Accumulation of nitrous oxide and depletion of oxygen in seasonally frozen soils in northern Japan — Snow cover manipulation experiments

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ARTICLE INFO

ABSTRACT

It has been suggested that soil-thawing and snow-melting are critical triggers for vigorous emissions of nitrous oxide (N_2O) from soils in cold regions. However, because soil freezing is affected by air temperature and snow cover, accurate predictions that estimate subsequent emissions of this important greenhouse gas are difficult to make. In this study, we measured in situ soil gas N_2O and oxygen (O_2) concentrations at two experimental sites in northern Japan over the period of a year, from November 2008 to October 2009, to clarify the factors stimulating N_2O production in soil at low temperatures. The sites were N-fertilized bare arable lands with different soil frost depths and snowmelt rates, according to the snow cover management imposed. Winter-to-spring net N_2O fluxes, ranging from −0.10 to 1.95 kg N_2O—N ha^{-1}, were positively correlated with the annual maximum soil frost depth (ranging from 0.03 to 0.41 m; $r = 0.951^{***}$). In the plots with deeper maximum soil frost, winter-to-spring N_2O fluxes represented 58% to 85% of the annual values. Soil N_2O production was stimulated when the soil frost depth was greater than 0.15 m or the daily mean soil temperature at 0.05-m depth was below −2.0 °C. In the soil with the greatest frost depth, soil gas N_2O concentrations at the depth of 0.10 m peaked at 46 ppm when soil gas O_2 concentrations fell down to 0.12 m^3 m^{-3} under soil temperature below 0.0 °C. Snowmelt acceleration had no stimulating effect on N_2O production in the soil during the winter-to-spring period.

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1. Introduction

Nitrous oxide (N_2O) is a trace greenhouse gas that contributes to 6% of global warming (Solomon et al., 2007) and is a dominant substance related to the depletion of the stratospheric ozone layer (Ravishankara et al., 2009). The atmospheric N_2O concentration has increased from less than 280 ppb in the pre-industrial era to about 320 ppb in 2006 and is higher in the northern than in the southern hemisphere (Ishijima et al., 2009). In northern high-latitude regions, large N_2O emissions from soils are found not only in the summer (Martikainen et al., 1993; Repo et al., 2009) but also in the winter-to-spring periods. Because N_2O fluxes during winter-to-spring periods sometimes account for more than 50% to 70% of the annual values (Duxbury et al., 1982; Flessa et al., 1995; Röver et al., 1998; Regina et al., 2004; Wagner-Riddle et al., 2007), significant attention has been paid to N_2O production in soil under cold conditions (Phillips, 2008).

The freezing and thawing of soils are important factors related to large N_2O fluxes in cold regions. Soil freezing is primarily affected by air temperature. However, the timing of snowfall and depth of the snowpack greatly affect the soil frost formation as well (Hirota et al., 2002, 2006). Wagner-Riddle et al. (2007) first demonstrated a positive correlation between the cumulative N_2O emissions from November to April and the soil freezing intensity estimated by the accumulated degree-hours below 0 °C at a 0.05 m depth of soil (referred to as ADH$_{0.05}$). They reported that no-tillage management, one of the recommended cultivation practices in Canada, reduced the N_2O emissions as a result of suppressing soil frost formation due to the accumulated snowpack that was entrapped by crop residues on the fields. On the other hand, in northern Japan, removal of the snowpack and an acceleration of the snowmelt are locally conducted as part of the agricultural management from

winter to spring for the purpose of weed control (Hirota, 2008) and to extend the growing season for crops (Hirota et al., 2008). These management practices can affect soil freezing-thawing dynamics and thus change N_2O fluxes in agricultural lands in cold regions.

Both laboratory incubation (Christensen and Tiedje, 1990; Röver et al., 1998; Sharma et al., 2006; Maljanen et al., 2009) and in situ stable isotope tracer studies (Müller et al., 2002; Wagner-Riddle et al., 2008), conducted to clarify the mechanisms of N_2O production in cold soil, have indicated that soil freezing triggers denitrification-derived N_2O production. This production causes the N_2O to accumulate in the surface soil layer. Because denitrification is mediated by facultative anaerobic microorganisms (Zumft, 1997), several studies have speculated the presence of anaerobic microsites in soil under freeze-thaw conditions (Teepe et al., 2001, 2004; Koponen et al., 2004; Öquist et al., 2004; Phillips, 2008). We suggest that a decrease in oxygen (O_2) concentration in soil gas in addition to an increase in the N_2O concentration would support the hypothesis that the soil freeze-thaw conditions enhance denitrification activity in soil. Therefore, we conducted snow cover manipulation experiments in which we created different snow cover, soil frost and snowmelt rate conditions in situ and followed simultaneously the changes in the net N_2O fluxes and soil gas concentrations of N_2O and O_2.

