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Nitrous oxide production in boreal soils with variable organic matter content at low temperature – snow manipulation experiment

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Abstract

Agricultural soils are the most important sources for the greenhouse gas nitrous oxide (N₂O), which is produced and emitted from soil also at low temperatures. The processes behind emissions at low temperatures are still poorly known. To simulate the effects of a reduction in snow depth on N₂O emission in warming climate, snow pack was removed from three different agricultural soils (sand, mull, peat). Removal of snow lowered soil temperature and increased the extent and duration of soil frost which led to enhanced N₂O emissions during freezing and thawing events in sand and mull soils. The cumulative emissions during the first year when snow was removed over the whole winter were 0.25, 0.66 and 3.0 g N₂O-N m⁻² yr⁻¹ in control plots of sand, mull and peat soils, respectively. Without snow cover the respectively cumulative emissions were 0.37, 1.3 and 3.3 g N₂O-N m⁻² yr⁻¹. Shorter snow manipulation during the second year did not increase the annual emissions. Only 20% of the N₂O emission occurred during the growing season. Thus, highlighting the importance of the winter season for this exchange and that the year-round measurements of N₂O emissions from boreal soils are integral for estimating their N₂O source strength. N₂O accumulated in the frozen soil during winter and the soil N₂O concentration correlated with the depth of frost but not with the winter N₂O emission rates per se. Also laboratory incubations of soil samples showed high production rates of N₂O at temperatures below 0°C, especially in the sand and peat soils.

1 Introduction

Nitrous oxide (N_2O) is a strong greenhouse gas having global warming potential of 298 times that of carbon dioxide (CO_2) in a time horizon of 100 years (Solomon et al., 2007). The concentration of N_2O in the atmosphere is increasing annually at the rate of 0.8 ppb and agriculture and land use change are globally the most important sources of this gas (Solomon et al., 2007).

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The emissions of N₂O in the Northern Hemisphere during winter are important because they may account more than half of the annual N₂O emissions (e.g. Regina et al., 2004; Maljanen et al., 2004). Several studies have linked the high emission periods to freezing and thawing events in soil (e.g. Goodroad and Keeney, 1984; Christensen and Tiedje, 1990; Müller et al., 2002; Röver et al., 1998; Matzner and Borken, 2008). However, the production of N₂O in soils occur at low temperatures without freezing-thawing episodes (e.g. Regina et al., 2004; Maljanen et al., 2007).

Recent laboratory experiments have shown that N_2O can be produced and emitted from soils at temperatures below 0°C, even down to -6°C (e.g. Holtan-Hartwig et al., 2002; Koponen et al., 2004, 2006a; Öquist et al., 2004). Teepe et al. (2001) suggested that high production of N_2O in frozen soil occurs in unfrozen water films covering soil particles. This unfrozen water, where soluble microbial subtrates are concentrated as a result of ice formation in soil, is surrounded by ice which limits oxygen supply to the water film thus supporting denitrification there. There is a rapid change in the temperature response of N_2O production rate close to 0°C (e.g. Koponen et al., 2004). According to Holtan-Hartwing et al. (2002) below 0°C enzymes facilitating N_2O reduction to N_2 are less active and Öquist et al. (2007) concluded that N_2O was the end product of dentrification in forest soils incubated at -4°C. However, the overall reasons for this shift are poorly known. Sharma et al. (2006) suggested that the freezing/thawing stress could change the microbial community but this has not been noticed in other study by Koponen et al. (2006b).

Snow cover is a good insulator and keeps soil temperature close to 0°C during winter even when air temperature falls far below zero (Hardy et al., 2001; Schürmann et al., 2002). According to Kubin and Poikolainen (1982) a clear negative correlation exists between the frost and snow depth, and frost formation is affected by the timing of the first snow in autumn together with temperature. Without snow cover the soil temperature drops in the top soil well below 0°C (Hardy et al., 2001; Maljanen et al., 2007) and lower temperatures may enhance N_2O emissions (Koponen et al., 2004; Maljanen et al., 2007; Groffman et al., 2006). With global warming thinner snow cover

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and more frequent freeze-thaw events could be expected in Northern Hemisphere in future (Solomon et al., 2007; Mellander et al., 2007) which possibly will result in higher N₂O emissions. It is not known how the low soil temperature enhances N₂O production in agricultural soils, especially in organic ones which are the most potential sources of N₂O (Kasimir-Klemedtsson et al., 1997; Regina et al., 2004; Koponen et al., 2004).

We hypothesized that the N_2O production and emission from boreal soils during winter depends not only on the snow cover, but also on soil characteristics, e.g. organic matter content. We therefore compared the N_2O production and emissions at low temperatures in three soils with variable organic matter content. We studied 1) the annual emissions of N_2O 2) the effect of thinner snow cover on soil characteristics (e.g. temperature, frost depth, soil moisture) and to the N_2O emissions/production in the soils 3) if the amount of unfrozen water in soil explains the differences in the N_2O production between various soils below $0^{\circ}C$.

2 Materials and methods

2.1 Study sites

Two of the study sites were located in Maaninka (63°09′N, 27°20′E), Eastern Finland and one in Kannus (63°54′N, 23°56′E), Western Finland. The mineral soil type (in Maaninka) was fine sand, according to FAO classification it was classified as dystric regosol, medium textured (Table 1). The sand soil profile (0–25 cm) consisted mainly of fine sand (71%), silt (15%), and clay (6%). The other two soils were mull (in Maaninka) and peat (in Kannus), classified as histosols. See Table 1 for detailed soil characteristic.

