Title

# $N_2O$ emissions during the freezing and thawing periods from six fields in a livestock farm, southern Hokkaido, Japan

Running title

$N_2O$ emissions during the freezing-thawing period

Authors

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Abstract

In many countries, high nitrous oxide ($N_2O$) emissions have been observed during soil freezing and thawing periods. Quantification of those emissions is crucial to evaluate annual $N_2O$ emissions. For this study, we measured $N_2O$ and NO fluxes along with soil $N_2O$ concentrations at a corn field and five grasslands during a winter-spring period in Southern Hokkaido, Japan. We also measured denitrification activities of the soils from those sites. During the observation period, the soils froze to a maximum depth of 370 mm under saturated conditions and the lowest soil temperature at a 50 mm depth was -4.5 °C. After 6 March 2005, daily air temperature rose above 0 °C, but the soil temperature remained approximately 0 °C for about two weeks. These two weeks were defined as the 'transition period,' while the periods before and after the transition period were defined as the 'freezing' and the 'thawing' periods, respectively. During the freezing and transition periods, $N_2O$ concentration increased in the frozen soils relative to the unfrozen soils and the highest values were observed in the frozen soils during the transition period. During the thawing period, the $N_2O$ concentration in the soils decreased. $N_2O$ emissions were much higher during the thawing period than during the freezing and transition periods, and remarkably higher $N_2O$ emissions were observed at the corn site compared to those at the grassland sites. NO emissions were also observed during the thawing period but at much lower levels than $N_2O$ emissions at all the sites. $N_2O$-N/NO-N ratio exceeded one at all the sites during the entire period, indicating $N_2O$ production through denitrification. At the corn site, denitrification activity was much lower and $N_2O/(N_2O+N_2)$ was much higher than at the grasslands. The result indicated that high $N_2O$ emissions at the corn site were caused by complimentary processes: (1) high accumulated $N_2O$ through denitrification in the frozen soil during the freezing and transition periods; and (2) low $N_2O$ reduction rate during the thawing period.

Key words: corn field, denitrification activity, freezing and thawing periods, grassland, nitrous oxide

## INTRODUCTION

Nitrous oxide ($N_2O$) is a major greenhouse gas that is responsible for destruction of stratospheric ozone (Crutzen 1970). Global $N_2O$ emissions are estimated at 17.7 Tg N $y^{-1}$ and of those, an estimated 6.7 Tg N $y^{-1}$ are from anthropogenic sources (Denman et al. 2007). Agricultural soils are the main source of $N_2O$ emissions, at an estimated 42% of total anthropogenic $N_2O$ emissions (Denman et al. 2007).

In soils, $N_2O$ is produced mainly by nitrification and denitrification. These biological processes are influenced by soil environmental factors, such as moisture condition, oxygen level, soil temperature, nitrogen (N) availability, organic matter content, and pH (Mosier 1998; Sahrawat and Keeney 1986). The soil freeze-thaw cycle has also been recognized as having an influence on soil $N_2O$ emissions, since Bremner et al. (1980) observed $N_2O$ emissions during a winter period. Kaiser and Ruser (2000) summarized annual $N_2O$ emission data and winter $N_2O$ losses observed on arable soils in Germany from 1995 to 1999. The annual $N_2O$ losses ranged from 0.53 to 16.8 kg N $ha^{-1}$ and $N_2O$ losses during the winter ranged from 7% to 89%. We also summarized the reported data and the $N_2O$ losses during the winter ranged from 0% to 93% (Table 1). These results indicate a high concentration of $N_2O$ emissions during the winter period and uncertainty of $N_2O$ emissions.

It has been assumed that $N_2O$ would be produced in an unfrozen soil layer beneath a frozen layer and released from the soil surface during a thawing period once the frozen soil cover has disappeared in situ (Bremner et al. 1980, Burton and Beauchamp 1994, and Kaiser et al. 1998). But based on a soil column experiment, Teepe et al. (2001) and Wagner-Riddle et al. (2007) hypothesized that $N_2O$ was produced and trapped in the frozen soil during a freezing period and then released during a thawing period. Recently, Yanai et al. (2011) found an increase in $N_2O$ concentration with a decrease in $O_2$ concentration in frozen soil during the freezing and

thawing periods.

To investigate the $N_2O$ production process during the freezing and thawing conditions, soil incubation experiments have been conducted by many researchers. Yanai et al. (2004b) evaluated the nitrification potential of frozen soils and reported that soil freeze-thaw cycles did not inhibit the nitrification potential of soils that experienced a smaller decrease in microbial biomass following the freeze-thaw cycles. The source of ammonium ($NH_4^+$), which is the base of nitrification, may be soil organic matter (McGarity 1962, Groffman and Tiedje 1989, Neilson et al. 2001) or destructed microbes or substrates from the destructed microbes (McGarity 1962, Groffman and Tiedje 1989, Schimel and Clein 1996, Yanai et al. 2004a). Christensen and Christensen (1991) evaluated soil carbon availability by denitrifiers, using an acetylene block method under freeze-thaw conditions. Müller et al. (2002) and Ludwig et al. (2004) reported that high $N_2O$ emissions were caused by the enrichment of $NO_3^-$ through $^{15}NO_3^-$ addition experiments. Based upon an experiment with a $^{15}N$ tracer, Wagner-Riddle et al. (2008) concluded that denitrification activity during a thawing period was the main process of $N_2O$ production. Mørkved et al. (2006) also conducted a $^{15}NO_3^-$ addition experiment and reported that denitrification was the main $N_2O$ source in freeze-thaw-affected soil and that soluble carbon could play a significant role in the freeze-thaw cycle. Öquist et al. (2004) observed higher $N_2O$ emissions after freezing at -4 $^oC$ in soils with a high moisture content compared to soils with a low moisture content.

Despite significant research on $N_2O$ emissions from soil, there are only a few studies that have measured both $N_2O$ concentration in soil and $N_2O$ flux at a field and evaluated denitrification potentials as well. The objective of this study was to determine the cause of high $N_2O$ emissions from soils during a thawing period. We measured $N_2O$ fluxes and $N_2O$ concentrations in soils at a livestock farm in southern

Hokkaido, Japan. The study was conducted after a cropping period in 2004, because high $N_2O$ emissions were observed from 2000 to 2003 (Table 1). Furthermore, an incubation experiment was conducted just after soil thawing to measure denitrification activities in soils.

## MATERIALS AND METHODS

### Study area

The study was conducted at the 458-ha Shizunai Experimental Livestock Farm of the Field Science Center for the Northern Biosphere, Hokkaido University, Japan (42.4317N,142.4817E). The site is an experimental station, but also a working production facility where young animals are reared to maturity (e.g., for beef production and preserving native species of Hokkaido horses) and crops (primarily corn and grass) are grown to support the animals. The farm is located in the watershed of the Kepau River, which flows through the farm from an upstream forested area. The average annual mean temperature at the study site is 7.9 °C, the minimum monthly temperature is –8.1 °C in February, and the maximum monthly temperature is 23.6 °C in August. The average annual precipitation is 1365 mm.

