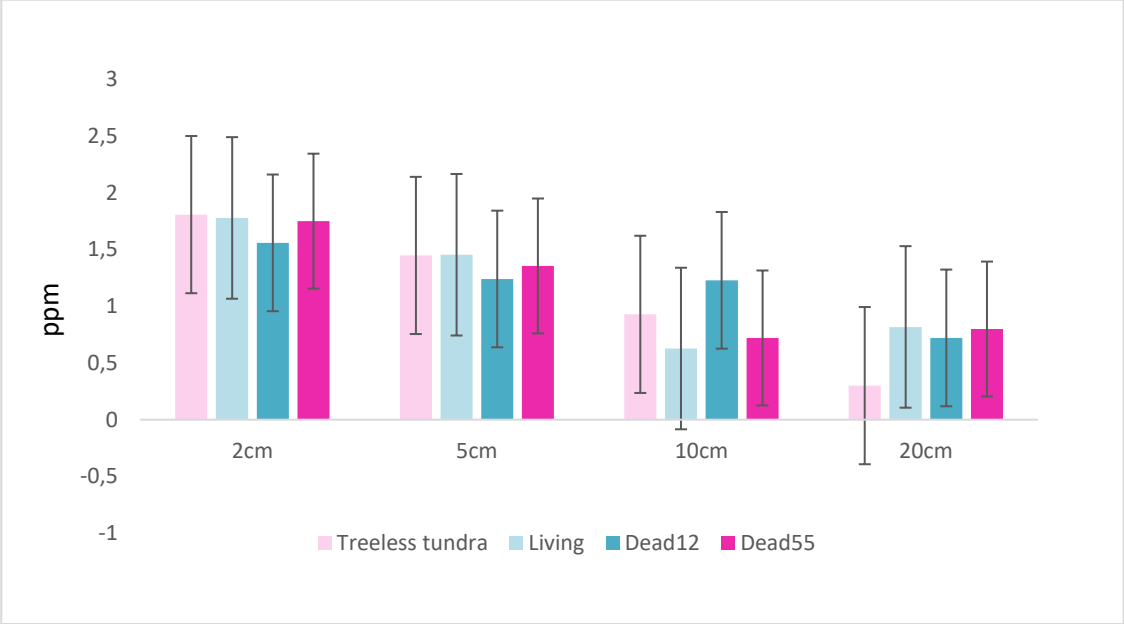

Figure 14. CH_4 soil gas concentrations from soil depths of 2, 5, 10, and 20 cm averaged across all 3 sites impacted by insect outbreak in subarctic tundra. For explanation of treatments, see caption of figure 1.

5.3.2 CO_2 concentrations

Carbon dioxide concentrations ranged from 485 to 1698 ppm, being highest at 20 cm depth and lowest at 2 cm depth (Fig. 15. & 16.). The concentration at 20 cm depth was significantly higher compared to all other depths (p = 0.000). (Fig. 16). Again, there were no significant differences between treatments at any depth.

Figure 15. CO_2 soil gas concentrations from soil depths of 2, 5, 10, and 20 cm from all 3 sites impacted by insect outbreak in subarctic tundra. For explanation of treatments, see caption of figure 1.

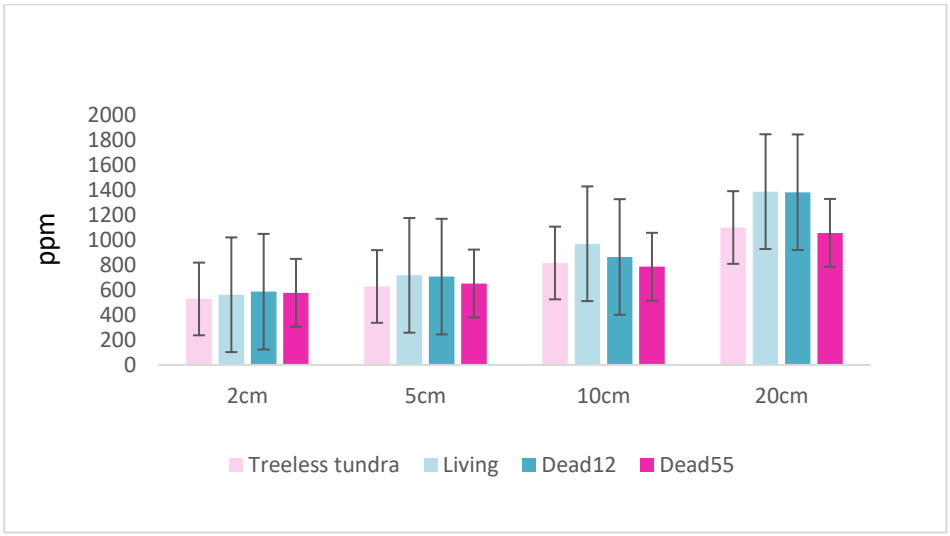

Figure 16. CO_2 soil gas concentrations from soil depths of 2, 5, 10, and 20 cm averaged across all 3 sites impacted by insect outbreak in subarctic tundra. For explanation of treatments, see caption of figure 1.

5.4 Environmental Parameters

In table 2. the soil characteristics are presented as average values per treatment averaged across the three sites. The lowest soil temperature at 5 cm depth was measured in living treatment and the highest temperature in treeless tundra treatment (Table 4.).

In soil moisture there was a clear difference between the treatments; the highest average WFPS was measured in treeless tundra (55.2%) followed by dead 12 (41.5%), living tree (39.4%), and dead 55 (34.1%). The pH ranged from 4.00 to 4.17 in organic soil and from 5.49 to 5.61 in mineral soil. The pH was similar between treatments. Electric conductivity ranged from 45.5 to 57.3 in organic soil and from 6.18 to 11.8 in mineral soil.

The correlation matrix (Appendix 1) shows the relationship between the above parameters. The matrix shows e.g., that WFPS % had a weak, negative correlation with NH_4^+ (-0.356) and Nmin (0.353).

Table 4. Soil characteristics per treatment averaged across all 3 sites impacted by insect outbreak in subarctic tundra. For explanation of treatments, see caption of figure 1.