2. Materials and methods

2.1. Experimental setup

The two study sites were located at the western and eastern part of Hokkaido prefecture, northern Japan (Supplementary Fig. 1a). Both sites were experimental research fields of the National Agricultural Research Center for Hokkaido Region, National Agriculture and Food Research Organization. The western site (141° 25′ E, 43° 05′ N: "932 field" in the Sapporo site) was characterized by heavy snowfall with shallow soil-freezing, whereas the eastern site (143° 04′ E, 42° 53′ N: "A3 field" in the Memuro site), some 200 km distant, was characterized by light snowfall with deep soil-freezing. The 30-year (1979−2008) mean annual temperature and precipitation for the Sapporo site were 8.8 °C and 1022 mm, respectively, compared to 6.0 °C and 941 mm, respectively, for the Memuro site. As described below, the experimental plots were mostly established in September 2008. At both sites, volcanic ash-derived Andosols were present. The physico-chemical properties of surface

Table 1
Physico-chemical properties of surface soils (0.0−0.1 m deep).

		Sapporo	Memuro
pH (H_2O)	[b]	5.2 (0.1)	5.5 (0.1)
pH (KCl)	[b]	4.3 (0.0)	4.9 (0.1)
Total C	g C kg^{-1} [a]	57.8 (7.6)	36.8 (5.1)
C/N ratio	[b]	15.2 (0.3)	12.2 (0.7)
NO_3^- content	mg N kg^{-1} [a]	28.5 (9.0)	22.8 (3.1)
NH_4^+ content		0.4 (0.5)	2.1 (1.0)
Available P	mg P_2O_5 kg^{-1} [a]	90 (7.8)	58 (24)
Exchangeable K	$cmol_c$ kg^{-1} [a]	0.57 (0.06)	0.33 (0.09)
Exchangeable Mg		1.35 (0.09)	0.54 (0.10)
Exchangeable Ca		11.6 (0.8)	6.1 (1.4)
Cation exchangeable capacity		39.4 (2.2)	21.1 (1.0)
Phosphate sorption coefficient	g P_2O_5 kg^{-1} [a]	1632 (56)	1680 (83)
Bulk density	Mg m^{-3}	0.88 (0.00)	0.74 (0.03)
Texture [c]	[b]	Clay loam	Loam

The standard deviation of three experimental plots (CO, SR, SM) is shown in parentheses except for bulk density, where a standard deviation of three replicates collected from the CO plot are presented.
CO: untreated control, SR: snow removal, SM: snowmelt acceleration.
[a] Oven dry basis.
[b] Dimensionless.
[c] Examined by touch, in detail in Supplementary Table 1.

soils, from 0 to 0.1 m deep, are shown in Table 1 (see also Supplementary Table 1).

Three snow cover management treatments (SR, removal of snow cover to enhance soil frost depth; SM, acceleration of snow cover melting; and CO, untreated control) were implemented on three 10 m × 10 to 15 m non-cultivated bare experimental plots for both sites (Supplementary Fig. 1a). Considering that the predominant wind direction during the winter is NNW to NW (Hayashi et al., 2005), these treatment plots were arranged to avoid cross-contamination of snow due to the snowdrift. Snow cover management activities were contingent on the weather conditions from January to March. On SR plots, snow removal was performed seven times at the Sapporo site but only two times at the Memuro site. Snow cover was removed using a combination of heavy machinery and manual shoveling. On SM plots, the snowmelt rate was accelerated by spreading 2 Mg ha^{-1} of charcoal (dark 4 mm-granular, 250 g kg^{-1} of moisture content, 922 g C kg^{-1}) on the snowpack surface, thereby lowering its albedo below 0.3 (Hirota et al., 2008). Charcoal application was performed once on March 9, 2009 (67th day-of-year in 2009: DOY 2009) at both sites because snowmelt had started on March 6 (64 DOY 2009). Although it snowed occasionally after the application of the charcoal in the Sapporo site, we did not perform any supplemental applications. The soil frost depth was determined by either the frost tubes containing 0.03% methylene blue solution or a soil temperature below 0.0 °C. Micrometeorological conditions were measured, including air temperature, precipitation, soil temperature, height of the snowpack and the albedo of the ground surface at each plot throughout the observation period according to the methods as described in Iwata et al. (2008). The ADH (°C h) of soil was calculated as follows:

$$ADH_z = \left| \sum (t_z \cdot 1) \right|$$

where, z and t, respectively, stand for a given soil depth (m) and soil temperature (hourly mean value, °C) less than or equal to 0.0 °C. Micrometeorological measurements at the Memuro CO and SR plots were established in October 2005 (Iwata et al., in press). A subplot for gas monitoring was added in November 2007 and then rebuilt in September 2008 as described below. For the other plots, all the measurements were newly established in September 2008.