Measurements were taken at a corn field ($C_2$), a grassland that was recently converted from a corn field in 2003 (CG), and four grasslands ($G_{2s}$, $G_{2c}$, $G_{2n}$ and $G_3$) which have been used as a meadow for more than three years. Three grasslands $G_{2s}$, $G_{2c}$, and $G_{2n}$ were divided from $G_2$ and named $G_{2s}$ for the southern site, $G_{2c}$ for the center site, and $G_{2n}$ for the northern site (see Supporting Information, Figure S1). These three grasslands were adjacent to one another and they were all managed in the same way. The soil types of the research sites (Katayanagi et al. 2008) are Vitric Andosols at CG, $G_{2s}$, $G_{2c}$, and $G_{2n}$ and Histosols at $C_2$ and $G_3$ (FAO 1988) (Table 2). The dominant vegetation type is corn (*Zea mays* L.) at $C_2$, reed canary grass (*Phalaris*

*arundinacea* L.) at $G_{2s}$, $G_{2c}$, $G_{2n}$ and $G_3$, and timothy-grass (*Phleum pratense* L.) at CG. Grass was harvested twice annually and corn was harvested once a year.

After the cropping season in 2004, manure was applied to CG, $G_3$, and $C_2$ on 27 October 2004 and slurry was applied to $C_2$ on 18 October 2004 (Table 3). Nitrogen surpluses at $G_2$, $G_3$, CG, and $C_2$ calculated by Katayanagi et al. (2008) are also provided in Table 3.

**Temperature, precipitation, and snow depth**

Daily precipitation measurements from the farm's Sasayama Weather Station (42.4333N, 142.4817E) (Fig. 1) were used for the analysis of gas data. Daily air temperature was also observed at the station but there were many missing data due to equipment problems. Snow depth was not observed at the station. Therefore, the data measured at the Shizunai Weather Station (42.3433N, 142.3617E) were used instead. For calculation of gas fluxes, air temperature inside a static chamber was measured by a digital thermometer during the gas measurement. Soil temperature at a depth of 50 mm was measured continuously during the study period at hourly intervals using a small waterproof temperature logger (TR-52, T&D, Nagano, Japan) for one replicate per field. Depth of soil freezing was measured using an acrylic tube that was filled with a 0.03% methylene blue solution. A polyvinyl chloride (PVC) pipe was used to protect the acrylic tube. Petroleum jelly was spread thinly on the acrylic tube to prevent it from sticking to the PVC pipe.

**Soil sampling**

Disturbed soil samples for measurements of soil chemical properties were collected from a 0−50 mm depth. The sampling was conducted with three to six replicates per site before and after freezing (08 or 18 December 2004 and 19 April 2005, respectively). Only a single sample was collected on 31 March 2005 when the

soil started to thaw because only surface soil from approximately 0 to 10 mm depth had thawed, and it was difficult to collect a large volume of soil samples at that time. The collected soil samples were preserved at 4 ℃ and analyzed within a week of sampling.

**Analysis of soil chemical properties**

The disturbed soil samples from each site were extracted in deionized water (at a 1:5 ratio of soil to water) in bottles and filtered through a 0.2 μm membrane filter using suction. Nitrate-N ($NO_3^-$-N), $NH_4^+$-N and dissolved organic carbon (DOC) concentrations in the extracted solution were determined using an ion-exchange chromatography (QIC analyzer, Dionex, Sunnyvale, CA, USA), colorimetry with indophenol-blue, and total organic carbon analysis (TOC-5000A, SHIMADZU, Kyoto, Japan), respectively. Soil pH was measured with a glass electrode (F-22, Horiba, Kyoto, Japan) using infiltrated samples remaining in the bottle after the filtration.

**Measurement of denitrification enzyme activity**

Using the disturbed soil samples collected on 31 March 2005, denitrification activity was measured by the acetylene block technique proposed by Tiedje (1994). A total of 15 g of fresh soil sample was thoroughly mixed with a 15 mL chloramphenicol solution (1 g $L^{-1}$) in a 200-mL serum bottle to prevent enzyme production (Tiedje 1994, Lowrance 1992, Murray and Knowles 1999). To determine the denitrification activity under field conditions, available C and N were not added to the soil samples. The serum bottle was evacuated and flushed four times with $N_2$ to ensure anaerobic conditions. For this study, the incubations were conducted using non-acetylene and acetylene treatments to determine the denitrification activities of $N_2O$ production and $N_2$+$N_2O$ production, respectively. $N_2O$ production was estimated using $N_2O$ emissions from the non-acetylene-treated samples and $N_2$+$N_2O$ production was estimated using

$N_2O$ emissions from the acetylene-treated samples because acetylene inhibits $N_2O$ reduction to $N_2$. Acetylene gas was added to a final concentration of 10% (10 kPa) in the headspace for the acetylene treatment. Incubations were conducted in triplicate at 5 °C, which was the soil temperature at the time of collection from the field. At 1 and 2 hours after the incubation start time, 15 mL of headspace gas was sampled from each bottle into an evacuated glass vial using a 25 mL syringe. $N_2O$ concentration of the gas samples, kept in evacuated glass vials, was analyzed by gas chromatography with an electron capture detector (GC 14B, Shimadzu, Kyoto, Japan) at 375 °C. PorapackQ filled in a stainless steel column (2 m length and 3 mm inner diameter) was used as stationary compound and 5% methane in argon was used as the carrier gas. The minimum detectable concentration of $N_2O$ was 0.007 ppmv, as determined from the deviation obtained by repeatedly analyzing the concentration of $N_2O$ standard gas (atmospheric level).

**Measurement of $N_2O$ and NO fluxes**

Measurements of $N_2O$ and NO were conducted once a month in December 2004 and February 2005 and once a week in March and April 2005. Each flux measurement was replicated at four locations per site for $G_3$, CG, and $C_2$ and at six locations per site for $G_{2s}$, $G_{2c}$, and $G_{2n}$.

The measurements were conducted using a closed-chamber technique (Rolston 1986). A cylindrical stainless steel chamber (diameter 300 mm, height 350 mm) was used for measurements. Detailed information about this chamber design is reported in a previous study (Katayanagi et al. 2008). The deepest snow depth on the monitoring days was only 150 mm, and the chambers were placed on the snow during the freezing period. During the soil freezing conditions, gas samples were collected at 0 and either 40 or 60 minutes after closing the lid; during the other periods, samples were collected

at 0 and 20 minutes after closing the lid. The chamber height from the snow surface to the top of the chamber was measured for four replicates to calculate gas flux. A 500-mL gas sample was taken using a 50 mL syringe and injected into a 500 mL Tedlar® bag. The bags were then brought to the laboratory and 20 mL of each gas sample was immediately transferred into a 10 mL evacuated glass vial, using a 25 mL syringe, for $N_2O$ analysis.

$N_2O$ concentrations of the samples kept in evacuated glass vials were analyzed using the gas chromatograph as described earlier. The gas samples kept in Tedlar® bags were analyzed by a chemoluminescence nitrogen oxide analyzer (MODEL-265P, Kimoto Electric, Osaka, Japan) within 24 hours of sampling to measure NO concentrations in the samples.