	Tsoil_5cm [°C]	**SM [%]**	**WFPS (%)**	**pH_org.**	**EC_org**	**pH_min.**	**EC_min.**
Treeless tundra	10.5	27.3	55.2	4.04	45.5	5.51	10.9
Living	8.25	22.8	39.4	4.17	57.3	5.50	11.2
Dead 12	8.53	23.7	41.5	4.16	47.4	5.61	11.8
Dead 55	9.51	22.4	34.1	4.00	56.7	5.49	6.18

6 Discussion

The main aim of this study was to identify if tree death has a significant effect on N_2O emissions and whether this is changing over time. I hypothesized that there will be significant N_2O fluxes after tree death since absence or damage of plants would trigger N_2O release due to lack of nutrient uptake by plants. Increased N turnover due to increased litter input from dead wood could even intensify that effect.

Based on my results, tree death does not have significant effect on N_2O emissions. However, all the measured N_2O fluxes were low, and it is thus difficult to make clear conclusions. There were certain trends, which support my hypothesis: treeless tundra had highest N_2O fluxes and showed also occasionally high N_2O concentrations within the soil profile. Thus, the primary hypothesis was to some extent confirmed. Nevertheless, this is a snapshot of N_2O emissions under in-situ conditions for one very dry growing season, which might have missed either hotspots or hot moments on the landscape scale. Further field studies or incubation experiments might therefore identify treatment differences or verify these findings presented here with changing moisture contents.

I repeat here the three main objectives of the thesis:

1. To measure and quantify N_2O fluxes from three different mountain birch sites in Finnish Lapland that have been affected by moth outbreak in the past.
2. To identify possible environmental drivers of these N_2O fluxes such as temperature, soil moisture, and nutrient availability.
3. To identify possible dynamic over time after the tree death by comparing localised tree statuses such as living, dead, and treeless tundra.

Since objective 1. resulted in fluxes below detection limit and therefore neglectable N_2O emissions, objective 2. and 3. are as well of limited conclusiveness. The following discussion of the results will therefore focus on trends and possible dynamics due to the site and changing

environmental conditions, which might drive seasonal N_2O activity in the sites impacted by insect outbreak. I will also briefly discuss results on CH_4 and CO_2.

5.5 Greenhouse Gas Fluxes

The fluxes measured in July 2019 were overall low compared to the fluxes reported in literature. However, N_2O results had not been reported for these ecosystems from the literature and therefore, it is difficult to compare our results with published values.

N_2O

Due to the N_2O fluxes being under the detection limit of the method, the significant differences are not absolutely for sure. The low fluxes could be due to the dry environmental conditions represented by soil moisture below 30 % and WFPS ranging between 34 to 55% (Table 4.) during the measurements time frame, as moisture content is one of the most important drivers of N_2O emissions (Butterbach-Bahl, 2013). According to Davidson et al. (2000), N_2O emissions are at an optimum level at 70 – 80% WFPS range, depending on soil type. However, N_2O emissions are also still possible during drier and wetter periods especially during shoulder seasons, when it is still warm enough for microbial activity (Groffmann et al., 2009). In my study there was a moderate positive correlation between N_2O and WFPS % meaning that in wetter seasons or after a rain event, significant fluxes might occur. The monthly rainfall in 2019 was only 29.6 mm when in previous years it was 130.3 mm (2017) and 93.4 mm (2018), and in July 2020 the rainfall was 94.3 mm (Table 1.). This could explain the low WFPS % and therefore low fluxes.

Even though N_2O emissions were low and no significant treatment effects were found, there were certain trends, which support my original hypothesis: treeless tundra had relatively highest N_2O fluxes and showed also occasionally high N_2O concentrations within the soil profile. Thus, the absence of roots seems to support the emissions of this strong GHG to some extent. In permafrost peatlands, bare surface soils have highest N_2O emissions (Repo et al., 2009). However, at my site this could have been also an indirect effect of water content, since water content was highest in treeless tundra and there was a positive correlation found between soil moisture and N_2O fluxes, as mentioned above. Highest WFPS of 55% was found in treeless tundra. Between 40 and 60 WFPS% the source for N_2O is most likely nitrification which releases much less N_2O than denitrification (Bollmann & Conrad, 1998). Thus, nitrification was most likely the predominant N_2O source in treeless tundra. There was no difference in N_2O emissions

between living tree treatment, dead 12 treatment, and dead 55 treatment, indicating that nutrient turnover was not affected by the insect damage. This was also supported by the data on nutrient content, which did not vary between the treatments. Again, discussion of the trends and interpretation should be taken with caution.

Again, it should be noted that there was in fact no significant difference in N_2O emissions between the treatments and emissions were also low (below detection limit). But it could well be that we did not catch the emission peaks of these ecosystems. The question is whether field studies and measurements should be conducted in other seasons rather than in summer. Would perhaps more accurate results be achieved if studies and measurements are conducted in shoulder seasons, such as spring and autumn. The question of was the measured site just a low emitting system or were measurements conducted at the wrong time also arises from these results and reflecting them to the literature and previous studies. Although, N_2O fluxes in Finland are not well documented and reported fluxes often are small and underestimated (Maljanen et al., 2006) but Maljanen et al. (2003) points out there is high seasonal variation with fluxes and in their measurements maximum fluxes occurred in spring and early summer.

As Groffmann et al. (2009) explains, various soil microbes are still active at temperatures around 0 °C and freeze-thaw processes lead to pulses of N_2O emissions which contribute significantly to the annual N_2O budget. A possible driver for this is the release of stored C during thawing. However, the easily available C could have been depleted by the time of conducting this study and these measurements. Due to dry conditions on the field site, not much C was made available since. Thus, N_2O production processes could have been C limited and would become active as soon as microbial activity increases again due to a rain event as microbial production processes are a dominant source of N_2O (Butterbach-Bahl et al., 2013). Understanding these transition effects are crucial in understanding the environmental controls of N_2O release (Butterbach-Bahl et al., 2013). Therefore, the need for N_2O emission studies during other seasons than summer and for long-term is evident. Studies have shown that annual budgets of NO and N_2O fluxes from different ecosystem soils are often dominated by defined periods, for example, <5-20 days, which have extremely high emissions (Groffmann et al., 2009). The periods

with extremely high N_2O emissions are usually at the end of winter, when the soil starts to thaw, in temperate and boreal regions.