The long term average annual temperature (1971–2001) is 2.4°C in Maaninka and 2.8°C in Kannus and the average annual precipitation is 609 mm and 561 mm in Maaninka and Kannus, respectively (Drebs et al., 2002). Of the total precipitation approximately 50% falls as snow on both sites. Snow cover typically appears in mid-November and melts in late April. The coldest month is February (long term average

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-9.6°C in Maninka and -9.2°C in Kannus), and the warmest July (long term average 16.5°C in Maaninka and 15.8°C in Kannus) (Drebs et al., 2002). The mean daily air temperature in Maaninka during the two-year study period varied from -32 to +24°C (Fig. 1), being on average 4.6°C (2.2°C above the long term average). In Kannus mean daily air temperature fluctuated between −29 and +23°C, mean being 3.9°C (1.1°C above the long term average).

The long term average maximum snow depth in Maaninka is 50 cm and in Kannus 44 cm (in the middle of March). The average ground water table depth during the growing season in sand soil was about 6 m, in mull soil from 1 to 2 m and in peat soil 0.6 m.

The study sites (about $20 \, \text{m} \times 10 \, \text{m}$) were separated from larger grass swards. The swards were established with a mixture of timothy (*Phleum pratense L.*) and meadow fescue (*Festuca pratensis Huds.*) in 2004. The study sites were used for hay production and were not grazed.

The sand soil site received a surface fertilization (kg ha⁻¹) of N 72 (52% as NH₄-N), P 12 and K 20 in 14 May 2005. The second application was given in 4th July 2005 with N 90 (56% as NH₄-N). In October 2005 the site was treated with glyphosate (41 ha⁻¹) to eliminate all plants from the site. The sand site was ploughed in 14th July 2006 and sown with a mixture of timothy and meadow fescue on 19th July 2006 with fertilization of (kg ha⁻¹) of N 70 (56% as NH₄-N), P 7.5 and K 22.5.

The mull soil site received a surface fertilization (kg ha⁻¹) of N62 (0.52% as NH₄-N), P10 and K17 on 21st May 2005 and N90 (56% as NH₄-N) on 4th July 2005. In October 2005 the site was also treated with glyphosate (41ha⁻¹). The mull soil site was ploughed in 14th July 2006 and timothy and meadow fescue were sown in July 2006 with fertilization (kg ha⁻¹) of N70 (0.56% NH₄-N), P7.5 and K22.5.

Peat soil in Kannus was not fertilized or treated with glyphosate during the study period.

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2.2 Experimental setup

The field measurements were made between September 2005 and October 2007. During the first year an area of 2×8 m in each site was kept free from snow to lower soil temperature in winter (referred here as bare soil) and the control area had undisturbed snow cover. During the first winter, all snow was removed from the bare plots with a spade on each day the snow height exceeded about 5 cm. On each soil type (sand, mull and peat) there were total of 12 collars for gas flux measurements (see later) of which six were located on manipulated soil (bare) and six were located on undisturbed (control) soil.

After the first year the Maaninka sites (sand and mull soils) were ploughed and grass was sown in July as described above. The collars were then re-installed on both sites. In Kannus site (peat) the soil was not ploughed and collars remained on the same positions as in the first winter.

During the second winter the bare plots (three areas in each site, about 2×3 m each) were kept free from snow from October to December while the control plots received undisturbed snow cover at that time. After December all plots received natural snow cover.

2.3 Environmental variables

Soil frost was measured using frost-depth gauge filled with methylene blue (Gandahl, 1957) and soil temperatures (depth 5 cm) were recorded with iButton temperature loggers (Dallas Semiconductor Corp., USA). Air temperature was recorded at a weather station, located at Maaninka study site and at weather station about 20 km from the Kannus study site. Soil moisture in sand and mull soils in Maaninka from September 2006 to August 2007 were recorded using Time-Domain-Reflectometry (TDR) method with soil moisture probes (CS625) and dataloggers (CR200) at the depth of 5 cm (Campbell Scientific, UK).

Soil samples for analysis of NO_3^- , NO_2^- and NH_4^+ were collected at depth of 0–10 cm

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26 times from Maaninka sites and 9 times from Kannus site between September 2005 and October 2007. NO_3^- and NO_2^- were extracted from soil samples with distilled H_2O and NH_4^+ with 1M KCl solution. The amount of NO_3^- and NO_2^- in the H_2O -extracts were analyzed by an ion chromatograph (DX 120, Dionex Corporation, USA) and NH_4^+ with a spectrophotometer (Ultrospec 3000 Pro, Biochrom, UK) using method by Fawcett and Scott (1960). Soil dissolved organic carbon (DOC) concentrations during the first study year were measured from 0.25 M K_2SO_4 -extracts with TOC analyzer (Shimadzu Scientific).

