Nitrous oxide emissions from drained organic and mineral soil: a study on hemi boreal Spruce forests

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Licentiate Thesis
University of Gothenburg
Department of Earth Sciences
Gothenburg, Sweden 2017

ISBN 978-91-639-2892-5

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ISBN 978-91-639-2892-5
Internet ID: http://hdl.handle.net/2077/51523
Printed by Kompendiet AB, Gothenburg, 2017

Distribution: Department of Earth Sciences, University of Gothenburg, Sweden

To my family

Mohammad Aurangojeb

Abstract

Terrestrial soils are the major source of N_2O, related to the soil N cycle and influenced by many factors. Forest soils have in general lower emission than soils in agricultural use. However higher emission than commonly found in agriculture can be found for some forest soils, as for the drained peat soil at the Skogaryd research site.

To understand the N_2O flux size and its controls two soil types existing in the Skogaryd area were investigated, drained peat and mineral soil, having high fertility and the same type of spruce forest. Thus we were able to keep weather conditions similar and only the soil types were different. Field measurements were conducted during three years, where soil emissions were sampled by manual closed chambers. To investigate soil gross N turnover processes at the organic site a ^{15}N tracer field study was conducted for control plots and plots without either only roots or both roots and ectomycorrhizae.

Over the years the average emission from the drained organic soil was six times higher than from the mineral soil ($4.2 \pm 0.1$and 0.7 ± 0.1 kg N_2O $ha^{-1}yr^{-1}$, respectively), despite slightly more fertile soil at the mineral site. The emissions varied over the year for both the organic and mineral soils where the large emissions were found during summer especially following precipitation after dry periods. Precipitation and temperature are thus influencing factors. The higher emission for the organic site was initially suggested connected to a larger soil organic matter (SOM) content of this soil, in percentage of top 0.30 m soil, however the SOM amount (kg/m^2) was similar for both sites, thus other suggestions were sought for. For the mineral site, both above ground biomass and mycorrhizae growth were much higher than those for the organic site resulting in a higher nitrogen demand and less N available for nitrification and denitrification. The importance of mycorrhizae was also shown in the trenching experiment at the organic site. Here the presence of roots and mycorrhizae stimulated microbial NH_4^+ immobilization more than NH_4^+oxidation to NO_3^-, explaining a lower N_2O emission.

In summary, the findings of this thesis showed that N_2O emission differed between drained organic and mineral soil with higher emissions from the former in same climate conditions. It also suggest that tree roots and mycorrhizae affect soil N cycle through uptake of mineralized N and by stimulating microbial N immobilization thereby keep the N_2O emission down.

Keywords: N_2O emissions, organic soil, mineral soil, spruce forest, soil organic matter, ^{15}N tracer, roots and mycorrhizae, mineralization, immobilization, nitrification, denitrification
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Populärvetenskaplig sammanfattning

Den största källan för tredje viktigaste växthusgasen lustgas (N_2O) är från marken, vilket är naturligt men påverkas av markanvändning. Lustgasen bildas främst i nitrifikation och denitrifikation och påverkas av många faktorer. Skogsmark har vanligtvis lägre emission än jordbruksmark, men även skogsmark kan ha höga emissioner. Till exempel så har dränerad torvmark vid Skogaryd forskningsstation visats ha hög avgång av lustgas.

För att förstå varför så mycket N_2O avgår och vad som påverkar flödet undersöktes två närliggande områden i Skogaryd vilka har olika typ av mark, dränerad torv och en mineraljord, med liknande bördighet och samma typ av granskog. Mätningar i fält gjordes under tre år, där gaser som avges från marken samlades upp manuellt med hjälp av kammare. Dessutom undersöktes med spårämnesanalys (^{15}N) de processer som omsätter kväve (N) i den organogena jordens kontrollyta, och ytor där antingen bara rötter exkluderats eller både rötter och mykorrhizasvampar.

Emission av lustgas var sex gånger högre från den dränerade torvjorden jämfört med mineraljorden, i medeltal 4.2 ± 0.1 respektive 0.7 ± 0.1 kg N_2O ha^{-1} $år^{-1}$, trots en något högre bördighet för mineraljorden. Emissionerna varierade också med årstiden på båda ytorna, där de största emissionerna ägde rum under sommaren och särskilt då i samband med regn efter en torr period. Nederbörd och temperatur visade sig vara viktiga faktorer som påverkar emissionen. Till en början förklarades den högre emissionen på den organogena ytan med att marken har en högre halt organiskt material i ytjorden, men den totala mängden av organiskt material i ytjorden var lika så en annan förklaring söktes efter. Något som skiljde sig åt mellan ytorna vara skogens tillväxt ovan jord samt tillväxt av mykorrhizasvampar i jorden, vilka båda var mycket högre på mineraljorden. Eftersom dessa efterfrågar mer kväve blir mindre kväve tillgängligt för nitrifikation och denitrifikation. Betydelsen av mykorrhiza visades också genom ett experiment där rötter eller både rötter och mykorrhizasvampar hållits undan från experimentjorden. Närvaro av både rötter och mykorrhizasvampar ökar på mikroorganismernas upptag av kväve och mindre blir då över för nitrifikation, vilket kan förklara en lägre N_2O emission.