Several factors influence N_2O emissions; temperature, precipitation, land use, N input, and soil properties such as pH, texture, and C:N ratio (Schaufler et al., 2010). These factors together with N_2O emissions having high spatiotemporal variability (Butterbach-Bahl et al., 2013) makes it difficult to pinpoint one exact reason for the cause or lack of emissions. Most likely it is a combination of factors. Additionally, N_2O emissions can also be affected by seasonal or spatial dynamics of soil moisture or temperature (Butterbach-Bahl et al., 2013). Temporary waterlogging, seasonal passing from drought to rewetting as well as transient zones between upland and wetland soils present ideal conditions for the transition from microbial oxygen to NO_3 respiration, and therefore can create hot moments and hot spots for N_2O emissions (Groffmann et al., 2009). As there are so many possible factors and causes for emissions and in this study flux measurements are only from one site over a three-week period, no conclusive outcomes can be stated. These measurements give an indication of the scale of emissions in this particular ecosystem, but further measurements and studies are needed to determine which factors affect fluxes the most. Comparisons between sites can be made only through the measured environmental parameters (Tsoil_5cm, SM & WFPS%, pH, and EC) as they have been measured from all sites. It seems however, from the data I collected that at the time of measurement (peak summer) in a relatively dry year, the tundra sites impacted by insect outbreaks are not a source of N_2O, and that there is no effect of tree dying or damage on the N_2O emissions.

In the environmental parameters there are no major differences between sites that stand out specifically. All the parameters are in the same range. What stands out is that site 2 treeless tundra has the highest WFPS, 65.56%, and the highest temperature, 12.45 °C, and the second highest N_2O; 38.0 μg N_2O m^{-2} d^{-1}, out of all the sites. This could be an indication of possible fluxes as soil moisture and temperature are the major drivers of N_2O emissions (Schaufler et al., 2010; Butterbach-Bahl et al., 2013). Lowest soil moisture and WFPS were in living tree treatment on sites 2 and 3. Soil moisture was 17.85% on site 3 and WFPS was 26.71% on site 2. The measured

fluxes showed a temporal decrease in living tree treatment (Fig. 1.) and this could be a possible explanation. Nutrient content was also not significantly different between the sites. Generally, NH_4^+ content was in the average range of nutrient content published from Northern soils (Repo et al., 2009, Marushchak et al., 2011, 2013) however, NO_3^- content was low. This indicates that nitrification is hampered, likely due to the relatively low soil moisture content. The conditions were not suitable for denitrification to take place. The C:N ratio range was on average between 27 to 34 (not significantly different between sites) and was thus slightly above the optimum C:N ratio for N_2O emissions (Klemedtsson et al., 2005). Also, this could be the reason for the low N_2O fluxes.

All in all, what these measured fluxes and environmental parameters give is great cause for further continuous studies in this ecosystem and site. Especially in the light of site 2 having highest WFPS% and temperature it would be very interesting to conduct flux measurements on all three sites and not just during the growing season but in shoulder seasons as well with the intention of either verifying or rejecting these indications of possible fluxes. Voigt et al. (2020, review) also highlight that the N_2O intensity of nearly 300 times the GWP compared to CO_2 makes it important to investigate year-round N_2O emissions.

CH_4

All measured CH_4 fluxes were low, and negative fluxes were found which indicate net CH_4 uptake. It is known that waterlogged soils emit large quantities of CH_4 (Liblik et al., 1997) and as the sites studied were upland tundra sites and not lowland sites, and on top of that July 2019 in the area was drier than previous years, this explains the low fluxes. However, according to Jorgensen et al. (2015) in dry, arctic tundra soils CH_4 uptake can be of immense importance for the regional CH_4 Balance and therefore further studies and measurements in this area are needed to confirm whether this ecosystem/area really is a CH_4 sink rather than source. This should also be studied and monitored in the long-term to detect whether increasing moisture content turns these ecosystems from sink to a source. The soil gas concentration measurements of CH_4 confirmed the results on CH_4 uptake, since CH_4 concentration decreased with depth.

CO_2

Carbon dioxide fluxes were mainly used as quality control for this study. Measured CO_2 fluxes showed stable increase over time which validates the static chamber method used (Maljanen et al., 2006). Fluxes were checked and any odd fluxes were not considered which also was one method for quality control. Since there were no differences in CO_2 fluxes between the treatments, it can be expected that there was no priming or increased microbial activity in the living tree or dead tree treatments. This is in line with the finding that N_2O emissions were not impacted by insect outbreak or treatment.

5.6 Effect of Autumnal Moth

This study focused on the autumnal moth's role from the perspective of how birch trees survive and behave after the moth attack. Trees do not recover well from moth attacks (Kallio & Lehtonen, 1973; Lehtonen & Yli-Rekola, 1979; Lehtonen, 1987). It is likely that trees survive and recover from the first attack, but not from the second or third anymore.

It is known that temperature is the dominant factor directly affecting herbivorous insects and the abundance and range of forest insect pests is predicted to increase due to global warming (Bale et al., 2002) which prompts the question of what will happen to these ecosystems if moth attacks increase in occurrence. The autumnal moth displays cyclic outbreaks at approximately 10-year intervals and causes extensive defoliation and occasional mortality of mountain birch forests in Fennoscandia (Kallio & Lehtonen, 1973). Mean annual temperatures have undergone a noticeable increase in the past 15 years in the region (Jepsen et al., 2008). This gives reason to believe that autumnal moth outbreaks will increase. It might be an increase in the extent of the outbreak or that the outbreaks occur more often, at shorter intervals than 10 years. Since the results of my study suggest only minor effects on N_2O emissions, the question arises if the shorter periods in outbreak will change this due to more substantial changes of the N cycle.

The study site had undergone moth attacks many years ago and documentation and studies of previously moth attacked areas in the region have been done about 30-50 years ago (Kallio & Lehtonen, 1973; Lehtonen & Yli-Rekola, 1979; Lehtonen, 1987). Those studies are extremely valuable, but it would be of great interest to document and study an area right after it has undergone a moth attack and follow it long-term. For example, a study during a moth attack with immediate nutrient and N_2O measurements could identify the exact factors affecting fluxes in this ecosystem.