2.4 Gas flux measurements

 N_2O flux rates from snow-free soil were measured with a static chamber method using aluminium chambers, equipped with a fan, $(60\times60\,\mathrm{cm},\,\mathrm{h})$ from 15 to 30 cm) and aluminium collars $(60\times60\,\mathrm{cm},\,\mathrm{h})$ pre-installed in the soil. After closing the chamber gas samples were taken with a 50 ml polypropylene syringe (Terumo) at 5, 10, 20 and 25 min intervals from the headspace of the chamber. Samples were injected within 24 h into pre-evacuated 12 ml vials (Labco Excetainer). Samples were analyzed for N_2O with a gas chromatograph (Agilent 6890N, Agilent Technologies, USA) equipped with an autosampler (Gilson, USA) and an electron capture (EC) detector. Compressed air containing 0.389, 3.0 or $50.1\,\mu\mathrm{II}^{-1}$ of N_2O was used for daily calibration. The flux rates were calculated from the linear increase or decrease of the gas concentrations in the headspace of the chamber. If there were any indications of failures in the gas sampling or gas analysis the results were discharged (less than 2% of all samples).

Gas fluxes from the snow covered plots were determined by measuring gas concentration gradients from the snow and by calculating associated diffusion rates in the snow (Sommerfeld et al., 1993; Maljanen et al., 2003a). Gas samples (40 ml) from the snow pack were drawn with a stainless steel probe (Ø 3 mm, length 100 cm). For the calculation of the diffusive fluxes, an ambient gas sample was taken above the snow pack and another sample inside the snow pack two cm above the soil surface.

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Simultaneously, snow samples were collected with a PVC tube (\emptyset 10.2 cm) for porosity measurements. The intact samples were weighed for calculation of the average porosity of snow using the density of pure ice (0.9168 g cm⁻³).

2.5 Gas concentrations in soil

N₂O concentrations in soil were measured simultaneously with the gas flux measurements. Samples of 30 ml were taken with syringes from pre installed silicon tubes (Ø 1.0 cm, wall thickness 0.3 cm, length 110 cm, V=86 cm³) inserted horizontally in sets at the depths of 5 and 20 cm in the soil (Maljanen et al., 2007) beside to the collars for the gas flux measurements. Sand and mull soil sites were both equipped with 6 sampling tubes at both depths and peat soil site with 5 sampling tubes at both depths. Samples were treated and analyzed with a gas chromatograph as described above.

2.6 Temperature dependence of N₂O and CO₂ production rates, laboratory incubations

 N_2O and CO_2 production rates in laboratory incubations were measured using soils collected in the autumn 2007. Samples were stored at +4.9°C before experiment. Five replicate samples, adjusted to 80% WFPS (water filled pore space), each consisting of 50 ml soil were placed in 550 ml infusion flasks and were incubated at +12.5, +4.9, -2.0 and -6.8°C. The flasks were closed with an aluminium foil during the incubation period except during gas sampling when the flasks were sealed with an air tight rubber septum. Immediately after sealing, 100 ml of air was added with a needle through the syringe into the flask to create overpressure for gas sampling. A total of four gas samples (20 ml) were taken from the headspace of the flasks between 4 to 24 h after closing. N_2O concentrations were analyzed as described above and CO_2 concentrations with HP 5893 gas chromatograph equipped with TC detector (see Nykänen et al., 1995). N_2O and CO_2 production rates were calculated from the linear increase of gas in the headspace. At the beginning of the experiment and after the total incubation

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time of 10 weeks concentrations of NO_3^-N , NO_2^-N and NH_4^+-N were analyzed from the soils as described earlier for field samples. The amount of unfrozen water at temperatures from -8 to $+2^{\circ}C$ from sand, mull and peat soils were measured with high-field 2H_2O NMR (Sparrman et al. 2004). The samples were placed in 10 mm NMR-tubes, stored for \geq 24 h at $+4^{\circ}C$, then analyzed using a Bruker Avance DRX 500 spectrometer (1H at 500.13 MHz), detecting 2H at 76.77 MHz with a 10 mm liquid broadband probe, in experiments with a single 90° -pulse of $19\,\mu s$ followed by a $40\,\mu s$ pre-acquisition delay as a T_2 -filter. Signals were acquired over $100\,kHz$ spectral width and there were $200\,scans/transients$ (eight dummy scans) with 1s relaxation delays.

10 2.7 Statistical analysis

The differences between various soils in the physical and chemical variables were analysed with One-Way Anova (SPSS 14.0. SPSS Inc.). The N_2O flux rates were compared with non parametric Mann-Whitney U-test.

3 Results

3.1 Soil frost

The maximum snow depths in the undisturbed areas in the sand, mull and peat sites were 49, 59 and 41 cm during the first winter and 30, 34 and 47 cm during the second winter. All snow was removed from the manipulated plots during the first winter. During the second winter, after the short-term manipulation of snow cover was ceased, sand, mull and peat sites reached the maximum snow depth of 32, 34 and 46 cm. The total period with snow cover on unmanipulated plots (Fig. 1) was shorter during the second winter (about 85 days) than during the first winter (about 135 days).

In the autumn 2005 soil frost appeared in early December and the top layer of sand and peat soils remained frozen until thawing between April and July. During the first

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winter soils without insulating snow cover developed deep soil frost; in sand, mull and peat soils the maximum soil frost depths were 120, 80 and 40 cm, respectively. Under snow cover the maximum soil frost depth remained shallower being 30, 10 and 20 cm in sand, mull and peat soils. In mull soil there was no continuous frost during that winter (Fig. 2).