**Measurement of soil $N_2O$ concentration**

The soil $N_2O$ concentration was measured concurrently with the gas flux measurement. Stainless steel tubes (inner diameter 10 mm and outer diameter 12 mm; the lengths of the tubes were the target depth plus 100 mm; there was no side hole on the pipe) were installed in the soil at 100, 200, 300, 400, 500, and 600 mm depths with three replications. A stainless steel stick (20 mm longer than the pipe) was inserted in the pipe during installation, in order to avoid clogging the pipe with soil and to provide air space (ca. 2 mL) at the bottom of the pipe. The stick was removed after installation. A three-way cock was connected to the aboveground part of an installed pipe using a vinyl tube (100 mm), a straight connector and a silicone tube (50 mm); 50 mL air was drained from inside the pipe through the cock just after the installation. The pipe was left in the place throughout the freezing and thawing periods, and the cock was closed and covered using a PET cap to avoid freezing the cock from snow and ice exposure. A 50 mL soil gas sample was collected from each pipe. The gas samples were collected slowly to prevent contamination by gas from non-target depths. However, the volumes

of the pipes were small and the gas under the target depth would be collected together with the gas at the target depth. The samples that were collected from the same depth were placed into a Tedlar® bag together. A 300 mL air sample from the soil surface was collected near the pipes at the same time. $N_2O$ was analyzed using the gas chromatography, as described earlier. Because of those operations, we had only one replicate sample per depth at each site; therefore, standard variations could not be reported in this paper.

**Calculation of $N_2O$ and NO fluxes**

$N_2O$ and NO fluxes were calculated by a linear regression using the following equation:

$$F = \rho \times (V/A) \times (\Delta c/\Delta t) \times [273/(273 + T)] \times P/760$$

where $F$ is the flux (mg N $m^{-2}$ $h^{-1}$), $\rho$ is the gas density ($\rho N_2O\text{-}N = 1.26 \times 10^6$ and $\rho NO\text{-}N = 0.63 \times 10^6$ mg N $m^{-3}$), $V$ is the volume of the chamber ($m^3$), $A$ is the area of the chamber ($m^2$), $\Delta c/\Delta t$ is the ratio of change in the gas concentration inside the chamber ($10^{-6}$ $m^3$ $m^{-3}$ $h^{-1}$), $T$ is the air temperature inside the chamber (°C) and $P$ is air pressure (mm Hg). For this study, we defined positive fluxes as emissions and negative fluxes as uptake by soil or snow and calculated $N_2O$-N/NO-N when both $N_2O$ and NO fluxes were positive during the observation period.

**Statistical analysis**

Statistical analyses were performed with Kyplot 5.0 (KyensLab Inc. Tama, Tokyo, Japan). The $NH_4^+$-N, $NO_3^-$-N, and DOC concentrations, measured before freezing and at the end of thawing, were compared using paired t-tests. Differences between $N_2O$ and $N_2$+$N_2O$ fluxes, which were the results of denitrification enzyme activity

measurement, were also compared using paired t-tests and differences of $N_2O$ and $N_2$+$N_2O$ fluxes among each site were compared by Tukey-Kramer test. Correlation analyses between the $NH_4^+$-N, $NO_3^-$-N, and DOC concentrations and the manure plus slurry N application and surplus N were performed using the same software.

## RESULTS

### Temporal variability of air temperature, soil temperature, precipitation, snow depth and frozen soil layers

The daily air temperature decreased from December to February and increased from March to April (Fig. 1). Daily mean soil temperature at 50 mm depth dropped below zero from 20 December 2004 and remained below zero until around 06 March 2005 (Fig. 2). The lowest recorded daily soil temperatures were -4.5, -1.8, -2.3, -4.7, -3.2, and -3.3 °C at $C_2$, CG, $G_{2s}$, $G_{2c}$, $G_{2n}$, and $G_3$, respectively. After 06 March 2005, the soil temperature did not increase and remained approximately 0 °C for about two weeks, even though air temperature rose above 0 °C (Fig. 2a). We defined these two weeks as the 'transition' period while the periods before and after were defined as the 'freezing' and 'thawing' periods, respectively. The freezing period started from 20 December under the definition and the period prior to 20 December 2004 was defined as the 'unfreezing' period.

The maximum snow depth during the whole period was 250 mm (Fig. 1) and soil freezing was observed from 24 January 2005. The depth of the frozen soil layer was getting deeper after soil freezing started (Fig. 2e); the maximum depths of the frozen layers were observed in March and were 370, 230, 220, 220, 340, and 350 mm at $C_2$, CG, $G_{2s}$, $G_{2c}$, $G_{2n}$, and $G_3$, respectively. The frozen soils were very hard during the freezing period due to the saturated soil conditions caused by high soil water content before freezing (water-filled pore space was 72, 92, 79, 81, 82 and 79% at $C_2$,

CG, $G_{2s}$, $G_{2c}$, $G_{2n}$, and $G_3$, respectively) and also by water supply from rainfall and melted snow when air temperature increased above 0 °C. Soil thawing was observed from 24 March 2005 at both the soil surface and the bottom of the frozen layers (Fig. 2e). Frozen soil layers were not observed after 19 April 2005 at all sites.

**$N_2O$ and NO fluxes**

$N_2O$ emissions during the thawing period were much higher than the emissions during other periods (Fig. 2b). During the thawing period, fluxes at all sites showed three patterns: i) remarkably high $N_2O$ emissions were observed at $C_2$ and CG; ii) $N_2O$ emissions increased through the transition and thawing periods at $G_{2s}$, $G_{2c}$, and $G_3$, and the fluxes at those sites were much lower than fluxes at $C_2$ and CG; and iii) $N_2O$ peaks were observed during the transition and thawing periods and high $N_2O$ uptake (negative $N_2O$ flux) was also observed at $G_{2n}$. $N_2O$ emissions were also observed during the unfreezing and freezing periods at all sites and were higher at $C_2$ than at the other sites (Fig. 2b), but the emissions were lower during the freezing period than during the thawing period.

During the study period, NO emissions at all sites showed a similar temporal variation: low NO emissions were observed at the beginning of the transition period and higher NO emissions were observed during the latter part of the transition and thawing periods (Fig. 2c). NO emissions were also observed at all sites during the unfreezing and freezing periods as well as during the thawing period (Fig. 2c). However, while NO emissions were observed during the whole observation period, the emission level was much lower than $N_2O$ (Fig. 2b, c).

$N_2O$-N/NO-N during the unfreezing and freezing periods was lower than the values during the thawing period (Fig. 2d). The values were distributed from 2.0 to 13.6 except at $C_2$ during the unfreezing period. The highest value at $C_2$ during the

unfreezing was 69.8. During the thawing period, higher $N_2O$-N/NO-N was observed at $C_2$ than at the other sites and the highest value at $C_2$ was 151. The values at the other sites were distributed from 8.5 to 63.3 during the thawing period. The temporal variation of $N_2O$-N/NO-N was similar to that of $N_2O$ at $C_2$, CG, $G_{2s}$, and $G_{2c}$ (Fig. 2b, d). At $G_{2n}$ and $G_3$, there was no apparent pattern due to a few samples and negative $N_2O$ emissions.

**Soil $N_2O$ concentrations**

During the freezing and transition periods, the $N_2O$ concentrations increased in the frozen soils and were higher during those periods than concentrations during the unfreezing and thawing periods at all sites except $G_{2c,}$ where gas samples in the frozen soil could not be collected (Fig. 2e). The highest $N_2O$ concentrations were observed in the frozen soils during the transition period at all the sites except $G_{2c}$. $N_2O$ concentrations also increased in the unfrozen soil layers below the frozen layers at $C_2$, CG, $G_{2s}$ and $G_{2n}$, but, the concentrations in the unfrozen soils were lower than those in the frozen soils (Fig. 2e). At $G_{2c}$ and $G_3$, the $N_2O$ concentration in the unfrozen soil layers just below the frozen soil layers and at the edge of the frozen soil layers was lower than that of the atmosphere (i.e., approximately 0.3 ppmv).