Autumnal moth has mostly short-term effects on mountain birch trees. Long-term effects are not big on N_2O but still there possibly is some effect, perhaps priming. The insect outbreak and insect death could boost N availability due to increased N input by insect feces, and leaf-litter in the topsoil which results in increased decomposition and easily available C (Sistla et al. 2013; Pausch

and Kuzyakov 2018). This leads to priming after the N from insects is depleted. As the nutrient mining interpretation of priming is based on that labile OM is used as an energy source that supports microbial activity and microorganisms co-metabolize SOM to release and obtain N from soil (Craine et al. 2007, Meier et al. 2017, Macdonald et al. 2018). What this means for N-poor soils in the Arctic and subarctic is that "microbial responses to inputs of labile OM may be driven by microbial demand for N" (Hicks et al., 2020). Hartley et al. (2010) discovered that labile OM had a priming effect on the decomposition of soil C and that this priming response was reduced when labile OM was added together with inorganic N, suggesting that microbial demand for N caused priming to occur. This is a possible explanation for my own results as N-poor soils are more susceptible to priming (Hicks et al., 2020). However, priming is extremely hard to measure in field conditions (Meyer et al., 2021, unpublished) nor have actual priming effects been observed in sterile conditions (Jansson, 1959). Conducting incubation studies would be of great interest so that the priming effect for these soils could be either verified or rejected. Interestingly, the treeless tundra soil had the highest SOM content in my study, suggesting that priming and increased nutrient turnover occurred in all other sites where N_2O emissions were lowest. Priming of C and N could be decoupled (Wild et al., 2017).

5.7 Mineral nitrogen, NH_4^+ and NO_3^-, and C:N ratio

In all sites and treatments, NH_4^+ was the dominant form of mineral N which highlights the immediate N availability for plant uptake as well as nitrifying microbes. Nitrate content was, however, very low suggesting low rates of nitrification. Nitrate can still be produced and immediately consumed after a light rain event, the increased NO_3^- would be subject to plant and microbe competition. Nitrate would be needed in excess amounts to be denitrified and N_2O emissions to occur. The magnitude of these possible emissions, however, remains unknown. However, figure 4. shows a difference in site 2; there the highest amount of NH_4^+ is in living treatment whereas sites 1 and 3 follow the same trend of having the highest amount in dead 55 treatment. Site 2 had the lowest NO_3^- but highest NH_4^+. Nevertheless, site 2 did not show any specific dynamics with respect to N_2O; it was site 3 where N_2O concentrations increased with depth (Fig. 11).

The N min content was highest in living treatment on sites 2 and 3 (Fig.4). Nitrogen availability can possibly be an indication of priming effect. Silfver et al. (2020) have shown that warming under natural herbivory increases soil N availability in living trees. It has been established that NH_4^+ causes larger priming effects than NO_3^- (Rennie and Rennie, 1973; Kowalenko and Cameron, 1978; Steele et al., 1980; Stout, 1995) and NH_4^+ was the dominant form on all three sites (Fig. 4.) so this could be an indication of priming taking place. Also differences between trees such as size, age, and root systems can influence priming.

On a final note, even though N_2O fluxes were low, N_2O could have been still produced in the studied sites. Nitrous oxide can be further reduced to N_2 and even though this usually happens under anaerobic conditions, also aerobic denitrification has been found to occur (Davidson et al., 2000). However, N_2O fluxes have been measured more than N_2 fluxes but they do not provide comprehensive information on denitrification (Groffmann et al., 2006a). Thus, N_2 flux measurements are also very much needed, and this should be the topic for further studies.

7 Conclusion and summary

Based on this study all measured N_2O fluxes from the tundra ecosystems impacted by insect outbreak were low. However, this was only a short measuring period and as July 2019 differed so much in monthly rainfall compared to previous years, it is difficult to make conclusions. Nonetheless, to make accurate global N_2O emission estimates, these findings are important and need to be reported. Also, this gives great reason and cause for continuing studies in the area, both short- and long-term. I highly recommend based on my results to conduct measurements and further studies in other seasons in this area.

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Appendices

APPENDIX 1.

Correlation matrix.

Correlations

		CO2_[mg/m2d]	N2O_[µg/m2d]	logCH4_[mg/m2d]	mg_NO3NkgDW	log_mg_NH4NkgDW	log_mg Nmin/kgDW	SM_[m3/m3]	WFPS_(%)
CO2_[mg/m2d]	Pearson Correlation	1	-0,162	-0,277	-0,038	.344*	.339*	-0,237	-0,303
	Sig. (2-tailed)		0,317	0,084	0,814	0,030	0,032	0,141	0,058
	N	40	40	40	40	40	40	40	40
N2O_[µg/m2d]	Pearson Correlation	-0,162	1	-0,097	-0,023	-0,247	-0,247	0,245	.451**
	Sig. (2-tailed)	0,317		0,551	0,886	0,124	0,124	0,127	0,003
	N	40	40	40	40	40	40	40	40
logCH4_[mg/m2d]	Pearson Correlation	-0,277	-0,097	1	-0,006	0,135	0,135	-0,135	-0,110
	Sig. (2-tailed)	0,084	0,551		0,969	0,359	0,359	0,365	0,460
	N	40	40	48	48	48	48	47	47
mg_NO3NkgDW	Pearson Correlation	-0,038	-0,023	-0,006	1	0,214	0,270	0,170	0,059
	Sig. (2-tailed)	0,814	0,886	0,969		0,145	0,063	0,253	0,695
	N	40	40	48	48	48	48	47	47
log_mg_NH4NkgDW	Pearson Correlation	.344*	-0,247	0,135	0,214	1	.998**	0,008	-.356*
	Sig. (2-tailed)	0,030	0,124	0,359	0,145		0,000	0,957	0,014
	N	40	40	48	48	48	48	47	47
log_mg Nmin/kgDW	Pearson Correlation	.339*	-0,247	0,135	0,270	.998**	1	0,013	-.353*
	Sig. (2-tailed)	0,032	0,124	0,359	0,063	0,000		0,931	0,015
	N	40	40	48	48	48	48	47	47
SM_[m3/m3]	Pearson Correlation	-0,237	0,245	-0,135	0,170	0,008	0,013	1	.863**
	Sig. (2-tailed)	0,141	0,127	0,365	0,253	0,957	0,931		0,000
	N	40	40	47	47	47	47	47	47
WFPS_(%)	Pearson Correlation	-0,303	.451**	-0,110	0,059	-.356*	-.353*	.863**	1
	Sig. (2-tailed)	0,058	0,003	0,460	0,695	0,014	0,015	0,000	
	N	40	40	47	47	47	47	47	47

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

APPENDIX 2.