In autumn 2006, the first cold period with snow and soil frost occurred in October–November. In the end of November all soils thawed again and remained unfrozen until the middle of December 2006. During the second winter, in all soils with snow cover, the soil frost was deeper than during the first winter (maximum depths 60, 40 and 30 cm in sand, mull and peat soils, respectively). There were no differences in the maximum frost depth between manipulated (short period in October–November) and natural plots during the second winter (Fig. 2).

3.2 NH_4^+ , NO_3^- , NO_2^- and DOC concentrations in soil

The mean (\pm SD) NO $_3^-$ -N concentrations during the study period were 4.44 \pm 12.0, 48 \pm 65 and 134 \pm 137 μ g N g $_{dw}^{-1}$ in sand, mull, and peat soils, respectively. The highest NO $_3^-$ concentrations were in early summer and the lowest ones in autumn. The mean NO $_2^-$ -N concentrations in soils were 0.07 \pm 0.17, 0.03 \pm 0.10, and 0.12 \pm 0.32 μ g N g $_{dw}^{-1}$ in sand, mull, and peat soils, respectively. The NO $_2^-$ -N concentrations were at their highest in January–February 2006. NH $_4^+$ concentrations in soils were 2.4 \pm 4.3, 7.4 \pm 11.5, and 27.5 \pm 24.0 μ g N g $_{dw}^{-1}$ in sand, mull, and peat soils, respectively. Maximum NH $_4^+$ concentrations occurred during spring thaw in 2007. There were no statistical differences between the bare and control soils in the NO $_2^-$, or NO $_3^-$ concentrations. Mean NH $_4^+$ concentrations in bare mull soil (10.5 μ g N g $_2^{-1}$) were significantly higher than those in mull soil under snow cover (4.3 μ g N g $_2^{-1}$) during the first year.

The mean (\pm SD) K₂SO₄ extractable DOC concentrations in soils during the first manipulation year were 0.04 \pm 0.01, 0.14 \pm 0.07 and 0.40 \pm 0.12 mg C g⁻¹ in sand, mull, and peat soils, respectively. There were no differences between bare and control soils if

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all data was analysed. Only during one sampling day in the end of February 2006 DOC concentration in the bare mull soil was almost twice (0.45 mg C g⁻¹) of that in the control soil (0.25 mg C g⁻¹). In the early winter 2005/2006 and after February 2006 K_2SO_4 extractable DOC concentration remained at rather constant level and did not increase during soil thawing.

3.3 N₂O dynamics and seasonal emissions

During the first year the N_2O emissions from sand and mull soils but not the peat soil increased slightly during the first freezing of the top soil in the autumn, but thereafter emissions decreased and were rather low until soil thawing (Fig. 1). During thawing the N_2O emissions from bare plots were remarkably higher than those from the plots which had undisturbed snow cover. A maximum emission ($10\,000\,\mu g\,N_2O-N\,m^{-2}\,h^{-1}$) took place in the bare peat soil in early May 2006. During the following summer the N_2O emissions were low, less than $500\,\mu g\,N_2O-N\,m^{-2}\,h^{-1}$ in the peat soil and less than $100\,\mu g\,N_2O-N\,m^{-2}\,h^{-1}$ in the sand and mull soils. In autumn 2006 when the top soil was frozen first time there was an increase in the N_2O emission from the sandy soil but not from the mull or peat soils. Thawing related N_2O emission peaks occurred also in autumn 2006 in the sand soil after a short cold period in November. However, the N_2O emissions did not increase when the the soils were frozen again in December until the thawing related high spring emissions in April 2007 (Fig. 1).

Soil temperature at depth of 5 cm ranged from -12 to 23° C (Figs. 1, 3). The N₂O emissions did not decrease linearly with decreasing soil temperature (Fig. 3). In the sandy and mull soils the highest N₂O emissions took place mostly at soil temperature close to 0° C. In the peat soil the highest N₂O emissions occurred at around 5° C. When soil temperature dropped below -1° C at the depth of 5 cm the N₂O emissions from all soils decreased (Fig. 3).

Soil moisture measured with TDR from the field during the unfrozen periods did not correlate with the N₂O emissions in the sand and mull soils (Fig. 4). When the

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soils were frozen first time in November enhanced N₂O emissions occurred with simultaneous decrease in water content. From the middle of January until April the N₂O emissions remained low as did soil moisture until a rapid increase in the soil moisture and N₂O emissions during thawing in April. After thawing there was a simultaneous rapid decrease in soil moisture and N₂O emissions in sand soils, but thereafter none of the variation in N₂O emissions could be explained by the variations in soil moisture.

Soil temperature above 0°C and soil moisture (sand and mull soils) had a significant negative correlation (R=-0.694, p<0.001), i.e. soil moisture decreased with increasing soil temperature. However, at soil temperatures below 0°C, soil moisture in sand soil did not correlate with soil temperature but in mull soil there was a positive correlation (R=0.420, p=0.010), i.e. amount of unfrozen water decreased with decreasing temperature.

The annual N_2O emissions from the all control plots were higher in the first year than in the second year (Table 2). The soils rich in organic matter (mull and peat) had the greatest difference in their N_2O emissions between the years. In the peat soil the annual N_2O emission in the second year was only about one third of that in the first year.