Sammanfattningsvis, resultat i denna avhandling visar att under samma väderförhållanden skiljer sig N_2O-emission mellan dränerad torvmark och mineralmark, där torvmarken hade högre emission. Resultaten pekar också på att trädens rötter och dess mykorrhiza påverkar kväve-cykeln genom att själva ta upp kväve och stimulera markmikroorganismers kväveupptag, och därigenom hålla nere N_2O emissionen.

List of papers

This thesis includes following two papers:

I. Aurangojeb, M., Klemedtsson, L., Rütting, T., Weslien, P., Banzhaf, S., Kasimir, Å. Nitrous oxide emissions from Spruce forests on drained organic and mineral soil. Submitted to Canadian Journal of Forest Research
M. Aurangojeb conducted field work, data collection, data analyses and writing of the paper together with supervisor

II. Holz, M., **Aurangojeb, M.**, Kasimir, Å, Boeckx, P., Kuzyakov, Y., Klemedtsson, L., Rütting, T. (2015). Gross nitrogen dynamics in the mycorrhizosphere of an organic forest soil. Ecosystems, 19(2): 284-295.

M. Aurangojeb was responsible for N_2O *data collection, data analyses, took part in situ* ^{15}N *labelling experiment and contributed to part of writing*

Table of content

Part I

Summary

Introduction

The earth's surface temperature has increased by 0.85 [0.65 to 1.06] °C, over the period 1880–2012, due to increased emissions of greenhouse Gases (GHGs) to the atmosphere (Hartmann et al., 2013). Carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) are the most important anthropogenic GHGs and the atmospheric concentration of these gases has increased significantly since the preindustrial time. Among these anthropogenic GHGs, the emission of N_2O is of particular concern because of its high global warming potential which is 265 times that of CO_2 in a 100 year perspective (Myhre et al., 2013). In addition to the global warming potential, the N_2O gas is projected as the largest stratospheric ozone-depleting substance for the remainder of this century (Ravishankara et al., 2009).

Nitrous oxide emissions from terrestrial soils are assumed to be the major source of atmospheric N_2O (IPCC, 2007). And the emissions from terrestrial soils are found to be largely influenced by anthropogenic activities including land use and land use change (Leppelt et al., 2014), e.g. agricultural soils are the largest source of N_2O emission but forest soils have in general low emission. Also, natural undrained peatlands are known as a minor source for N_2O emission (Martikainen et al., 1993; Von Arnold et al., 2005). However when peatlands are drained for agriculture and forestry, the decomposition rate of the organic matter of peat increases releasing both carbon (C) and nitrogen (N) and thus leading to enhanced N_2O emission (Kasimir-Klemedtsson et al., 1997; Martikainen et al., 1993). Globally around 60 Mha of peatland have already been drained for agriculture or forestry which is 0.3 percent of world's land cover (FAO, 2012). In Sweden, 1.5 Mha of forested drained peatland exists which is 6 % of total of the total 23 Mha productive forestry area and N_2O emission from these forests on drained peat were found to be of the size 15% of the total anthropogenic N_2O emission from Sweden (Ernfors et al., 2008).

High emissions of N_2O have been recorded from forested drained peat soils in temperate and boreal region (Klemedtsson et al., 2005; Maljanen et al., 2012). A review study compiling data on emission measurement of N_2O in forested soils showed emission from mineral soils to be low compared to drained organic soils (Maljanen et al., 2010). However, our knowledge on flux difference between soil types is primarily based on individual studies which focused on either the drained peat or mineral soils. Diverse environmental conditions make it difficult to compare N_2O emissions from different studies since in diverse conditions other factors than soil type could be important for emission. Therefore, to compare N_2O emissions between different types of soil, it is important to try to keep other conditions such as weather and vegetation as similar as possible.

In this thesis the N_2O emissions from two different types of forested soils: a drained organic soil (Histosol) and a drained mineral soil (Umbrisol) were investigated (**Paper I**). The sites were closely located (within 1 km) and both of them were afforested with Norway spruce after abandonment of agricultural activities, thus exposed to the same land use history, climate conditions and vegetation composition. The work hypothesis was N_2O emission from forested drained organic soil are higher compared to mineral soil in same climatic conditions since drained organic soil have higher soil organic matter (SOM) content (in percentage) compared to the mineral soil.

The emission of N_2O from terrestrial ecosystems is directly related to the soil N cycle, which is complex in nature as it includes several simultaneously occurring processes (Hart et al., 1994; Myrold and Tiedje, 1986; Nason and Myrold, 1991). Briefly, the soil N cycle is described here. The soil N cycle includes mineralization, immobilization, nitrification and denitrification processes. The major terrestrial reservoir of N is SOM. In the mineralization process the organic N compound of SOM is transformed into ammonium (NH_4^+) which is then either taken up by plants or immobilized by microbes (Booth et al., 2005), or used by nitrifiers for nitrification. In nitrification, oxidation of NH_4^+ (autotrophic nitrification) or organic N compound (heterotrophic nitrification) produce nitrate (NO_3^-) via nitrite (NO_2^-) and N_2O is produced as a byproduct of the reactions (Wrage et al., 2001). Denitrification is the reduction of NO_3^- to molecular N_2 via N_2O and is a heterotrophic process which takes place under anaerobic conditions as heterotrophic denitrifiers use NO_3^- as a terminal electron acceptor only when O_2 is unavailable. The microbial nitrification and denitrification are the major N transformation processes involved in N_2O production in soil (Firestone and Davidson, 1989). In addition, nitrifier denitrification and chemodenitrification are known N_2O producing processes in soils (Wrage et al., 2001). These processes of the N cycle are influenced by the environment, such as ecosystem type, soil type, land management, weather and climate, and living communities of plants and heterotrophs (Canary et al., 2000; Chapman et al., 2006; Gödde and Conrad, 2000; Mary et al., 1996).