Correlation matrix.

Correlations										
			CO2_[mg/m2d]	N2O_[µg/m2d]	logCH4_[mg/m2d]	mg_NO3NkgDW	log_mg_NH4NkgDW	log_mg Nmin/kgDW	SM_[m3/m3]	WFPS_(%)
Spearman's rho	CO2_[mg/m2d]	Correlation Coefficient	1,000	-0,120	-0,197	0,000	0,295	0,300	-0,215	-0,282
		Sig. (2-tailed)		0,459	0,223	0,999	0,064	0,060	0,183	0,078
		N	40	40	40	40	40	40	40	40
	N2O_[µg/m2d]	Correlation Coefficient	-0,120	1,000	-0,196	-0,046	-0,234	-0,209	0,171	.338*
		Sig. (2-tailed)	0,459		0,226	0,776	0,146	0,197	0,292	0,033
		N	40	40	40	40	40	40	40	40
	logCH4_[mg/m2d]	Correlation Coefficient	-0,197	-0,196	1,000	0,135	0,172	0,195	0,058	0,010
		Sig. (2-tailed)	0,223	0,226		0,362	0,241	0,184	0,697	0,949
		N	40	40	48	48	48	48	47	47
	mg_NO3NkgDW	Correlation Coefficient	0,000	-0,046	0,135	1,000	.441**	.494**	0,142	0,036
		Sig. (2-tailed)	0,999	0,776	0,362		0,002	0,000	0,341	0,808
		N	40	40	48	48	48	48	47	47
	log_mg_NH4NkgDW	Correlation Coefficient	0,295	-0,234	0,172	.441**	1,000	.991**	0,137	-0,189
		Sig. (2-tailed)	0,064	0,146	0,241	0,002		0,000	0,358	0,204
		N	40	40	48	48	48	48	47	47
	log_mg Nmin/kgDW	Correlation Coefficient	0,300	-0,209	0,195	.494**	.991**	1,000	0,122	-0,182
		Sig. (2-tailed)	0,060	0,197	0,184	0,000	0,000		0,414	0,222
		N	40	40	48	48	48	48	47	47
	SM_[m3/m3]	Correlation Coefficient	-0,215	0,171	0,058	0,142	0,137	0,122	1,000	.869**
		Sig. (2-tailed)	0,183	0,292	0,697	0,341	0,358	0,414		0,000
		N	40	40	47	47	47	47	47	47
	WFPS_(%)	Correlation Coefficient	-0,282	.338*	0,010	0,036	-0,189	-0,182	.869**	1,000
		Sig. (2-tailed)	0,078	0,033	0,949	0,808	0,204	0,222	0,000	
		N	40	40	47	47	47	47	47	47

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).N1:X28

APPENDIX 3

Field protocol:

Determination of soil N_2O fluxes by a diffusion gradient method

Version 1

1. Contact information

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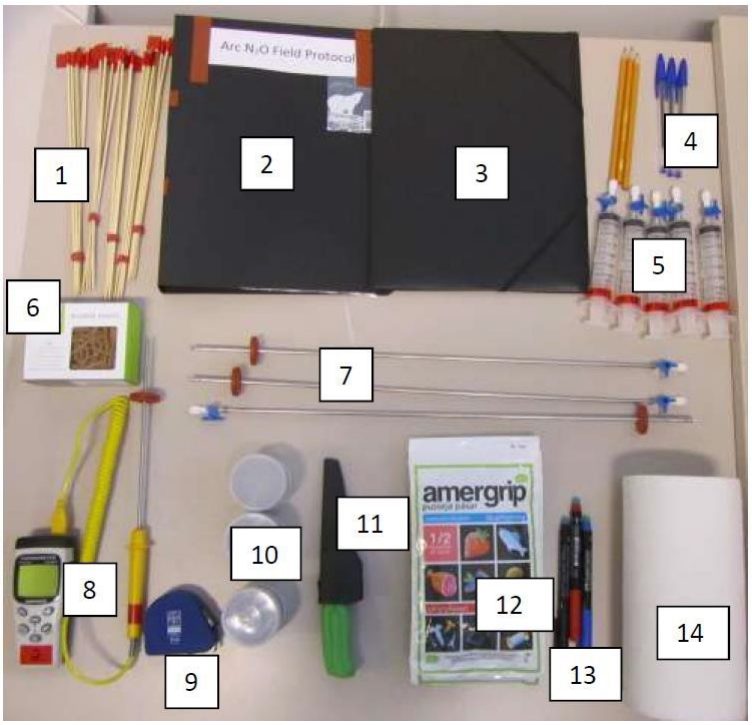
Contents

2. Equipment

<u>Field equipment shipped from Kuopio:</u>



1. 25 x Marking sticks for the plots
2. Folder with field protocols
3. Folder with field templates
4. Pencils & pens
5. 50 x Syringes with 3-way valves
6. Bag of rubber bands
7. 3 x Soil gas probe
8. Thermometer with a soil probe

9. Measuring tape
10. 3 x Soil rings (Ø=6 cm, h=6 cm)
11. Knife
12. Resealable plastic bags
13. Markers
14. Paper towels
15. Aluminium weighing boats

Field equipment from the station:

- Camera
- GPS
- Spade
- Thaw depth probe
- Water bottle for cleaning the soil gas probe in case of blockage with soil

Lab equipment shipped from Kuopio:

16. 100 x 12 ml glass exetainers
17. 50 x Injection needles
18. Paper bags for dried soil samples

3. Principle of the method, short description and aim of the project

Non-water saturated soils producing nitrous oxide (N2O) display elevated concentrations of this gas, whereas soils consuming N2O have concentrations below the ambient N2O concentration. The surface flux from the soil to the atmosphere or vice versa is driven by the physical diffusion process from a larger concentration to a smaller concentration.

The simple field method based on soil gas concentration described here can be used to check gas concentrations (N2O, CO2, CH4) in the soil profile, and to estimate the fluxes of these gases based on their soil concentrations, soil porosity and published gas diffusion coefficients.

In this method soil gas is withdrawn into a syringe via a metal probe. The method works well for non- waterlogged soils with medium porosity. Dense soils with low porosity may be difficult to sample, but in most cases sampling is possible also from these soils by increasing the time of sample withdrawal. In water-logged soils the soil gas concentration can be determined based on the concentration of N2O dissolved in the soil pore water.