During the first study year the annual N_2O emissions from the sand and mull soils without snow were higher than those from the control (undisturbed snow) plots (Table 2). Lower soil temperature (snow removal) did not affect annual emissions from the peat soil. The complete removal of snow had stronger effect on the N_2O emissions than the partial removal of snow during the second winter. During the second year with only a short manipulation period the N_2O emissions were slightly higher from the manipulated plots than from the controls (Table 2). The N_2O emissions during winter (here winter period is considered to last from the beginning of November to the end of April) were surprisingly high in all sites, with or without snow manipulation. The cumulative N_2O emissions in winter (6 months) were on average 81, 77 and 78% of the annual emissions in sand, mull and peat soils respectively.

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3.4 N₂O concentrations in the soil

Concentrations of N_2O in all soils and treatments started to increase immediately after the top soil was frozen, and remained at a high level until thawing when soil N_2O concentrations rapidly decreased close to the atmospheric concentration in a few weeks (Fig. 2). Accumulation of N_2O was faster in plots without snow than in the plots with undisturbed snow cover. There was no statistical difference in the N_2O concentrations between the depths 5 and 20 cm in any of the soils. The highest N_2O concentration occurred in early April in both years, just before the soil thawing began. The N_2O concentrations increased with increasing depth of the soil frost in all sites. Soils without snow also had higher N_2O concentration in winter than soils with a natural snow cover. During winter the average concentrations of N_2O were highest in peat soil (max. $5800 \, \mu I \, I^{-1}$) and lowest in mull soil, thus the accumulation of N_2O was not clearly dependent e.g. on soil OM content. During the unfrozen periods the N_2O concentrations in soil were close to the ambient concentration, about $0.3 \, \mu I \, I^{-1}$ (Fig. 2).

3.5 Production of N_2O and CO_2 in laboratory incubations

In the beginning of the incubation experiment all soils produced N_2O at $4.9^{\circ}C$ (Fig. 5) and the production rates were rather similar, on average $0.05\,\mathrm{ng}\,\mathrm{N_2O}\,\mathrm{cm^3}\,\mathrm{h^{-1}}$. In sand soil the N_2O production rate at $-2^{\circ}C$ increased after two weeks, in contrast to the other temperatures, and remained thereafter higher than those at other temperatures. In mull soil the N_2O production rates decreased with time at all temperatures. After 10 weeks incubation at $+4.9^{\circ}C$ the N_2O production rates in mull soils were less than half of that in the beginning of the experiment. Mull soils incubated at -6.8 and $-2.0^{\circ}C$ had very low production rates or they even consumed some N_2O (Fig. 5). Peat soils incubated at $+12.5^{\circ}C$ produced N_2O at high rate during the first weeks, but thereafter production rates decreased to the initial value measured at $+4.9^{\circ}C$. At $+4.9^{\circ}C$ N_2O was produced in peat soils at almost constant rate during the 10-week incubation period. In contrast

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to the soils incubated above 0°C, N_2O production rates of peat incubated at -6.8 and -2.0°C increased with time and the final production rate at -2.0°C, 4.7 ng N_2O cm³ h⁻¹, was even higher than the maximum rate measured at +12.5°C (Fig. 5).

 CO_2 production rate was rather constant in all soil during the incubation of 10 weeks. CO_2 production rates at +4.9°C increased with increasing soil OM content, being 380, 450 and 710 ng CO_2 cm³ h⁻¹ in sand, mull and peat soils, respectively. At 12.5°C the mean rates were 690, 610 and 1300 ng CO_2 cm³ h⁻¹. At -2°C all soils produced still CO_2 with rates of 69, 28 and 170 ng cm³ h⁻¹ in sand, mull and peat soils. At -6.8°C CO_2 production was not detected in sand and mull soils but in peat soil it was still 30 ng CO_2 cm³ h⁻¹.

In all soils and all incubation temperatures the amount of NO_3^- after the 10-week incubation period was higher than the initial values (Table 3). The NO_3^- concentration was highest in soils incubated at +12.5°C and lowest at -6.8°C, whereas NO_2^- concentrations increased only in soils incubated at -2.0 and -6.8°C and were under detection limit in other temperatures (Table 3). The amount of NH_4^+ decreased during the incubation of 10 weeks at +4.9 and +12.5 but increased at -2.0 and -6.8°C.

There was unfrozen water in all soil types down to -8° C which was on average from 10 to 20% of the water at $+4^{\circ}$ C. The amount of unfrozen water decreased with decreasing temperature in the temperature range from -2 to -8° C. There was a significant difference between all temperatures, except almost significant difference (p=0.056) for temperatures -6° C and -4° C in the mull soil (Fig. 6).

4 Discussion

During the first study year the total removal of snow enhanced annual N_2O emissions from sand (1.5-fold) and mull soils (2-fold) compared to the control soils. These observations support the results from Maljanen et al. (2007) and Groffman et al. (2006), who report similar responses to snow removal treatments. In the study by Maljanen

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et al. (2007), the removal of snow disturbed also the growth of perennial plants during the following growing season and the higher N₂O emissions from the bare plots were suggested to be a result of higher availability of NO₃ for denitrification in the absence of plants. Here, during the first winter, plants were killed in the autumn from all plots of the sand and mull soils. Therefore, the higher N₂O emissions after thawing from snow-free plots can not be explained by the absence of plants. In peat soil the effect of snow removal on N₂O emissions was minor. N₂O emissions from control and snow free-plots were similar, although the control plots had intact vegetation cover and perennial grasses were partly deceased in the manipulated plots.