Plants affect soil N cycling through several mechanisms; uptake of N, retain in tissues which are then slowly released via roots turnover in rhizosphere. Roots turn over and associated mineralization are known to be a major component of soil available N in rhizosphere (Frank and Groffman, 2009). Also, plants host a variety of microbial communities in their rhizosphere and enhance the growth and activity of microorganisms through exudation of labile carbon (C) via roots (Frank and Groffman, 2009; Hütsch et al., 2002). Additionally, the exudation of labile C via plant roots stimulate production of microbial enzymes for degradation of complex soil organic N compound which in turn facilitated nutrient availability for plant uptake (Frank and Groffman, 2009). Plant and microbes interaction, thereby, influence soil N dynamics and higher gross N mineralization in the rhizosphere compared to bulk soil, has already been observed in previous studies conducted in laboratory condition (Herman et al., 2006; Landi et al., 2006).

Most of the plant roots in temperate and boreal forests host ectomycorrhizae fungi (ECM) (Taylor et al., 2000) which play a key role in uptake of nutrients through enhancing the availability N to plant (Powell and Klironomos, 2007). A few studies have investigated the effect of roots/ECM on gross N transformation rates in situ (Holub et al., 2005; Ross et al., 2001), however the effect of ECM on gross N transformation is not clear. In a previous study by Ernfors et al. (2011), using trenching experiment on organic soils at Skogaryd, noticed two times higher N_2O emissions after exclusion of roots and mycorrhizal mycelia. This higher emission was explained by increased N availability for N_2O producing microorganisms due to reduced plant uptake of N from soil through the mycorrhizal fungi. In the **paper II** of this study, the gross rates of N transformation on the same site was investigated using in situ ^{15}N tracer study to elucidate how internal N transformation rates changed as a consequence of exclusion of roots and roots plus ECM which resulted in enhanced N_2O emissions. The understanding of the influence of soil-plant's roots and ECM interaction on soil N turnover could improve our understanding of plants control on N_2O emissions. This understanding is included into some models. One example is the process based CoupModel which recently been calibrated on the Skogaryd organic site and used to simulate N_2O emission (He et al., 2016a; He et al., 2016b). Here the ground water levels together with nutrient uptake by roots were the most influential factors. However, here the ECM interaction was not yet included which may have improved the result. Modelling N_2O emission is always difficult due the many influential factors, processes and thresholds needed to pass for high emissions to occur.

In contrast to this study gross soil N dynamics in soil are traditionally determined by ^{15}N pool dilution experiments in the laboratory, where soils are often mixed and /or sieved which may alter factors that influence soil N transformations, such as N pool sizes and mobility and, root biomass and the microbial community structure, especially ectomycorrhizal hyphae (Frank and Groffman, 2009). For instance, Booth et al. (2006) noticed that soil mixing promotes gross mineralization and NH_4^+ consumption. Therefore, the virtual core approach proposed by Rütting et al. (2011) was used in the study of **paper II** which allowed us investigating gross N dynamics under field conditions in minimum disturbed soils.

In this study process-related gross N transformation rates were quantified by a numerical data analysis based on a ^{15}N tracing model where parameters are optimized using the Markov chain Monte Carlo (MCMC) parameter optimization technique (Müller et al., 2007). The advantage of the ^{15}N tracing model with numerical data analysis is that numerical ^{15}N tracing model provides the advantage to estimate gross nitrogen transformation rates from several simultaneously occurring gross nitrogen transformation processes, while analytical equations quantify only total gross production and consumption rates of the labelled N pools (Barraclough and Puri, 1995; Schimel, 1996). Moreover, this approach allows longer study periods (1–2 weeks) than commonly used isotope dilution experiments with analytical data analysis (usually 1–2 days)(Rütting et al., 2011). Combining this calculating method and the virtual core approach allowed us to reveal the interaction between soil, plant roots and their associated microbial communities including mycorrhizae and N transformation rates in field condition. As root exudates stimulate microbial activity, here the hypothesis was that trenching reduces both gross mineralization and $NH_4^{\ +}$ immobilization rates; and due to decreased NH_4^+ immobilization the relative importance of nitrification for NH_4^+ consumption increases which results in higher soil N_2O emissions after exclusion of roots and ECM.