The aim here is to “screen” Arctic soils for possible N2O emissions, or N2O uptake potential. We thus would like you to take random samples from the sites, where you work or where you have access to. However, watch out for bare soils particularly on peatlands, for soils with disturbed vegetation, thermokarst features (signs of thawing) and whenever you think (or know) that soil nutrient concentrations are high (e.g., discharge areas). Based on previous experience, perma-frost peatlands with uplifted and partly bare peat surfaces are the most potential sources of N2O among Arctic soils (Repo et al. 2009, Marushchak et al. 2011, Voigt et al. 2017a&b), but it has been shown that permafrost thaw can induce N2O emissions also from mineral soils (Elberling et al. 2010, Abbott & Jones 2015). Since N2O fluxes have not been comprehensively studied across Arctic soil and vegetation types, it is likely that there are significant Arctic N2O sinks and sources yet unidentified.

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4. Field sampling

A. Plot selection

1. Select five representative sampling plots per surface type (n = 5) and mark them with wooden sticks (1). **NOTE! The distance between replicate plots should be ~5 m or more.**
2. Take a photo with an overall view of the surface types, showing the 5 replicate sampling locations. Mark the number of the photo in the field template (3) ('Overview photo no.').
3. Take photos of all the 5 replicate sampling plots. Mark the number of the photos in the field template ('Plot photo no.').
4. Take GPS coordinates of each plot and write down to the field template ('Coordinates')

B. Ambient air sampling

Demonstration video:

Video 1 in: https://www.youtube.com/playlist?list=PLOc4avq1UBb1H-aQnPxvwGtq1TxB-jECGd

General:

Three ambient air samples should be taken per each surface type at ~15 cm above the soil surface.

1. Hold the syringe (5) (connected to a 3-way valve) horizontally at ~15 cm above the soil surface and flush the syringe 2-3 times by quickly pulling and pushing the syringe piston.
2. Pull the syringe piston until the syringe is filled with ~30mL of ambient air, and close the valve (Picture A).
3. After the field day in the lab, transfer 25mL of gas to glass exetainers as described below in Section 5, Lab work after field.

NOTE! Avoid breathing to the direction where you are taking the ambient air sample. This would cause elevated CO2 concentrations in ambient samples.

Picture A. Syringe with 30 mL of ambient air & closed 3-way valve.

C. **<u>Soil gas sampling</u>**

Demonstration video:

Videos 2&3 in: https://www.youtube.com/playlist?list=PLOc4avq1UBb1H-aQnPxvwGtq1TxBjECGd

General:

Soil gas samples are taken, if possible, from four depths: **2 cm, 5 cm, 10 cm, and 20 cm.** The four sampling depths are marked with thin lines on the soil gas probe (7).

Depending on moisture conditions soil gas samples are obtained via two different methods: 1) sampling of gas in well-drained soils, and 2) sampling of soil pore water in water-saturated soils and analyzing the gas concentration in the headspace equilibrated with the water sample.

Method 1: Well-drained soils

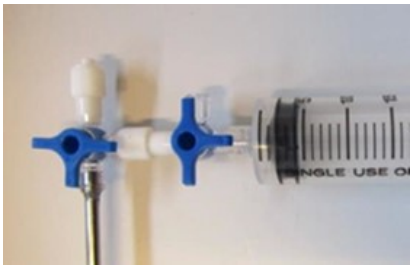
1. Before starting the sampling it is recommended to sort the syringes (5) in ascending order according to their codes (e.g. N-1, N-2, N-3, N-4 for sampling depths 2, 5, 10, 20 cm). Mark down the codes in the field template (3).
2. Connect a syringe (5) to the soil gas probe (7) and turn both the 3-way valves so that gas can flow from the soil gas probe to the syringe (Picture B).

3. Flush the soil gas probe 2-3 times with ambient air (to remove dirt and exchange the air inside the tube) by quickly pulling and pushing the syringe piston. This way you can also test whether the 3-way valves are in the correct position for the gas flow to move from the soil gas probe into the syringe.
4. Set the red rubber disk on the soil gas probe to the first sampling depth (2 cm) (Picture C). **NOTE! The depth is measured from the soil surface including dense ground vegetation (mosses, lichens) when present.**

Picture B. Syringe connected to the gas probe.

Picture C. Rubber disk set to first sampling depth.

5. Push the soil gas probe carefully into the soil down to the sampling depth with the syringe connected to the probe.
6. Slowly pull the syringe piston backwards and sample 1-2mL of gas. Open the 3-way valve connected to the syringe to the atmosphere (Picture D) and press the piston to remove the gas for flushing the soil gas probe.



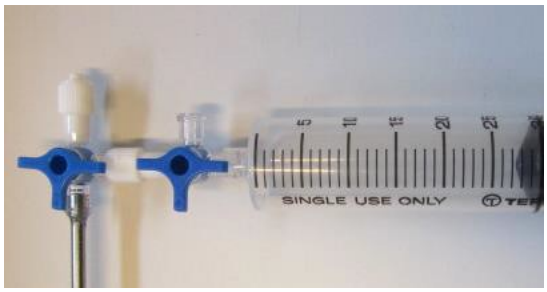
Picture D. Discarding the 1-2 ml of air removed from the tube before taking the sample.

7. Turn the 3-way valve back to its previous position (Picture B) and gently pull the syringe piston backwards until the gas volume in the syringe is ~30 ml (Picture E).

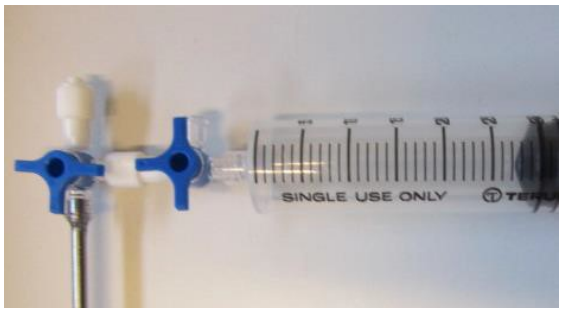
   NOTE! In soils with high density, withdrawing of gas can be difficult and may cause underpressure in the syringe, causing the piston to move back when you release it. In this case, pull the syringe piston slowly and hold it in place until ~30mL are sampled and the piston is no longer moving when released. This could take a while.