Annual N₂O emissions from the studied soils types (sand, mull and peat) increased with increasing organic matter content, N concentration and C/N ratio and with decreasing pH and bulk density of the soil. The annual emissions were in the range reported earlier for similar soils in Finland (Regina et al., 2004; Syväsalo et al., 2004; Maljanen et al., 2003b, 2004, 2007). However, there was a high variation between the years, especially in mull and peat soils. Therefore, for the comparison of annual N₂O emissions from different soils you should use a multi-year averages. The highest annual emission from the peat soil during the first year resulted mainly from the thawinduced N₂O emission peak in the spring. This thawing episode during about one month was responsible for 50% of the annual emission from the peat soil. The lower annual emission from the peat site during the second year was mostly explained by the lower emissions during thawing in that year. However, with the temporal resolution used for gas flux measurements we may have missed some of the second year N₂O peaks. With manual sampling the N₂O emissions are always slightly biased, because possible episodic high emissions of N₂O can be missed. This is true especially during thawing, when gas accumulated in frozen soil is suddenly released and a simultaneous production of N₂O occurs (Wagner-Riddle et al., 2008; Teepe et al., 2001). This study also confirmed results from previous laboratory microcosm experiments showing that not only soil thawing but also freezing can spike N₂O emission from soil (Koponen et al., 2004). This spiking of N2O during soils freezing may be result of physical pro-

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cesses and change of the soil porosity similarly as Mastepanov et al. (2008) reported for methane fluxes in tundra soil. However, in contrast to the results from a laboratory experiments by Koponen et al. (2004), the freezing peaks here were evident only from the sand soil and were always lower than those measured during thawing. Because of the longer sampling intervals with the peat soil, some freezing induced N_2O emission peaks may have been missed in our study, pointing out the need of continuous measurements of N_2O during these critical periods. In our study the N_2O emissions during the unfrozen season were on the average only 20% of the estimated annual emission, showing that most of the N_2O emissions originated from the winter period of 6 months. In addition, the proportion of winter emissions in our study were higher than those reported earlier for boreal agricultural soils (Maljanen et al., 2003b, 2007; Regina et al., 2004; Mäkiranta et al., 2007).

N₂O concentrations in soil were always higher in frozen than unfrozen soil. The depth of frost in soil was closely linked to the accumulation of N₂O in the soil and N₂O concentrations increased with frost depth. The gas sampling tubes were installed at depths of 5 and 20 cm and the effect of frost was seen at those depths even the frost depth went below these depths, down to 1 m. Thus, N₂O can be produced throughout the frozen soil layers. It is important to note that after 2 to 4 weeks under frozen conditions soil oxygen content is low because soil microbes are still active and consume oxygen in soil but the transport of oxygen from the atmosphere to the soil is limited resulting from the slow diffusion rate of oxygen in frozen soil (Öquist et al., 2004). Low O₂ concentration favours denitrification which is the most likely source of N₂O at low temperatures (Wagner-Riddle et al., 2008; Öquist et al., 2007; Phillips, 2008). The accumulation of N₂O in frozen soil could have been favoured by the inhibition of N₂O reductase activity at low temperatures (Holtan-Hartwig et al., 2002; Melin and Nommik, 1983). N₂O can be produced within unfrozen water films in soil, where microbial nutrient concentrations are high because they concentrate from freezing water into these unfrozen water films (Teepe et al., 2001). Enhanced denitrification in bare soil may be related to more severe frost and therefore release of microbially available organic car-

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bon which stimulates respiration and depletion of O_2 (Christensen and Tiedje, 1990; Mørkved et al., 2006) thus providing favourable conditions for denitrification. However, the environmental controls behind the N_2O emissions from soils are still poorly known.

During winter, the N₂O emissions can be low if an ice layer on the top of soil traps gases in the soil (van Bochove et al., 2001). There obviously was high N₂O production in our investigated soils (as shown by the accumulation of gases in frozen soil) and this N₂O was then released during thawing. We suggest, that during thawing the first burst of N₂O originated from accumulated N₂O (Teepe et al., 2001). After this release of the N₂O storage emissions dropped until they increased again when the soil was completely thawed. This second burst likely resulted from the production of N₂O as a result of enhanced microbial activity (Wagner-Riddle et al., 2008). This N₂O dynamics was seen in all three soils during the second spring.

In laboratory incubations peat soil had the greatest potential to produce N_2O at $-2^{\circ}C$, while the mull soil had a minor capacity to produce N_2O at any temperatures. The lower production rate in the mull soil than the sand soil was opposite as expected from the results from the field measurements. The reasons for the low N_2O emissions from mull soil, even the soil moisture was adjusted to 80% WFPS, which should be favourable for denitrification (Davidson, 1991), are unknown. There was nitrate available in the end of the 10-week incubation period at all temperatures from -6.8 to $+12.5^{\circ}C$. Ammonium, the key substrate for chemolithotrophic nitrification, was available also at temperatures $<0^{\circ}C$ as shown by the accumulation of ammonium. Therefore, NO_3^- did not limit the N_2O production in denitrification in frozen soil here.