After the start of soil thawing, $N_2O$ concentrations in the thawed and unfrozen layers decreased at $C_2$, CG, $G_{2s}$ and $G_{2n}$. At $G_{2c}$ and $G_3$, the $N_2O$ concentrations increased to and above the level in the atmosphere (Fig. 2e).

***Soil $NH_4^+$-N, $NO_3^-$-N and DOC concentrations during soil freezing***

Figure 3 shows the soil $NH_4^+$-N, $NO_3^-$-N and DOC concentrations during the unfrozen period measured on 08 or 18 December 2004, at the beginning of the thawing period measured on 31 March 2005, and at the end of thawing period measured on 19 April 2005.

Soil $NH_4^+$-N values were remarkably high at the beginning of the thawing period compared with the values during the unfreezing period and at the end of the thawing period for all sites (Fig. 3); however, the data couldn't be statistically compared with the other data. The value at the end of the thawing period ($p$ = 0.004) was significantly higher than before freezing at $C_2$, while there was not a significant difference in the values at the other sites (Fig. 3). The $NH_4^+$-N concentration did not show any correlation with slurry + manure N and N surplus, as shown in table 3.

Soil $NO_3^-$-N values at the beginning of the thawing period were higher than those before the freezing period at $C_2$, CG, and $G_{2n}$; the values at $G_{2s}$, $G_{2c}$ and $G_3$ did not show appreciable differences (Fig. 3) but the data couldn't be statistically compared with the other data. The highest $NO_3^-$-N concentrations were observed at the end of the thawing period for all sites, and the values at $C_2$, CG, $G_{2n}$ and $G_3$ were significantly higher than those before the freezing period (Fig. 3; the $p$ values were 0.0005, 0.0384, 0.0499, and 0.0034, respectively). The $NO_3^-$-N concentration showed a positive correlation with the application rates of slurry + manure N (Table 3) for all sampling dates ($r$ = 0.9031 and $p$ = 0.0136 on 08 and 18 December 2004, $r$ = 0.9272 and $p$ = 0.0078 on 31 March 2005, and $r$ = 0.9035 and $p$ = 0.0135 on 19 April 2005, respectively).

The soil DOC concentrations measured at the beginning of the thawing period were higher than those measured during the unfreezing period and at the end of the thawing period for all sites (Fig. 3). There was no significant difference between the values for the freezing period and those for the end of the thawing period. The DOC concentrations did not show any correlation with slurry + manure N and N surplus.

**Denitrification activities**

$N_2O$ production was significantly lower at $C_2$ and CG and higher at $G_3$ ($p < 0.05$); $N_2O+N_2$ production was significantly lower at $C_2$ and CG and higher at $G_{2c}$ and

$G_3$ compared with the other sites (Table 4). $N_2O + N_2$ production was significantly higher than $N_2O$ production at $G_{2c}$ and $G_3$ and lower than that at CG ($p < 0.05$) (Tables 4). $N_2O + N_2$ production was also higher than $N_2O$ production at $G_{2s}$ and $G_{2n}$, but it was not significant because of the high standard deviation (Table 4). The ratio of $N_2O/(N_2O+N_2)$ was lower at $G_{2c}$ and $G_3$ than at the other sites, but it was not significant (Table 4). A regression analysis of $N_2O$ and $N_2O + N_2$ productions and the amount of manure + slurry N and surplus N was also conducted, but the results did not show any relationship.

## DISCUSSION

### $N_2O$ production and accumulation in frozen soil during the freezing and transition periods

During the freezing period, production and accumulation of $N_2O$ in the frozen soils resulted from inhibition of gas diffusion by the frozen soil lids and from $N_2O$ reduction to $N_2$. Higher $N_2O$ concentrations in the soils, relative to the atmosphere (Fig. 2), indicated $N_2O$ accumulation in the soils and disturbance of gas exchange by the frozen saturated soil, which functioned as a “lid” for all the sites. In the soil layers, the highest $N_2O$ concentration was observed in frozen rather than unfrozen soils (Fig. 2); this indicated that higher $N_2O$ production and/or lower $N_2O$ reduction occurred in the frozen soils compared to unfrozen soils and that the produced or remaining $N_2O$ in the frozen soils accumulated there. The results show that $N_2O$ production occurs in the frozen soil *in situ* as well as in a laboratory (Christensen and Christensen 1991, Müller et al. 2002, Yanai et al. 2004a and b, Öquist et al. 2004, Öquist et al. 2007), and support the hypothesis of Teepe et al. (2001) and Wagner-Riddle et al. (2007) that $N_2O$ is produced and trapped in frozen soils during a freezing period.

Denitrification was identified as an important $N_2O$ production process in the

frozen soils, as demonstrated by the higher-than-one ratio of $N_2O$-N/NO-N during the freezing period (Fig. 2). Lipschultz et al. (1981) reported that the $N_2O$-N/NO-N ratio is considered an indicator of the contribution of nitrification and denitrification; when $N_2O$-N/NO-N is less than one, the main process of $N_2O$ production is considered to be from the nitrification process. Alternatively, when $N_2O$-N/NO-N is larger than 100, the main process of $N_2O$ production is considered to be the denitrification process. The lid mentioned before must disturb $O_2$ diffusion from the atmosphere into the soils and $O_2$ concentration in the soils must be low during the freezing period. Under such conditions, nitrification must be restrained by deficiency of $O_2$ as an electron acceptor and $N_2O$ production by denitrification would be accelerated. Yanai et al. (2011) observed an increase in $N_2O$ concentration corresponding with a decrease in $O_2$ concentration in frozen soil during freezing and thawing periods and Wrage et al. (2001) reported that low $O_2$ concentration inhibited $N_2O$ reduction to $N_2$.

Nitrate, which is a substrate of the denitrification, was supplied from slurry and manure, because application rates of slurry + manure N (Table 3) showed a positive correlation with $NO_3^-$-N on all the sampling dates. $NH_4^+$-N concentrations were higher in the surface soils just after thawing than before freezing (Fig. 3); this indicates that $NH_4^+$, which is a substrate of nitrification, was supplied through mineralization during the freezing period and high $NO_3^-$-N was caused by the nitrification activity. The source of the $NH_4^+$ was not determined in this study. However, it would likely be the applied slurry and manure, soil organic matter (McGarity 1962, Groffman and Tiedje 1989, Neilson et al. 2001), destructed microbes or substrates from destructed microbes (McGarity 1962, Groffman and Tiedje 1989, Schimel and Clein 1996, Yanai et al. 2004a). Yanai et al. (2004b) evaluated the nitrification potential of frozen soils and reported that a decrease in microbial biomass after freeze-thaw cycles was less substantial and that the nitrification potential was not inhibited by the soil freeze-thaw

cycles. Neilson et al. (2001) reported that soil freezing did not disturb nitrification. The nitrification process would also produce $N_2O$, but Mørkved et al. (2006) observed a low contribution of nitrification: $N_2O$ produced through nitrification was -0.7% to 4.35% of $N_2O$ emissions during the thawing period.