8. When the syringe is filled with ~30mL of gas, close the 3-way valve of the syringe so that the syringe can be disconnected from the probe without leakage (Picture F).
9. Mark down to the field template the type of sample (“gas”).
10. Continue taking soil gas samples from the remaining two depths as described above. Remem-ber to flush the probe with ambient air between the samplings (Point 3.).

NOTE! Between sampling depths, please check that the holes in the gas probe are open and you can withdraw gas through the probe. The holes may get blocked by fine soil material entering to the tube while sampling the soil gas. You can clean the probe by cleaning the holes with a sharp item and pushing ambient air through it with a sampling syringe (see above point 3). Sometimes you may need some water for flushing, so it is good to keep a bottle of clean water with you in the field.



Picture E. 30 ml sample is taken…



Picture F. …and the valve is closed.

11. Use a rubber band to bundle together the four soil gas syringes sampled at one plot.
12. After the field day in the lab, transfer the gas from syringes to glass exetainers as described in Section 5., Lab work after field.

Method 2: Water saturated soils (if sampling of soil gas is not possible because you get water to the syringe instead of gas)

6. Follow steps 1-5 as described above.
7. Slowly pull the syringe piston backwards and sample 1-2mL of water. Remove the water by opening the 3-way valve connected to the syringe to the atmosphere and press the piston (Picture G).

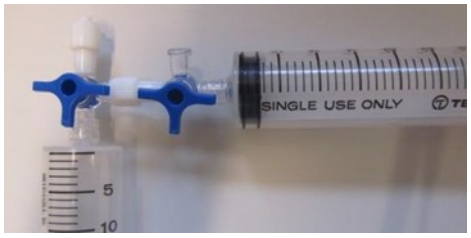
Picture G. Discarding the 1-2 ml of water before taking the soil water sample.

8. Turn the 3-way valve back to its previous position (Picture B) and gently pull the syringe piston backwards to sample soil pore water, until the water fills up ~7mL of the syringe.
9. When the syringe is filled with ~7mL of pore water, close the 3-way valve of the syringe and remove the syringe from the soil gas probe (Picture H).
10. Continue by adding ~28mL of ambient air to the syringe right in the field (Picture I) Take ambient air from ~15 cm above the soil surface as described in 'Ambient air sampling' (steps 1.-2.) You will obtain a total volume of ~35 ml (7mL gas + 28 mL water). Close the 3-way valve of the syringe.

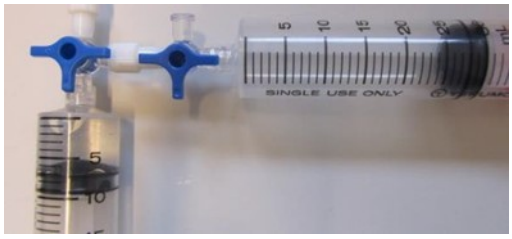
Picture H. Syringes with 7 ml water.

Picture I. 28 ml of ambient air taken to the syringe with the water sample.

11. Equilibrate the gas concentration in the pore water with the headspace gas concentration by shaking the syringe vigorously for 1 minute. By vigorous shaking, the gases dissolved in the soil water sample are transferred from the water phase into the gas phase
    NOTE! Sometimes you may get both water and gas into the syringe. If the amount of water is minimal (<3 mL) then it is ok to sample only the gas phase directly without filling air and shaking.
12. Connect a second syringe to the syringe containing the sample via the side valve (Picture J) and move the ~28mL of headspace gas to the second syringe (Picture K). Be careful not to draw pore water from the first to the second syringe! Close the 3-way valve of the second syringe and disconnect the two syringes from each other.



Picture J. An empty syringe connected to the syringe with the soil water sample.



Picture K. 28 mL headspace has been moved to the second syringe.

13. Mark down to the field template the type of sample (“water”).
14. Use a rubber band to bundle together the four soil gas syringes sampled at one plot.
15. After the field day in the lab, transfer the gas from syringes to glass exetainers as described in Section 5., Lab work after field.

D. Soil temperature measurement

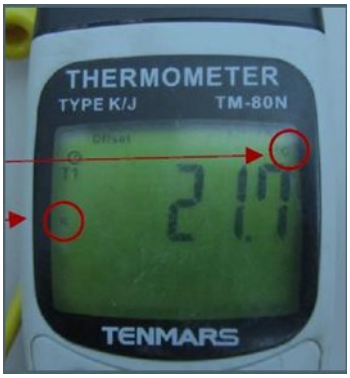
General:

Soil temperature is measured close to each soil gas sampling plot at 5 different depths: **2 cm, 5 cm, 10 cm, 15 cm and 20 cm**. The four first sampling depths are marked with thin lines on the soil temperature probe, while 20 cm is the full length of the probe.

The thermometer manual is inside the black thermometer case.

Before the first measurement: The thermometer (8) is powered by a 9 V battery, which is located inside the thermometer with contacts shielded with a tape. Remove the tape and connect the battery to the thermometer.

1. Switch the thermometer on from the -button.
   NOTE! Check that the screen has similar texts as in the picture. Sensor type should be K. Change from the K/J-button if needed! Unit should be °C. Change from the °C/°F-button if needed!



2. Set the red rubber disk on the temperature probe to the first sampling depth (2 cm).
   NOTE! The depth is measured from the soil surface including dense ground vegetation (mosses, lichens) when present.
3. Push the temperature probe to the soil until the probe tip is at the desired depth.

4. Keep the probe in the soil until the reading is stable (reading does not change, or changes back and forth between two readings.
5. Write the temperature down to the field template ('Temperature ('°C')') with accuracy of one decimal.
6. Repeat the steps 3.-6. at 5 cm, 10 cm, 15 cm and 20 cm.

E. Thaw depth measurement

1. If the site has permafrost, measure the thaw depth close to each gas sampling plot with a metal probe. Write down to the field template ('Thaw depth (cm)') with accuracy of 0.5 cm.

F. Soil sampling

General:

Since gas diffusion between the soil and the atmosphere is dependent on soil density and water content, we will determine these parameters in triplicate (n = 3) from each studied surface type.

Soil samples with a known volume are taken from the soil layers above each soil gas sampling depth.