Sparrman et al. (2004) reported that amount of unfrozen water increased with increase in soil organic matter content. In our study there was also similar trend but not linear relationship (Fig. 6). The probable reason is that we used, in contrast to the study of Sparrman et al. (2004) soils with variable mineral fractions. Clay content (here 6% in sand soil) has shown to increase the amount of unfrozen water in soil at minus degrees (Patterson and Smith, 1981). Therefore, the effect of mineral soil fractions could overlap the effect of organic matter. In peat and sand soils N₂O production was always

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higher at -2° C than at -6.8° C. The amount of unfrozen water (g soil dw⁻¹) decreased with decreasing temperature. In the unfrozen water the water potentials, important to microbes, can be very different at -2° C and at -6.8° C (Harryson Drotz et al., 2009) greatly affecting the water availability per se.

The production rate of N_2O in peat soils incubated at $-6.8^{\circ}C$ increased slowly with time and was in the end of the experiment even higher than that at $+12.5^{\circ}C$, which is in accordance with Öquist et al. (2004). Thus, the peat soil had a remarkable potential to produce N_2O for several weeks at temperatures $<0^{\circ}C$. In the same experiment CO_2 production rate, indicating heterotrophic microbial activity, decreased with decreasing temperature. At $-6.8^{\circ}C$ the CO_2 production rate was only 2% of that at $+12.5^{\circ}C$ and did not correlate with N_2O production rate. However, for CO_2 production it is likely that the observed rate is indicative of the rate of the microbial activity, while this may not be the case for N_2O production. High N_2O production rate in the frozen peat soil was also seen in situ as accumulation of N_2O in soil.

In contrast to many earlier findings (e.g. Bateman and Baggs, 2005) soil moisture during the unfrozen period did not correlate with the N_2O emissions. Therefore, e.g. modeling of N_2O emissions based on soil moisture with these soils is not possible. However, during winter the N_2O bursts during freezing and thawing events seemed to follow rapid changes in soil moisture and temperature. It is possible that rapid freezing concentrates e.g. nitrate in smaller volume of unfrozen water having low O_2 content which favours N_2O production in denitrification.

Denitrification has been considered as the main source of N_2O during freezing and thawing (e.g. Mørkved et al., 2006). However, there could be also other sources than biological denitrification for N_2O at low temperatures. The laboratory incubations showed that the production of N_2O from frozen soil continues several weeks and the production rates can even increase after the initial freezing. Nitrite accumulated during the incubation at temperatures below $0^{\circ}C$, especially in the sand soil. Also in the field NO_2^- was detected only from the frozen soils. Nitrite accumulation in frozen soils has been reported earlier (Smith et al., 1997), which could support chemical denitrification re-

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quiring nitrite (Christianson and Cho, 1983). Therefore, it can well be that part of the N₂O produced in frozen soils originates from chemodenitrification. However, Öquist et al. (2007) concluded that any abiotic N₂O production observed at -4°C in the organic surface layer of a boreal forest soil was negligible in comparison to the biogenic sources.

The results of this study support the findings by Groffman et al. (2006) and Maljanen et al. (2007) that lowering of soil temperature below 0°C by reducing the snow cover can increase N_2O emissions in northern soils, both from mineral and organic soils. Therefore the decrease in snow cover, a possible trend with warming climate, may enhance N_2O emissions from soils in the Northern Hemisphere. An important finding was that the proportion of winter time N_2O emissions of annual emissions was equal (about 80%) regardless of soil type. Another important finding was that the soil N_2O concentration did not correlate with the N_2O emissions if the soil is frozen and covered by ice. Thus, N_2O concentration cannot be used to predict N_2O emissions from soils during winter in all conditions. Therefore, more studies on the physics and chemistry of frozen soils are needed to improve the modeling of the N_2O production and emissions in the northern terrestrial ecosystems.

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Table 1. Soil characteristics (sampled from depth 0–10 cm).

Site	OM (%)	N (%)	C (%)	C/N	pH (H ₂ O)	BD (g cm ⁻³)
Sand	5.0	0.21	1.8	9.0	6.6	1.32
Mull	28	1.0	13	12.7	5.8	0.72
Peat	86	3.0	55	18.2	5.0	0.22

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Table 2. Annual N_2O emissions (g N m⁻²) and their standard error of the mean (n=6) from the study sites. During the first winter (2005/2006) snow was removed from the bare plots for the whole winter period and during the second winter (2006/2007) only from October to December.

	Sand		N	/ lull	Peat	
	Snow	Bare	Snow	Bare	Snow	Bare
2005/2006	0.25±0.04	0.37*±0.03	0.66±0.02	1.27**±0.09	2.97±0.7	3.30±1.0
2006/2007	0.35±0.02	0.38 ± 0.03	0.60 ± 0.06	0.70 ± 0.04	0.68 ± 0.1	1.14±0.3

^{*=}p<0.05, **=p<0.01 (significant difference between treatments)

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Table 3. Concentration of NO_3^- , NO_2^- , and NH_4^+ ($\mu g N g_{dw}^{-1}$) in incubation experiment at different temperatures (+12.5, +4.9, -2.0 and -6.8°C) in the beginning of the experiment (Wk 0) and after incubation of 10 weeks (10 Wk).