Aims

The aims of this thesis were to:

- Quantify and compare N_2O fluxes from afforested drained organic and mineral soils (**Paper I**)
- Elucidate how plants and their mycorrhizal symbionts control soil N cycling and affect N_2O emissions from forest soils (**Paper II**)

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Materials and Method

Site descriptions

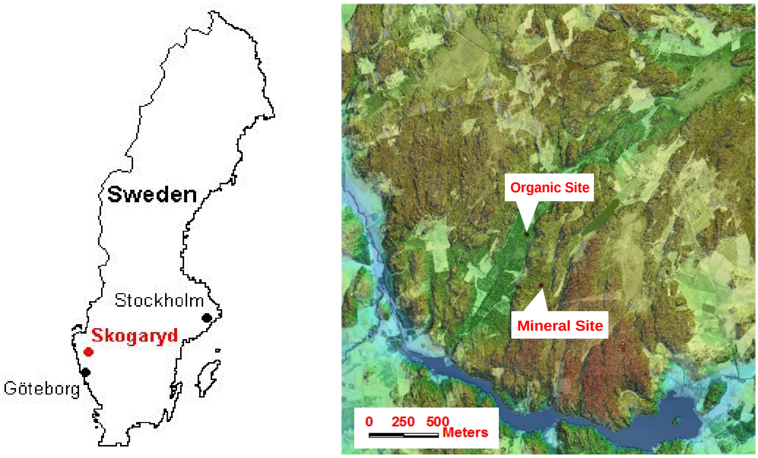

Fig. 1: Organic and mineral site at Skogaryd Research Catchment

For the work described in **paper I**, field measurement were conducted at two closely located sub sites at the Skogaryd catchment, a part of the SITES station network (www.fieldsites.se), located in southwest Sweden (58°23′N, 12°09′E) (Fig. 1). The subsites were on two different types of soil; Histosol and Umbrisol (FAO 2015) referred to as organic site and mineral site, respectively in this thesis. . Both are similarly drained (described in paper I). Experiments for **paper II** were performed on the organic subsite only. The sub sites were drained in the 1870s and used for agriculture until afforested with Norway spruce in the 1950s. At both sites Norway spruce (*Picea abies*) dominates the forest, with some Birch trees (*Betula verrucosa*) and a sparse field and bottom layer. Important characteristics of the sites are given in table (Table 1). The long term (1961–1990) mean annual temperature was 6.4°C and mean annual precipitation 709 mm, recorded at a nearby weather station in Vänersborg, situated 12 km from the study area (Alexandersson and Eggertsson Karlström, 2001).

At each sub site the N_2O fluxes were measured from three measurement stations: O1, O2, and O6 at the organic site, established in a previous trenching experiment (Ernfors et al., 2011), and M1, M2, and M3 at the mineral site, established during this study. The distances between stations were 11-28 m at the organic site and 8- 29 m at the mineral site. Each station was comprised of two flux measurement plots and there were three collars installed with a maximum distance of 1.5-5 m apart in each plot. Thus, a total of 18 collars were present at each site (Fig. 1, **paper I**) for N_2O measurement. In the trenching experiment by Ernfors et al. (2011), the three collars of each plot at the organic site were randomly assigned to one of the three treatments: (a) control (ctrl), (b) roots excluded (exclR) and (c) roots and ECM excluded (exclRM). Detailed description of the trenching can be found in Ernfors et al. (2011). For comparability with the mineral site, N_2O emissions data only from the control chambers of organic site were used in **paper I**.

Table1. Some important characteristics of organic and mineral site

	Organic Site	Mineral Site
SOM content (%)†	74(± 8)	17(± 3)
SOM amount (kg/m^2)†	50 ± (6)	55± (12)
Bulk Density (g/cm^3)†	0.2(± 0.0)	1.1(± 0.1)
Tree age in 2010	60 years	60 years
Above-ground biomass (ton dry weight ha^{-1})	180±0.9	300±1

Note: †, mean over 0.05-0.30 m depth

N_2O Flux Measurements

The flux measurements were conducted using dark stainless steel chamber as described in Ernfors et al. (2011). During August 2010 to July 2013, I conducted N_2O flux measurement from all plots at both organic and mineral site and flux data from control chambers were used in the work described in **paper I.** For the work of the **paper II**, flux data from all control and trenched chambers measured during 2010-2013 at the organic site and the flux data measured by Ernfors et al. (2011) for the period of July 2006 to Dec 2009 were used. Fluxes of N_2O at the soil surface were generally measured biweekly during the morning or early afternoon. A detailed description of the chamber and the procedure of gas sampling is given in **paper II** and Ernfors et al. (2011). The collected gas samples were analyzed by gas chromatography (Agilent 7890A, Agilent Technologies, Santa Clara, CA, USA) equipped with an auto-sampler (7697A). The N_2O fluxes were calculated from the slope of the linear regression of gas concentrations plotted against time.