**$N_2O$ production and emission during the thawing period**

High $N_2O$ emissions during the thawing period were caused by diffusion of accumulated $N_2O$ after disappearance of the frozen soil lids and a low $N_2O$ reduction rate during the thawing period. At $C_2$ and CG, higher $N_2O$ concentrations in the surface soils were observed during the freezing and transition periods, and high $N_2O$ emissions were observed during the thawing period after disappearance of the frozen soil lid (Fig. 2). However, the denitrification activity was much lower and $N_2O/(N_2O+N_2)$ was much higher at those sites compared with other sites (Table 4). From these results, it is evident that the accumulated $N_2O$ was not reduced to $N_2$ by denitrification activity and high $N_2O$ emissions were caused by the high $N_2O$ concentration in soils and the high gas diffusion coefficients (Table 2) at the sites. Compared with the $N_2O$ emissions at $C_2$ and CG, $N_2O$ emissions at $G_{2s}$ and $G_3$ were much lower, even though $N_2O$ concentrations in the surface soils were high (Fig. 2). The low emissions must be attributed to high denitrification activities, which reduced $N_2O$ to $N_2$ and resulted in low $N_2O/(N_2O+N_2)$ (Table 4). The low gas diffusion coefficient at the sites (Table 2) would also accelerate $N_2O$ reduction to $N_2$ before the emission. The $N_2O$ concentration in the frozen soil at $G_{2c}$ was not clear, but the low $N_2O$ emissions during the thawing period would be attributed to the same reason as $G_{2s}$ and $G_3$; the $N_2O$ profile (Fig. 2), the low gas diffusion coefficient (Table 2), the high denitrification activity and the low $N_2O/(N_2O+N_2)$ (Table 4) at $G_2$ were similar to the values at $G_3$. At $G_{2n}$, low $N_2O$ emissions as well as $N_2O$ uptake were observed during the thawing period (Fig. 2b). This would be caused by a relatively low $N_2O$ concentration in the frozen soil (Fig. 2)

and high denitrification activity (Table 4). In a soil column experiment, Teepe et al. (2001) and Wagner-Riddle et al. (2007) showed that $N_2O$ trapped in frozen soil was released during a thawing period, which is consistent with our findings.

$N_2O$ produced through nitrification and denitrification during the thawing period would also contribute to the $N_2O$ emissions during the period, but the contribution must be low at the sites. The $N_2O$-N/NO-N values above one resulted from an increase in $N_2O$ emissions during the thawing period (Fig. 2) indicated a low contribution of nitrification. The contribution of denitrification to $N_2O$ emissions was considered from the results, but high denitrification activity decreased $N_2O$ to $N_2$ in the sites. Therefore, the contribution of $N_2O$ production through denitrification during the thawing period would be low. Nitrification and the diffusion of accumulated $N_2O$ was the main source of $N_2O$ emissions during the period.

## CONCLUSION

$N_2O$ produced through denitrification activity during the freezing and transition periods accumulated in the frozen soils; this resulted from inhibition of $N_2O$ diffusion by frozen soil lids and from $N_2O$ reduction to $N_2$. $N_2O$ emissions during the thawing period were caused by diffusion of the accumulated $N_2O$ in the surface soil. During the thawing period, the contribution of $N_2O$ produced by nitrification and denitrification to the emissions was low at the study sites.

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## REFERENCES

Bremner JM, Robbins SG, Blackmer AM 1980: Seasonal variability in emission of nitrous oxide from soil. *Geophys. Res. Lett.* **7**, 641-644.

Burton DL, Beauchamp EG 1994: Profile nitrous oxide and carbon dioxide concentrations in a soil subject to freezing. *Soil Sci. Soc. Amer. J.* **58**, 115-122.

Christensen S, Christensen BT 1991: Organic matter available for denitrification in different soil fractions: effect of freeze/thaw cycles and straw disposal. *J. Soil Sci.* **42**, 637-647.

Crutzen PJ 1970: The influence of nitrogen oxides on the atmospheric ozone content. *Quart. J. Roy. Meteorol. Soc.* **96**, 320–325.

Denman, KL, Brasseur, G, Chidthaisong, A, Ciais, P, Cox, PM, Dickinson, RE, Hauglustaine, D, Heinze, C, Holland, E, Jacob, D, Lohmann, U, Ramachandran, S , da Silva Dias, PL, Wofsy SC, Zhang X. 2007: Couplings Between Changes in the Climate System and Biogeochemistry. In: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Ed. Solomon, SD, Qin, M, Manning, Z, Chen, M, Marquis, KB, Averyt, MT, Miller, HL. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Food and Agriculture Organization of the United Nations (FAO) 1988: Revised Legend of the FAO-UNESCO Soil Map of the World. 119

Flessa H, Dörsch P, Beese F 1995: Seasonal variation of $N_2O$ and $CH_4$ fluxes in differently managed arable soils in southern Germany. *J. Geophys. Res.* **100**, 23115-23124.

Goossens A, Visscher AD, Boeckx P, Cleemput OV 2001: Two-year field study on the emission of $N_2O$ from coarse and middle-textured Belgian soils with different

land use. *Nutr. Cycl. Agroecosyst.* **60**, 23-34.

Groffman PM, Tiedje JM 1989: Denitrification in north temperate forest soils: Spatial and temporal patterns at the landscape and seasonal scales. *Soil Biol. Biochem.* **21**, 613-620.

Kaiser E-A, Kohrs K, Kucke M, Schnug, Munch JC, Heinemeyer O 1998: Nitrous oxide release from arable soil: importance of perennial forage crops. *Biol. Fert. Soils* **28**, 36-43.

Kaiser E-A, Ruser R 2000: Nitrous oxide emissions from arable soils in Germany - An evaluation of six long-term field experiments. *J. Plant Nutr. Soil Sci.* **163**, 249-260.

Katayanagi N, Sawamoto T, Hayakawa A, Hatano R 2008: Nitrous oxide and nitric oxide fluxes from cornfield, grassland, pasture and forest in a watershed in Southern Hokkaido, Japan. *Soil Sci. Plant Nutr.* **54**, 662-680.

Koga N, Tsuruta H, Sawamoto T, Nishimura S, Yagi K 2004: $N_2O$ emission and $CH_4$ uptake in arable fields managed under conventional and reduced tillage cropping systems in northern Japan. *Global Biogeochem. Cycles*, 18, GB4025.

Lipschultz F, Zafirou OC, Wofsy SC, McElroy MB, Valois FW, Watson SW 1981: Production of NO and $N_2O$ by soil nitrifying bacteria. *Nature* **294**, 641-643.

Lowrance R 1992: Groundwater nitrate and denitrification in a coastal plain riparian forest. *J. Environ. Qual.* **21**, 401-405.

Ludwig B, Wolf I, Teepe R 2004: Contribution of nitrification and denitrification to the emission of $N_2O$ in a freeze-thaw event in an agricultural soil. *J. Plant Nutr. Soil Sc.* **167**, 678-684.

McGarity JW 1962: Effect of freezing of soil on denitrification. *Nature* **196**, 1342-1343.

Mørkved PT, Dörsch P, Henriksen TM, Bakken LR 2006: $N_2O$ emissions and product

ratios of nitrification and denitrification as affected by freezing and thawing. *Soil Biol. Biochem.* **38**, 3411-3420.