	0 Wk	10 Wk						
	+4.9	+12.5	+4.9	-2.0	-6.8			
$NO_3^- (\mu g N g_{dw}^{-1})$								
Sand	2.38	18.2	12.5	5.65	4.12			
Mull	17.4	39.1	38.5	22.9	18.6			
Peat	80.9	330	231	109	79.4			
$NO_2^- (\mu g N g_{dw}^{-1})$								
Sand	0.072	0.00	0.00	0.246	0.192			
Mull	0.011	0.00	0.00	0.003	0.443			
Peat	0.004	0.00	0.00	0.000	0.105			
NH_4^+ (μ g N g_{dw}^{-1})								
Sand	2.59	0.30	0.53	4.52	7.00			
Mull	1.03	0.30	1.32	1.66	5.34			
Peat	4.80	4.15	4.42	10.8	14.2			

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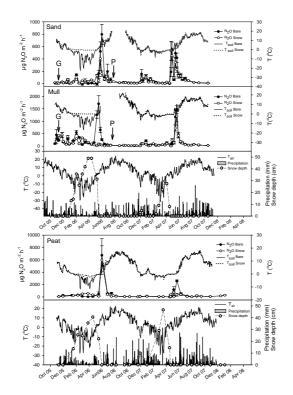
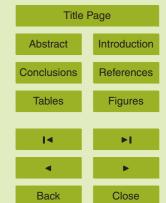


Fig. 1. N_2O dynamics and soil temperature at depth of 5 cm in sand, mull and peat soils (manipulated snow cover with black symbols or solid line and control soil with open symbols and dotted line) from September 2005 to October 2007. Mean daily air temperature, daily precipitation sum and snow depth on gas sampling days in Maaninka (sand and mull) and Kannus (peat) are also shown. Error bars indicate standard error of the mean. Arrow with G indicates the time of glyphosate treatment and arrow with P indicates time of ploughing and fertilization. Peat site was not fertilized or ploughed.

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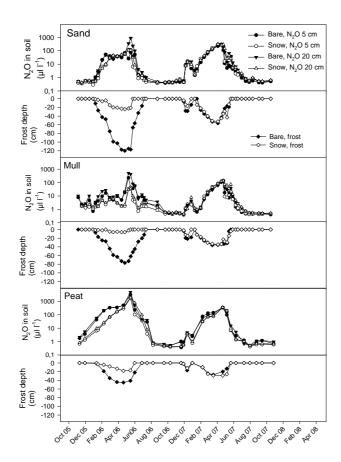


Fig. 2. N_2O concentration in soil at the depth of 5 cm (circles) and 20 cm (triangles) and the depth of soil frost (diamonds) in sand, mull and peat soils. Black symbols refer bare soil without snow and open symbols soil with undisturbed snow cover during the winter. Error bars show the standard error of the mean. Note the log scale in y-axis (N_2O in soil).

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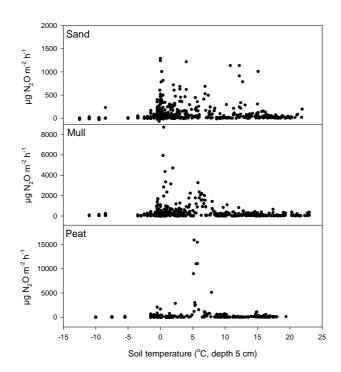


Fig. 3. N_2O emissions from all individual measurements from sand, mull and peat soils plotted against soil temperature at depth of 5 cm.

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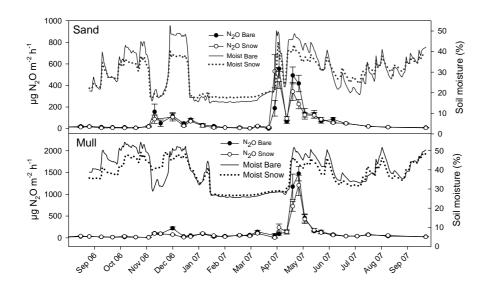


Fig. 4. N₂O fluxes (black symbols bare soil, open circles under undisturbed snow cover) and volumetric soil moisture content (solid line bare and dotted line undisturbed snow cover) from sand and mull soils measured with TDR from autumn 2006 to autumn 2007.

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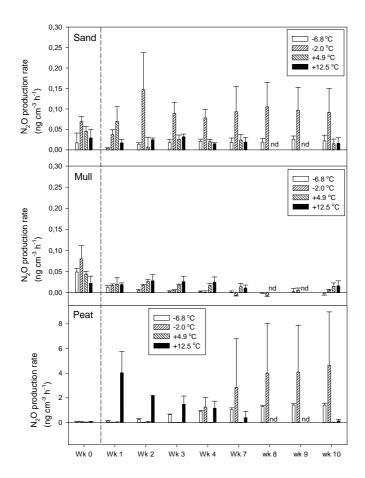


Fig. 5. N_2O production rates in laboratory incubations with sand, mull and peat soil (n=5) at temperatures of -6.8, -2.0, 4.9 and 12.5°C. Wk 0 refers the beginning of the incubations at +4.9°C at soil WFPS 80%. Error bars show the standard deviation of the mean. (nd=not determined).

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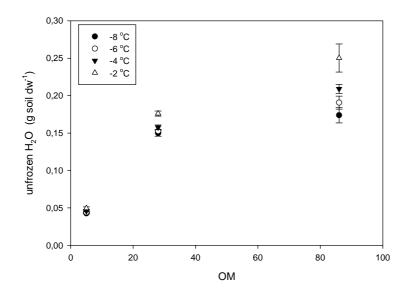


Fig. 6. Amount of unfrozen H_2O (g soil dw^{-1}) plotted against soil organic matter (OM) at different temperatures with standard deviation (n=6).

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