Measurement of abiotic variable and Soil properties

Air temperature data were collected with Campbell 107 Temperature Probes (Campbell Scientific Inc) at a level of 2 m above the ground at the organic site. Soil temperatures were manually measured at two depths (0.1 and 0.2 m) at both sites, concurrently with the gas sampling **(paper I, II)**. Groundwater level (GWL) was only measured at the organic site since the mineral site had a compact hard soil layers at a depth of around 0.4 m which made its difficult to install the tubes. The measurements were performed manually using a plumb line lowered into perforated tubes inserted to a soil depth of 1.5 m next to each chamber. Soil samples were collected close to the chambers for determination of soil SOM, pH (KCl), total carbon (C), total nitrogen (N) content and C/N ratio. The SOM content was determined by loss-on-ignition where the soil samples were dried at 65°C for 48 hours and then burned at 550° for 6 hours. The SOM content was calculated from the weight loss of the soil samples. To determine the total N and C, the oven dried soil samples were milled in order to homogenize and the samples were then analyzed with an elemental analyzer couple to isotope ratio mass spectrometer (IRMS) (20-20, Sercon Ltd, Crewe, Cheshire, UK).

^{15}N labelling, soil sampling and Analysis of ^{15}N

In **paper II**, for investigating the gross soil N dynamic under field conditions the soil at organic site was labelled with ^{15}N using the virtual soil core approach (Rütting et al., 2011; Staelens et al., 2012) in May 2013. The ^{15}N labelling was conducted by injecting either $^{15}NH_4NO_3$ or $NH_4{}^{15}NO_3$ (99%) into the soils and the detail description of ^{15} N labelling and soil sampling is available in **paper II**. Gross N mineralization and nitrification was calculated using the ^{15}N tracing model *Ntrace*. Detailed description of this process is given in the **paper II.**

Result and discussion

N_2O emission from forested drained organic and mineral soils

Both, drained organic and mineral soils were overall net sources for N_2O emission with the median value of flux rates 38 and 6 µg m^{-2} h^{-1} at the organic and mineral site respectively, although at a few occasions both soils also act as a sink . A pronounced seasonal pattern in N_2O emissions was observed at both sites with occasions of high fluxes to occur in summer (Fig. 4 in **paper I**). The annual emissions for all years were higher at the organic site (Fig. 2) and over the three years period this site showed in average six times higher N_2O flux than the mineral site (Table 2 in **paper I**), which agrees with the result reported by Maljanen et al. (2010) showing higher N_2O emission from drained organic soils compared to the mineral soils in a review study on N_2O emissions.

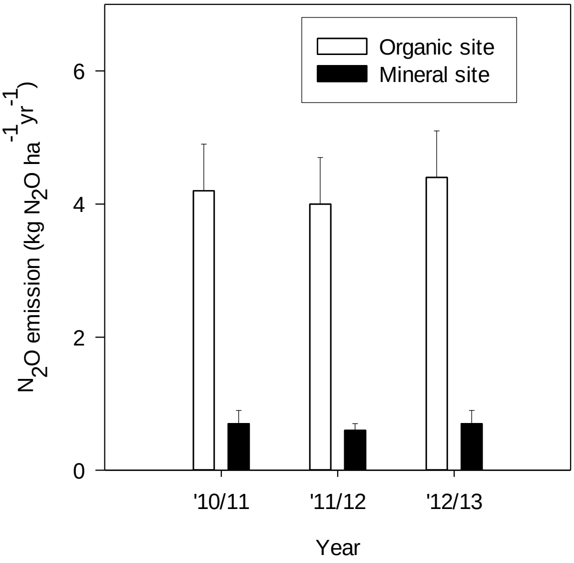

Fig 2: Annual nitrous oxide (N_2O) emission from organic and mineral soil in Skogaryd catchment in south-western Sweden and error bars show standard errors of the mean

The annual N_2O fluxes from the organic site ranged between 4.0 and 4.4 kg N_2O ha^{-1} during the period 2010 to 2013, which is within the range of the reported values for drained organic soils (0.4–8.1 kg N_2O ha^{-1} yr^{-1}:(Alm et al., 2007; Maljanen et al., 2003; Von Arnold et al., 2005; Yamulki et al., 2013). However, the emissions were smaller and with a lower variability between years (year to year) compared to the earlier period measured by Ernfors et al. (2011), where the annual emissions were between 5.4 and 11.2 kg N_2O ha^{-1} in the period 2006 to 2009 for exactly the same plots. Between years, the variation in emissions can be explained by summer months which in the earlier studies were found to be both drier and warmer compared to the years of the present study. This is also supported by the relatively higher N_2O fluxes in the latest study year (2013) which experienced warmer and drier summer and compared to the preceding two years. Drawdown of ground water table as a consequence of drought increases the thickness of aerobic surface peat layer (Alm et al., 1999) which can undergo faster decomposition and subsequent mineralization leading to increase availability of mineral N. This could be the cause of higher emission at the organic site during the warmer years as we can see in the **paper II** that increase availability of mineral N resulted in higher N_2O emission. However, the high variability in N_2O emissions between years underpins the need to compare sites when conditions are similar, since even for the same site the annual emissions vary.

The annual N_2O emissions from the mineral site ranged between 0.6 to 0.7 kg N_2O ha^{-1} yr^{-1}. To my knowledge, no data on N_2O emissions from Umbrisol is available. Nevertheless, the emissions were in the range (0- 1.0 kg N_2O ha^{-1} yr^{-1}) to what Gundersen et al. (2012) found when summarizing on N_2O emissions from temperate forest located on a range of mineral soils including Podzol, Cambisol, Gleysol and Arenosol.