1. Select locations for three soil sampling pits. In ideal case, the soil samples are taken right from the soil gas sampling plots after the soil sampling is done. If this is not possible, select nearby spots with similar vegetation cover, soil type and moisture conditions.
2. Using a spade, dig a small hole with a depth of ~30 cm. Keep the soil profile on one side as intact and "clean" as possible – avoid mixing the soil horizons!
3. Take a picture of the clean soil profile and write the number to the field template (Page 2, 'Soil pit photo no.')
4. Use the measuring tape (9) to find the right sampling depths:

- Soil layer 0-5 cm - take a sample through the whole layer

- Soil layer 5-10 cm - take a sample around 7.5 cm
- Soil layer 10-20 cm - take a sample around 15 cm

NOTE! Do not mix clearly different soil horizons to a single soil sample. If the transition from organic to mineral soil is in the uppermost 20 cm of the soil, take separate samples from both and write down the depth of the organic layer and the actual sampling depths to the field template.

5. Select a suitable method for the soil sampling:

A. **Sampling with volumetric soil rings** – Default method; suitable for mineral soils and highly decomposed organic soils & soils with few roots
B. **Cutting with a knife** – Use this method if it is not possible to get a volumetric sample by method A; suitable for poorly decomposed organic soils & soils with many roots

Method A. Sampling with volumetric soil rings (Default)

1. **Layer 0-5 cm:** Select a spot close to the intact wall of the soil pit, remove above ground vegetation with a knife and push a volumetric soil ring (10) vertically to the soil.

   Layers 5-10 cm & 10-20 cm: Push a volumetric soil ring horizontally to the intact wall of the soil pit, with the center point at the sampling depth.
2. While still in the soil, turn the soil ring around to disconnect the soil inside the core from the surrounding soils.
3. Gently pull the soil ring out from the soil. Pay attention to keep the full sample inside the soil ring!

OR

Method B. Cutting with a knife

1. Use a knife to cut out a cubic soil sample from the side of the intact soil profile.
2. Measure the dimensions of the soil sample. Try to keep the original shape and density of the soil sample

6. Write down the type of the soil sample (Page 2, ‘Cylinder’ OR ‘Cube’) to the field template.
7. Write down the dimensions of the soil core (Page 2, ‘Dimensions’) to the field template. If you have the full soil ring, you can just write ‘default’. In other case (part of the soil ring empty), write down the exact dimensions.
8. Place the soil sample into a resealable plastic bag, and mark with surface type and soil pit No.
9. Clean the soil ring and knife carefully with paper towels before the next sampling.

NOTE! Be careful not to lose any soil, since we need the complete weight of a volumetric sample for determination of the bulk density.

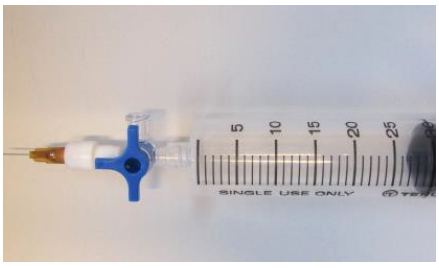
5. Lab work after field

A. <u>Transfer of gas samples in glass exetainers</u>

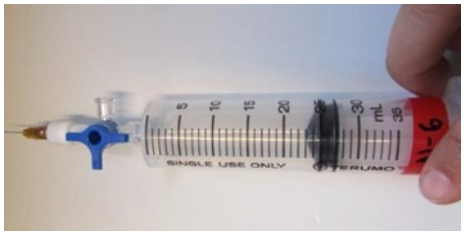
Demonstration video:
Video no. 4 in: https://www.youtube.com/playlist?list=PLOc4avq1UBb1H-aQnPxvwGtq1TxBjECGd

Transfer into exetainers should be done on the same day of sampling. Gas samples should not be stored in the syringes longer than 10-15h due to possible leakage.

1. Sort the exetainers (15) in the ascending number order according to their codes (e.g., N-1, N-2, N-3 and N-4 for sampling depths 2, 5, 10 and 20 cm) Mark down the codes to the field template.
2. Fix an injection needle (16) to the 3-way valve of the first syringe (Picture L).
3. Open the 3-way valve so that the gas flow is directed from the syringe through the needle. Gently press the syringe piston to flush the needle until 25mL of gas are left in the syringe (Picture M).

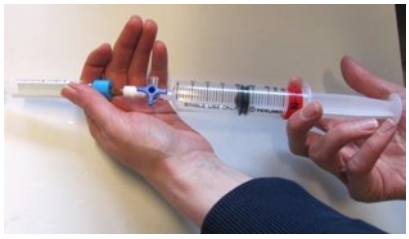


Picture L. Needle connected to syringe.



Picture M. Valve open, needle flushed with ~5 ml of sample.

4. Inject the 25mL of gas into a 12 ml glass exetainer. Since the exetainers are evacuated the piston will move automatically until the vial is filled with ~12mL. Press the remaining gas into the exetainer (thus creating overpressure), and remove the vial while holding the syringe piston (Picture N).
5. Place the exetainer with the gas sample in the cardboard sampling box upside down. This helps you to keep track on which vials have been used already.



Picture N. Needle connected to vial, inserting 25 ml of sample gas to the exetainer.

B. <u>Determination of bulk density and soil water content</u>

1. Take the fresh weight of the fresh soil sample using the aluminium drying boats (15), and write down to the field template (Page 2, 'Fresh weight (g)').
2. Dry the soil sample in a drying oven for 24 h or until a constant weight is reached. Different temperatures should be used for organic and mineral soils:

- Organic soils: 65°C
- Mineral soils: 105°C

3. After 24 h drying, let the samples cool down in a desiccator, if available. If not, let them cool down in room air.
4. Take the dry weight of the soil sample and write down to the field template (Page 2, 'Dry weight').
5. Pack the dry soil sample into a paper bag (17). Mark the paper bag with surface type and soil pit number (1, 2 or 3) and close it for shipping.
6. **Shipping information**

   Ship the gas samples, dried soil samples and sampling equipment to:

   Shipping address:

   Lona van Delden
   University of Eastern Finland
   Department of Environmental and Biological Sciences Yliopistonranta 1E
   FI-70210 Kuopio
   E-mail: lona.vandelden@uef.fi
   Tel. +358 50 449 3427

Please inform Lona van Delden (ADD) and Christina Biasi (christina.biasi@uef.fi) about the timing of the shipment and give a tracking code, when possible!

Thank you for collaboration!