Mosier AR 1998: Soil processes and global change. *Biol. Fertil. Soils*, **27**, 221–229.

Müller C, Martin M, Stevens RJ, Laughlin RJ, Kammann C, Ottow JCG, Jäger H-J 2002: Processes leading to $N_2O$ emissions in grassland soil during freezing and thawing. *Soil Biol. Biochem.* **34**, 1325-1331.

Murray RE, Knowles R 1999: Chloramphenicol inhibition of denitrifying enzyme activity in two agricultural soils. *Appl. Environ. Microbiol.* **65**, 3487-3492.

Neilson CB, Groffman PM, Hamburg SP, Driscoll CT, Fahey TJ, Hardy JP 2001: Freezing effects on carbon and nitrogen cycling in northern hardwood forest soils. *Soil Sci. Soc. Amer. J.* **65**, 1723-1730.

Öquist MG, Nilsson M, Sörensson F, Kasimir-Klemedtsson A, Persson T, Weslien P, Klemedtsson L 2004: Nitrous oxide production in a forest soil at low temperatures - processes and environmental controls. *FEMS Microbiol. Ecol.* **49**, 371-378.

Öquist MG, Petrone K, Nilsson M, Klemedtsson L 2007: Nitrification controls $N_2O$ production rates in a frozen boreal forest soil. *Soil Biol. Biochem.* **39**, 1809-1811.

Osozawa S 1998: A simple method for determining the gas diffusion coefficient in soils and its application to soil diagnosis and analysis of gas movement in soil (in Japanese with English summary). *Bulletin NIAES* **15**, 1-66.

Regina K, Syvasalo E, Hannukkala A, Esala M 2004: Fluxes of $N_2O$ from farmed peat soils in Finland. *Eur. J. Soil Sci.* **55**, 591-599.

Rolston DE 1986: Gas flux. In Methods of Soil Analysis Part 1: Physical and Mineralogical Methods, 2nd edn. Ed. A Klute, pp. 1103–1119. American Society of Agronomy, Madison.

Röver M, Heinemeyer O, Kaiser E-A 1998: Microbial induced nitrous oxide emissions

from an arable soil during winter. *Soil Biol. Biochem.* **30**, 1859-1865.

Ruser R, Flessa H, Schilling R, Beese F, Munch JC 2001: Effect of crop-specific field management and N fertilization on $N_2O$ emissions from a fine-loamy soil. *Nutr. Cycl. Agroecosyst.* **59**, 177-191.

Sahrawat KL, Keeney DR 1986: Nitrous oxide emission from soils. *Adv. Soil Sci.*, **4**, 103–148.

Schimel JP, Clein JS 1996: Microbial response to freeze-thaw cycles in tundra and taiga soils. *Soil Biol. Biochem.* **28**, 1061-1066.

Sehy U, Ruser R, Munch JC 2003: Nitrous oxide fluxes from maize fields: relationship to yield, site-specific fertilization, and soil conditions. *Agr Ecosyst. Environ.* **99**, 97-111.

Syvasalo E, Regina K, Pihlatie M, Esala M 2004: Emissions of nitrous oxide from boreal agricultural clay and loamy sand soils. *Nutr. Cycl. Agroecosyst.* **69**, 155-165.

Teepe R, Brumme R, Beese F 2000: Nitrous oxide emissions from frozen soils under agricultural, fallow and forest land. *Soil Biol. Biochem.* **32**, 1807-1810.

Teepe R, Brumme R, Beese F 2001: Nitrous oxide emissions from soil during freezing and thawing periods. *Soil Biol. Biochem.* **33**, 1269-1275.

Tiedje JM 1994: Denitrifiers. *In* SSSA Book Series 5, Methods of Soil Analysis. Part 2-Microbiloogical and Biochemical Properties, Eds. Weaver RW, Angle S, Bottomley P, *Appl. Environ. Microbiol.* pp. 245–257. SSSA, Madison, WI.

Wagner-Riddle C, Thurtell GW, Kidd GK, Beauchamp EG, Sweetman R 1997: Estimates of nitrous oxide emissions from agricultural fields over 28 months. *Can. J. Soil Sci.* **77**, 135-144.

Wagner-Riddle C, Furon A, Mclaughlin NL, Lee I, Barbeau J, Jayasundara S, Parkin G, Von Bertoldi P, Warl J 2007: Intensive measurement of nitrous oxide emissions

from a corn-soybean-wheat rotation under two contrasting management systems over 5 years. *Glob. Change Biol.* **13**, 1722-1736.

Wagner-Riddle C, Hu QC, van Bochove E, Jayasundara S 2008: Linking nitrous oxide flux during spring thaw to nitrate denitrification in the soil profile. *Soil Sci. Soc. Amer. J*. **72**, 908-916.

Wrage N, Velthof GL, van Beusichem ML, Oenema O 2001: Role of nitrifier denitrification in the production of nitrous oxide. *Soil Biol. Biochem*. 33, 1723-1732

Yanai Y, Toyota K, Okazaki M 2004a: Effects of successive soil freeze-thaw cycles on soil microbial biomass and organic matter decomposition potential of soils. *Soil Sci. Plant Nutr.*, **50**, 821-829.

Yanai Y, Toyota K, Okazaki M 2004b: Effects of successive soil freeze-thaw cycles on nitrification potential of soils. *Soil Sci. Plant Nutr.* **50**, 831-837.

Yanai Y, Hirota T, Iwata Y, Nemoto M, Nagata O, Koga N 2011: Accumulation of nitrous oxide and depletion of oxygen in seasonally frozen soils in northern Japan - Snow cover manipulation experiments. *Soil Biol. Biochem.* **43**, 1779-1786

Figure legends

Figure 1. Temporal variability of precipitation observed at the Sasayama Weather Station in the Shizunai Experimental Livestock Farm and air temperature and snow depth observed at the Shizunai Experimental Station from December 2004 to April 2005.

Figure 2. Temporal variability of a) soil temperature at a 50 mm depth, b) $N_2O$ flux, c) NO flux, d) $N_2O$-N/NO-N ratio, and e) $N_2O$ concentration in soil with soil freezing depths at $C_2$, CG, $G_{2s}$, $G_{2c}$, $G_{2n}$, and $G_3$ from December 2004 to April 2005. The lines in e) indicate the edge of the frozen soil layers and the soil between the lines froze.

Figure 3. $NH_4^+$-N, $NO_3^-$-N, and dissolved organic carbon (DOC) concentrations in soil at a 0−50 mm depth at the research sites in the Shizunai Experimental Livestock Farm. Measurements were carried out on 8th ($C_2$, CG and $G_3$) or 18th ($G_{2s}$, $G_{2c}$, and $G_{2n}$) December 2004 before soil freezing, on 31st March 2005 just after the start of soil thawing, and on 19th April 2005 at the end of the thawing period. Paired t-test was used to test for statistically significant differences between the data observed on 8th Dec 2004 and 19th April 2005 for each site. Levels of significance was as follows: * <0.05, ** <0.01, *** <0.001.