Factors influencing soil N_2O emissions

The pattern of N_2O emission was similar at both sites i.e. increases/decreases of N_2O emission occurred simultaneously most of the time (Fig. 4 in **paper I**). Most of the high flux occasions of N_2O were observed in summer between late May and early September at both sites and these high fluxes often occurred following rainfall after a dry period. High N_2O fluxes were also observed in March and April at the organic site and these high fluxes were related to thawing of frozen soils when the air temperature raised to above $0^{\circ}C$ (Fig. 4 in **paper I**). In contrast to the organic site the N_2O emission from mineral site was not found to increase during thawing.

Within site the SOM was the most important factor influencing the overall size of N_2O emissions from forested drained organic and mineral soil. Table 2 gives an overview of the station specific SOM and emissions data. The greatest N_2O emission was found from the station having highest SOM content (%) at both sites. This finding was also confirmed by the observed correlation between the N_2O emissions and SOM content (%) within both organic ($\rho=0.99$, $p<0.01$) and mineral site ($\rho=0.55$, $p<0.05$) (Table 3 in **paper I**).

Since weather conditions as well as forest age and vegetation were similar, the difference in emission between two sites; organic and mineral site, could be related to their soil properties. The 4-fold higher SOM content (%) of the organic site compared to the mineral site could be taken as an explanation for the higher emissions. However, because of higher bulk density of mineral soils the total SOM amount (kg per volume of soil) in the upper 0.30 m soil was found to be similar (Table 2) to that of the organic sites even though the soil had lower SOM content (%). Thus it could be asked why the mineral site had lower emissions than the organic site. One possible explanations could be that the mineral soil SOM is protected against microbial decomposition by association with minerals establishing physical barriers between SOM and microbes or enzymes (Six et al., 2004), or intermolecular interactions between organic and inorganic substances which decrease the availability of the organic substrate for decomposition (Guggenberger and Kaiser, 2003). On the other hand, in peat little inorganic substances exists (Päivänen, 1995) why this inhibition is less and the SOM in peat is highly decomposable when water-level is lowered (Clymo, 1984) following drainage for forestry. The forests are not fertilized, thus, N-deposition and the mineralization of SOM are the sources of inorganic N delivering to the trees as well as for microbial nitrification and denitrification. Therefore, it can be argued that the lower emissions for our mineral soil might result from lower mineralization of mineral soil SOM. However, the higher tree growth as indicated by its higher above ground biomass at this site (Table 1) does not support low mineralization of SOM at this site.

Table 2: N_2O fluxes and abiotic factors at the stations of organic (O1, O2, O6) and mineral site (M1, M2, M3)

Site	**Station**		
Organic site	**O1**	**O2**	**O6**
N_2O (kg ha^{-1} yr^{-1})	3.7 (± 0.5) ^{b}	6.20 (± 0.7) ^{a}	3.3 (± 0.9) ^{b}
SOM content (%)	66 (± 1)	89 (± 5)	65 (± 6.0)
SOM amount (Kg/m^{-2})	40 (± 3.0) ^{b}	60 (± 3.0)	45 (± 5.0) ^{b}
C/N ratio	28 (± 4.0)	24 (± 1.0)	25 (± 0.3)
pH	3.9 (± 0.1) ^{a}	3.2 (± 0.0) ^{b}	3.7 ± (0.1) ^{a}
Mineral site	**M1**	**M2**	**M3**
N_2O (kg ha^{-1} yr^{-1})	1.6 (± 0.0) ^{a}	0.3 (± 0.0) ^{b}	0.3 (± 0.0) ^{b}
SOM content (%)	22 (± 3) ^{a}	18 (± 2) ^{a}	10 (± 6.0) ^{b}
SOM amount (Kg/m^{-2})	75 (± 9.0) ^{a}	60 (± 6.0) ^{a}	35 (± 2.0) ^{b}
C/N ratio	16 (± 0.5)	19 (± 0.4)	20 (± 0.3)
pH	3.8 (± 0.1)	3.8 (± 0.0)	3.8 (± 0.0)

Note: Parameters that were significantly different between stations within each site are followed by different letters within a row of the site.
Numbers in parentheses are the standard errors of the mean

The availability of mineral N in forest ecosystems were found to be controlled by the competition between plants and soil microorganisms and it is recognized that plants can successfully compete with microorganisms for N (Schimel and Bennett, 2004). Plants are growing more vigorously at the mineral site, as seen in its higher above ground biomass

(300±1 ton dry weight ha^{-1}) compared to the organic sites (180±0.9 ton dry weight ha^{-1}), despite the age of the forests in two sub sites are similar. Thus, it is likely that the forest on the mineral site had a larger N uptake compared to the organic site. As for the above ground biomass, root associated ectomycorrhizal mycelia growth (43.3 and 7.5 g/m^2 at the mineral and organic site, respectively, measured in 2009 to 2010) was also found to be higher at the mineral site. The nutrient absorbing surface area for a plant can be increased (Chapman et al., 2006) by increasing the production of its roots associated mycorrhizal hyphal surface area (Simard et al., 2002) which even can lead to a decreased N availability in soil (Högberg et al., 2006). Thus, the higher root associated ECM production at the mineral site probably resulted in lower N availability in soil and thereby lower N_2O emissions. This suggestion is supported by the findings of the trenching experiment of **paper II** where N_2O emissions were found to be higher in the plots where roots and ECM were excluded. Also, He et al. (2016a) showed that plants uptake of N has significant influence on soil N availability and thereby on N_2O emission in a recent study investigating the factors affecting N_2O emission at our organic site using a process-based model, CoupModel.

Over the entire 6 years period in **paper II**, the mean N_2O emission rate, was higher by 62% in the exclR treatment (mean: 9.5 kg N_2O ha^{-1} yr^{-1}) compared to the control (mean: 5.9 kg N_2O ha^{-1} yr^{-1}) and the exclRM treatment showed almost tripled (mean: 17.1 kg N_2O ha^{-1} yr^{-1}) N_2O emissions compared to control. However, the rate of the gross mineralization was higher in control and the value was almost triple of that found in the exclRM treatment (4.49 and 1.34 µg N g^{-1} d^{-1} for the control and exclRM plot, respectively (Table 3). The gross mineralization rate did not differ between control and exclR treatment. Thus this finding indicates that the mineralization itself is not controlling the N_2O emissions at our organic site. The gross mineralization rate found in control chambers was similar to the gross mineralization rates, 3.5 to 5.9 µg N g^{-1} d^{-1} observed for organic soils forested with birch and poplar (Münchmeyer, 2001; Willison et al., 1998).

As stated above, the SOM mineralization is the main source for mineralized N at the organic site. Consequently, one obvious mechanism by which N_2O emission was decreased despite higher gross mineralization in control plots could be plant uptake of mineralized N leading to decreased N availability for the microbes to produce N_2O. Also, the microbial NH_4^+ immobilization (I_{NH4}) rate was highest at the control and after trenching the rate was decreased by 20% and 64 % in the exclR and exclRM treatment, respectively (Table 3). The decreased microbial I_{NH4} after trenching probably resulted from the declined microbial activity because of reduced input of labile C via roots (Kaiser et al., 2011).Thus, the combined N uptake by plants and microbes reduced concentration of mineral N as the substrate for N_2O emission in the control plots.

Besides immobilization of NH_4^+ (I_{NH4}), Oxidation of NH_4^+ to NO_3^- by nitrifier (autotrophic nitrification notes as O_{NH4}) is another the process that competes for ammonium produced from mineralization. The relative dominance of the processes competing for NH_4^+ , immobilization (I_{NH4}) and autotrophic nitrification (O_{NH4}), can be expressed in the ratio N/I. Using all treatments, including control and trenched, strong correlation between N_2O

emissions (averages over 6 years) and the N/I ratio was found (Fig. 5 in **paper II**). We cannot tell in which process the N_2O was produced, where nitrification is one suggestion. However, NO_3^- produced by nitrification is needed for denitrification which could be the main pathway of N_2O production in our investigated organic site (Björk et al., 2010; He et al., 2016a), the N/I ratio could then be a proxy for predicting N_2O emissions.

Table 3: Gross N transformation rates (mean ± standard deviation) for an organic forest soil in south-western Sweden, for control soil and soil with exclusion of roots (ExclR) or exclusion of roots and ectomycorrhiza (ExclRM).

N transformation		Kinetic	Transformation rate (µg N g^{-1} d^{-1})		
			Control	ExclR	ExclRM
M_{SON}	Mineralization of SON	0	4.49 (0.55) ^{a}	4.46 (0.78) ^{a}	1.34 (0.32) ^{b}
I_{NH4}	Immobilization of NH_4^+	1	2.21 (0.44) ^{a}	1.77 (0.33) ^{b}	0.80 (0.21) ^{c}
O_{SON}	Oxidation of SON to NO_3^-	0	5.67 (0.54) ^{a}	3.91 (0.51) ^{c}	4.59 (0.38) ^{b}
I_{NO3}	Immobilization of NO_3^-	1	0.95 (0.29)	n.d.	n.d.
O_{NH4}	Oxidation of NH_4^+ to NO_3^-	1	1.68 (0.36) ^{a}	2.75 (0.37) ^{b}	1.72 (0.47) ^{a}
D_{NO3}	Dissimilatory NO_3^- reduction to NH_4^+	1	1.42 (0.18) ^{b}	2.04 (0.39) ^{a}	0.85 (0.23) ^{c}
L_{NH4}	Losses of NH_4^+	1	1.34 (0.30) ^{b}	2.63 (0.30) ^{a}	n.d.
L_{NO3}	Losses of NO_3^-	1	4.48 (0.39) ^{a}	2.63 (0.31) ^{c}	3.99 (0.29) ^{b}

Note: Gross rates that were significant different between treatments are followed by different letters within a row. Kinetics were either zero order or first order; n.d. = not detected.