Table 1 $N_2O$ emissions during the winter and cropping periods and $N_2O$ losses during the winter period for the investigated experiments

| Reference | Country | $N_2O$ emissions during winter period (kg N $ha^{-1}$) | Annual $N_2O$ emissions (kg N $ha^{-1}$ $y^{-1}$) | Emission during winter (%) |
|---|---|---|---|---|
| Grassland | | | | |
| Wagner-Riddle et al. 1997 | Canada | -0.1 – 0.09 | -0.07 – 0.12 | -75 – 143 |
| Kaiser et al. 1998 | Germany | 0.57 – 0.92 | 1.29 – 2.37 | 39 – 53 |
| Goossens et al. 2001 | Belgium | 0.59 – 2.36 | 2.55 – 31.73 | 7 – 23 |
| Regina et al. 2004 | Finland | 0.06 – 6.7 | 2.6 – 9.9 | 1 – 68 |
| Syvasalo et al. 2004 | Finland | 0.54 – 2.24 | 1.5 – 3.9 | 33 – 59 |
| Katayanagi et al. 2008† | Japan | 0.35 – 2.39 | 7.30 – 82.1 | 2 – 33 |
| Cropland | | | | |
| Goossens et al. 2001 | Belgium | 0.17 – 0.59 | 0.78 – 1.54 | 11 – 76 |
| Wagner-Riddle et al. 2007 | Canada | 0.42 – 2.91 | 0.89 – 3.32 | 24 – 88 |
| Regina et al. 2004 | Finland | 3.30 – 18.9 | 6.20 – 24.1 | 53 – 79 |
| Syvasalo et al. 2004 | Finland | 1.55 – 6.98 | 3.70 – 7.5 | 42 – 93 |
| Flessa et al. 1995 | Germany | 1.09 – 4.63 | 9.36 – 16.78 | 11 – 46 |
| Röver et al. 1998 | Germany | 1.43 – 2.34 | 1.84 – 3.5 | 67 – 78 |
| Kaiser et al. 1998 | Germany | 1.66 – 2.01 | 3.33 – 6.16 | 30 – 61 |
| Teepe et al. 2000 | Germany | 2.80 | 4.80 | 58 |
| Ruser et al. 2001 | Germany | 0.73 – 3.32 | 1.34 – 6.93 | 40 – 58 |
| Sehy et al. 2003 | Germany | 0.30 – 1.7 | 3.10 – 10.1 | 10 – 20 |
| Koga et al. 2004 | Japan | 0.00 – 2.1 | 0.09 – 2.36 | 0 – 89 |
| Bremner et al. 1980 | US | 0.03 – 0.44 | 0.34 – 1.97 | 8 – 26 |
| Katayanagi et al. 2008† | Japan | 0.01 – 0.77 | 1.12 – 11.7 | 1 –24 |

† The winter period emission and the emission rate during the winter are the unpublished data.

Table 2 Soil properties at a 0-100 mm depth at the study site in Shizunai Experimental Livestock Farm.

| Site | Land-use type[†] | Total C[‡] (g kg$^{-1}$) | Total N[‡] (g kg$^{-1}$) | Bulk density[‡] (Mg m$^{-3}$) | D/D$_0$[§] |
|---|---|---|---|---|---|
| $C_2$ | C | 60.8 | 4.5 | 0.60 | 0.0635 |
| CG | G | 41.8 | 3.5 | 0.78 | 0.00684 |
| $G_{2s}$ | G | 32.2 | 3.0 | 0.81 | 0.00113 |
| $G_{2c}$ | G | 42.4 | 3.4 | 0.68 | 0.00429 |
| $G_{2n}$ | G | 49.7 | 4.3 | 0.63 | 0.00132 |
| $G_3$ | G | 75.2 | 5.4 | 0.65 | 0.00143 |

[†]Cornfield: C; Grasland: G

[‡]The values were reported by Katayanagi et al. (2008)

[§]Relative gas diffusion coefficients (D/D$_0$) of soil core samples were measured using the method proposed by Osozawa (1998). The data was measured using soils collected on 19 April 2005.

Table 3 Slurry and manure application after the cropping season and nitrogen surplus in 2004 at the study site in Shizunai Experimental Livestock Farm. Slurry was applied on 19 October 2004 and manure was applied on 1st November 2004.

| Site | Slurry (kg N $ha^{-1}$) | Manure (kg N $ha^{-1}$) | Slurry + Manure (kg N $ha^{-1}$) | Nitrogen Surplus† (kg N $ha^{-1}$) |
|---|---|---|---|---|
| $C_2$ | 9 | 123 | 132 | 16 |
| CG | 0 | 38 | 38 | 99 |
| $G_{2s}$ | 0 | 0 | 0 | 24 |
| $G_{2c}$ | 0 | 0 | 0 | 24 |
| $G_{2n}$ | 0 | 0 | 0 | 24 |
| $G_3$ | 0 | 43 | 43 | -12 |

†Katayanagi et al. (2008)

Table 4 $N_2O$ and $N_2O+N_2$ fluxes and $N_2O/(N_2O+N_2)$ ratio measured by denitrification enzyme activity in soils collected from the study site at Shizunai Experimental Livestock Farm just after the soil thawing on 31 March 2005.

| Site | $N_2O$ flux† ($\mu g\ N\ kg^{-1}\ h^{-1}$) | | $N_2O+N_2$ flux† ($\mu g\ N\ kg^{-1}\ h^{-1}$) | | $N_2O/(N_2O+N_2)$ † | *P* values‡ $N_2O \times N_2O+N$‡ |
|---|---|---|---|---|---|---|
| | *n* | Mean ± S.D. | *n* | Mean ± S.D. | | |
| $C_2$ | 3 | $0.478 \pm 0.122^{a}$ | 3 | $0.519 \pm 0.107^{a}$ | $0.98 \pm 0.41^{a}$ | $0.688^{ns}$ |
| CG | 3 | $0.466 \pm 0.106^{a}$ | 3 | $0.229 \pm 0.0485^{a}$ | $2.0 \pm 0.48^{a}$ | 0.0244* |
| $G_{2s}$ | 3 | $1.60 \pm 0.899^{a,b}$ | 3 | $14.0 \pm 11.4^{a,b}$ | $0.39 \pm 0.58^{a}$ | $0.132^{ns}$ |
| $G_{2c}$ | 2 | $3.84 \pm 1.53^{a,b}$ | 2 | $73.6 \pm 11.9^{c}$ | $0.051 \pm 0.013^{a}$ | 0.0144* |
| $G_{2n}$ | 3 | $5.45 \pm 4.31^{a,b}$ | 3 | $19.3 \pm 32.3^{a,b}$ | $7.6 \pm 8.0^{a}$ | $0.503^{ns}$ |
| $G_3$ | 3 | $6.63 \pm 0.720^{b}$ | 3 | $51.3 \pm 15.0^{b,c}$ | $0.14 \pm 0.046^{a}$ | 0.00681** |

† The values are means with standard deviation (S.D.). Different letters indicate significant differences among the means for each site by Tukey-Kramer test at $p < 0.05$. There was no significant differences among $N_2O/(N_2O+N_2)$ at all sites.

‡The difference between the means of $N_2O$ and $N_2O+N_2$ was tested by t-test for each site. Levels of significance was as follows: * <0.05, ** <0.01, *** <0.001, ns, not significant.

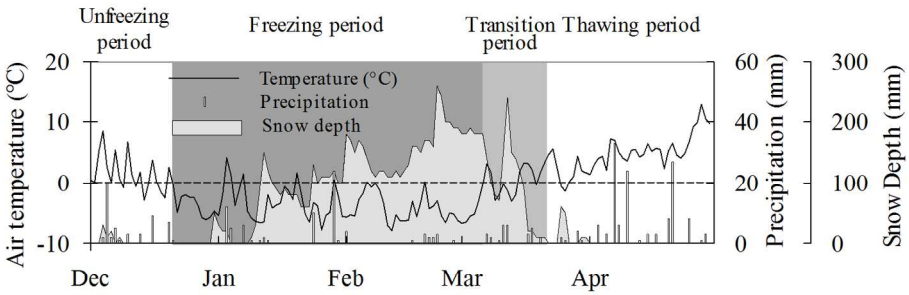


Figure 1. Temporal variability of precipitation observed at the Sasayama Weather Station in the Shizunai Experimental Livestock Farm and air temperature and snow depth observed at the Shizunai Experimental Station from December 2004 to April 2005.
144x52mm (300 x 300 DPI)

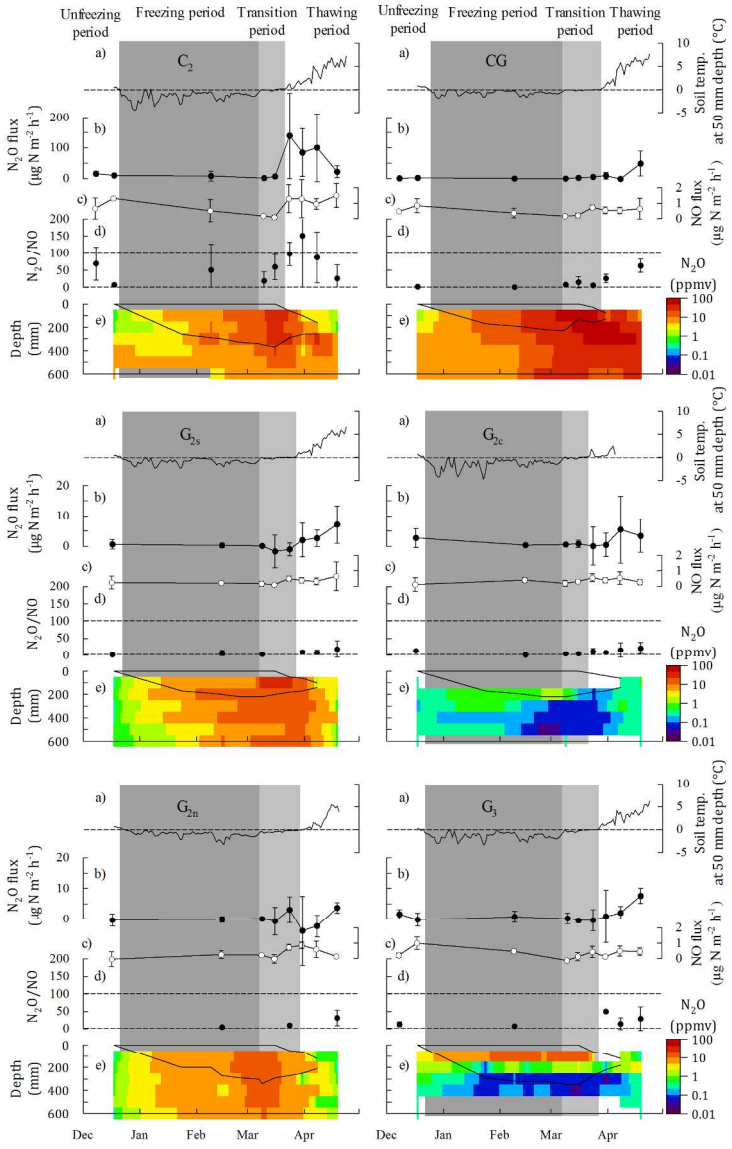


Figure 2. Temporal variability of a) soil temperature at a 50 mm depth, b) N2O flux, c) NO flux, d) N2O-N/NO-N ratio, and e) N2O concentration in soil with soil freezing depths at C2, CG, G2s, G2c, G2n, and G3 from December 2004 to April 2005. The lines in e) indicate the edge of the frozen soil layers and the soil between the lines froze.
170x260mm (300 x 300 DPI)

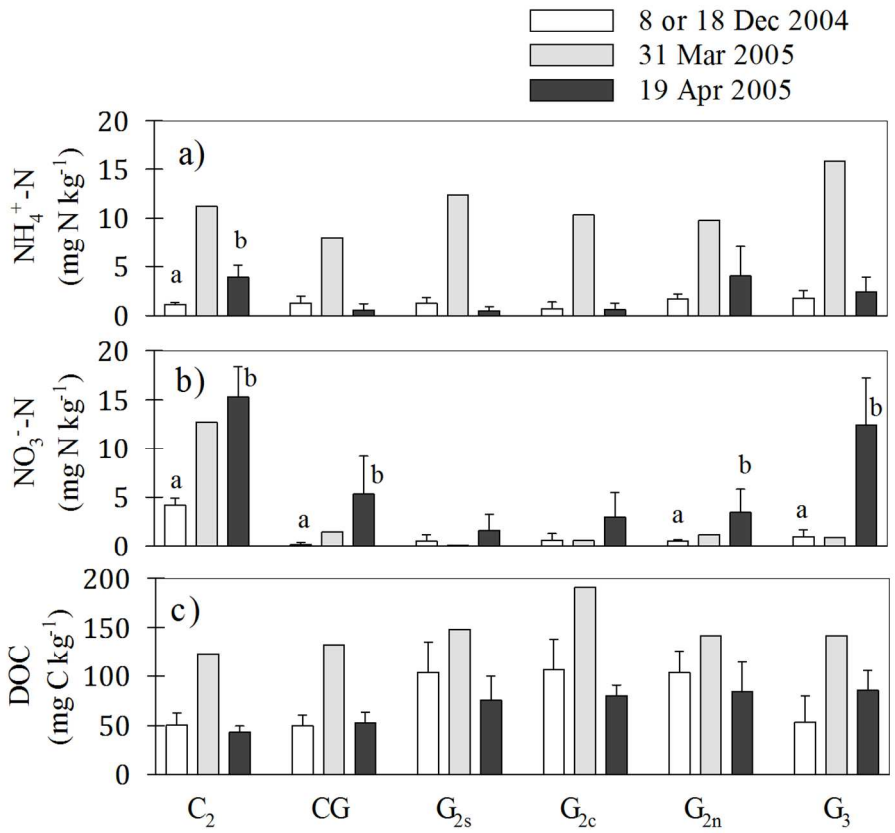


Figure 3. NH4+-N, NO3--N, and dissolved organic carbon (DOC) concentrations in soil at a 0−50 mm depth at the research sites in the Shizunai Experimental Livestock Farm. Measurements were carried out on 8th (C2, CG and G3) or 18th (G2s, G2c, and G2n) December 2004 before soil freezing, on 31st March 2005 just after the start of soil thawing, and on 19th April 2005 at the end of the thawing period. Paired t-test was used to test for statistically significant differences between the data observed on 8th Dec 2004 and 19th April 2005 for each site. Levels of significance was as follows: * <0.05, ** <0.01, *** <0.001.
121x113mm (300 x 300 DPI)