Similar to gross mineralization rate, the total gross nitrification was highest in the control plot which was followed by the exclR and exclRM treatment. These findings are consistent with the observed positive relation between these two processes by Booth et al. (2005). However, this result is different from the findings of Ross et al. (2001) and Kaiser et al. (2011), who found increased gross nitrification despite decreased gross mineralization after trenching (Ross et al., 2001) or tree girdling (Kaiser et al., 2011). In controls we observed a total gross nitrification, production of NO_3^- from soil organic N (O_{SON}) and NH_4^+ pool (O_{NH4}), rate of 6.3–7.4 µg N g^{-1} d^{-1} at Skogaryd (**paper II**) that were similar or within the range of values, 0–10 µg N g^{-1} d^{-1}, reported in the literature for temperate forests (Booth et al., 2005). Heterotrophic nitrification (O_{SON}) had the largest contribution, 77% of the total nitrification. Such dominance of O_{SON} for NO_3^- production in forest soils has also been demonstrated by others (Hart et al., 1997; Rütting et al., 2008; Staelens et al., 2012; Zhu et al., 2011). Even though the total nitrification rate was lower in exclR treatment, the rate of autotrophic nitrification was found to be higher by 41% compared to the control (Table 3). Thus, the relative importance of autotrophic nitrification to the total nitrification was increased in this in exclR treatment. The increase in autotrophic nitrification rate in the exclR treatment was probably dependent to some extent on the decrease production of root derived (or released) nitrification inhibitors (Paavolainen et al., 1998; White, 1986). It could also be that in absence roots competition, more NH_4^+ is available for microbes for autotrophic nitrification. However, the rate of O_{NH4} did not differed significantly between the exclRM and control treatment.

Nitrate immobilization (I_{NO3}) was only detectable in the control plots but not in the trenched treatments. Nitrate immobilization generally increases with increasing C availability (Booth et al., 2005; Hart et al., 1994) due to increased microbial activity and, hence, increased microbial N demand. The absence of I_{NO3} in the trenched plots (Table 3) thus, indicates reduced C availability.

Overall the findings of the study in **paper II** indicate roots and ECM have large influence on soil N turnover rates and that gross rates of N transformation, gross mineralization and nitrification in forested drained organic soil is enhanced in presence of plant roots and ECM. These enhanced rates of gross mineralization are however balanced by the high rates of N immobilization in presence of roots and ECM resulting in relatively low N availability for nitrification and denitrification. Hence soil N_2O emissions decreased in presence of plants roots and ECM.

Conclusions

From the studies presented in this thesis I conclude that,

- Nitrous oxide emission differs between forested drained Histosol and Umbrisol soil. Drained Histosol is a stronger source for N_2O emissions than Umbrisol.
- Plant roots and their mycorrhizal symbionts influence N transformation rates in forested soil. Gross mineralization–ammonium (NH_4^+) immobilization turnover increase in presence of roots and mycorrhizae probably due to high inputs of labile C stimulating microbial activity.
- The N_2O emission is positively related to the ratio of NH_4^+ oxidation (autotrophic nitrification) to NH_4^+ immobilization in forest soils.
- Plants uptake of mineral N as well as the stimulated microbial NH_4^+ immobilization in presence of roots and ECM reduce the importance of nitrification for NH_4^+ consumption and thereby decrease N_2O emissions from forested soils.

Acknowledgement

First of all, I am grateful to the Almighty **Allah (su)** who gives His blessings not only to accomplish this research work successfully but for all my life. He is the one who made me capable to complete this study.

I would like to thank to my supervisor Åsa Kasimir for her guidance and all advice concerning academic thinking, writing and research throughout this study. I want to thank Prof. Leif Klemedtsson for his valuable advice and suggestions during my study. Thanks also to my examiner Prof. Roland Barthel for his valuable suggestions in completing this thesis. I am grateful to Tobias Rütting for all the positive criticisms and insightful comments and for all helps to complete the studies. I am also grateful to Christine Achberger for her encouragement in the beginning of my research work at GEO.

I would like express a large thanks to Mark Johnson, the Head of the department, for his valuable discussions and for his help when needed.

I want to thank Per Weslien for sharing your great experiences and knowledge in solving difficulties in wide range of problems, and Robert Björk for nice and very helpful discussion about mycorrhizae. I am grateful to David Allbrand who has been always with me out in the field, and Aila Schachinger who help me in lab works.

It has been pleasure for me to meet all the people at GEO and to have a chat with them now and then. I want to thank Prof. Rodney Stevens for your generosity and for your wonderfully supportive attitude. Thanks to Fredrik Lindberg and Alexander Walther. It has been pleasure for me to work with you in GIS teaching. Special thanks to Anna-Karin, Tage Vowles, Yumei Hu and Tinghai Ou for sharing family life. I would like to thank Tariqul Islam and Hongxing He for their company during coffee break. Thanks also to Ewa Steen Stackelius for patiently organizing many administrative things in order for me during this study.

BECC (Biodiversity and Ecosystem services in a Changing Climate) is gratefully acknowledged for financing support.

I would like to thank to my parents who live far away from me but they always motivated and fervently encouraged me to complete my work. My warmest and deepest thank to my son Ayaan Nazeef. After a long and hard day’s work or sometimes when everything feels difficult and suffocating, there is nothing better than come to home and see your smiling face. Finally, thanks to my wife, Dr. Shamima Nazmin Akhi, for all her never-ending love and encouragement and being my friend all the way through.

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