Within each treatment plot, there was a subplot for monitoring gas dynamics (2 m × 2 m; Supplementary Fig. 1b). Undisturbed 1 m × 1 m space was set at the center of the subplot, and a pit (1 m wide, 1.5 m long, 0.6 m deep) was dug at one side of the space. Across the belowground part of the space, horizontal holes (18 mm OD) were bored to insert diffusion chamber type soil gas samplers (Kammann et al., 2001) without disturbing the soil. In order to collect long range-averaged soil gas samples, a 1.0-m-long probe consisting of a silicone tube (10 mm ID; 13 mm OD) was inserted at each depth of 0.10, 0.20, 0.30 and 0.40 m for N_2O concentration measurements, whereas a 0.50-m-long probe was inserted at each depth of 0.10 and 0.30 m for O_2 concentration measurements. To avoid deformation, the silicone tube was covered with a perforated polyvinylchloride (referred to as PVC) pipe (13 mm ID; 16 mm OD with 8-mm OD holes at 20-mm intervals; Supplementary Fig. 1c). One end of the silicone tube was sealed with a silicone rubber septum, and the other was connected to 2-m-long nylon tubing equipped with a two-way cock to allow gas sampling from above ground. The pit was carefully backfilled and, a month later, 144 g of potassium nitrate was applied to the surface of the subplot (equivalent to 50 kg N ha^{-1}) to mimic post-cultivation conditions in local farmlands. Then, a circular stainless steel collar (275 mm OD) with a water groove (20 mm in height) was embedded at the center of the subplot to monitor net soil N_2O emission rates based on the closed-chamber technique.

The soils were kept bare (no vegetation) to mimic the typical winter conditions for local farmland, except for winter wheat or rapeseed cultivation. To minimize disturbance on the height of snow cover in narrow size of the treatment plot, we did not have replication in measuring N_2O emission rate from soil. Furthermore, because of the limited availability of land, we could not replicate snow cover manipulation treatments at both sites. Instead, this study focused on the relationship among the development of soil gas concentrations (N_2O and O_2), precise time series in micrometeorological conditions and net N_2O fluxes during winter to spring period.

2.2. Gas sampling and analyses

A soil gas sample (volume = 30 ml) was collected from inside an individual 1.0-m-long silicone tube using an individual plastic syringe, to avoid cross-contamination of the sample. The gas sample was injected into a 20-ml pre-evacuated vial equipped with a rubber septum (Maruemu Co., Japan). Immediately after the gas was collected, an equal volume of dinitrogen was added to the silicone tube to maintain the internal pressure of the tube to the atmospheric level (Jacinthe and Groffman, 2001). Within a week after sampling, the N_2O concentration was determined using a gas chromatograph (Shimadzu 14B, Shimadzu Co. Ltd, Japan) equipped with an electron capture detector.

Net N_2O emission rate was measured using chambers made of colored PVC with a 0.25-m diameter, 0.40 m-height and 19.6-l volume. To keep the area between the chamber and the ground surface airtight, the groove on the collar was filled with liquid water as a seal. Samples (volume = 40 ml) drawn from the chamber headspace at the onset of the measurements at 1, 11, 21 and 31 min thereafter were individually injected into pre-evacuated 20-ml vials. The N_2O concentration was determined by gas chromatography as described above, and emission rates ($\mu g\,N\,m^{-2}\,h^{-1}$) were derived as the slope of a linear regression computed for measured N_2O concentration data in the chamber versus time (ppm h^{-1}). During the period when the collar was under snow cover, the chamber was placed directly onto the snowpack surface to minimize disturbance of the snow cover height. At that time, the quality of the air-tightness between the chamber and the snowpack surface was uncertain; therefore, the slope values were statistically tested for all datasets. In this study, any slope that did not differ significantly from 0 (Student's t-test, $\alpha = 0.20$, thus $p \geq 0.10$) was regarded as zero ppm h^{-1}, i.e., no net N_2O emissions occurred. Cumulative emissions over the monitoring period (N_2O fluxes; kg N ha^{-1}) were calculated by linear interpolation of the observed emission rates. The gas sampling was simultaneously done for soil gas N_2O concentrations and net N_2O emission measurements at 2- to 3-day intervals during the snowmelt period (March to early April 2009) and weekly for the other period.

The O_2 concentration inside the 0.50-m-long tubes was monitored using a Galvanic cell-type O_2 sensor (KE-25, GS YUASA Co. Ltd., Japan) connected to data loggers (CR 23X or CR1000, Campbell Sci. Inc., US). The output of the O_2 sensor (10−13 mV at the ambient condition) was occasionally calibrated with the O_2 concentration inside the silicone tubes, which was determined by a dual thermal conductivity detector equipped gas chromatograph that can completely separate O_2 from argon (Yoh et al., 1998).

In total, we had three experimental plots, with one chamber collar and six soil gas samplers (four for N_2O concentration and two for O_2 concentration measurements) at both of the sites (Sapporo and Memuro) in northern Japan. Field observation was conducted from November 2008 to October 2009. Correlation analysis was first done to examine the relationship between cumulative N_2O emissions from winter to spring and the soil frost intensity parameters; then, a linear regression equation was obtained to compare to the result reported by Wagner-Riddle et al. (2007).

3. Results

3.1. Weather conditions and N_2O emissions

The annual mean air temperature and annual precipitation at the Sapporo site were 8.0 °C and 910 mm, respectively, whereas those at the Memuro site were 6.1 °C and 1003 mm, respectively, from November 2008 to October 2009 (Fig. 1). The lowest and highest daily mean air temperatures were −8.5 and 23.4 °C, respectively, for the Sapporo site, and −16.5 and 24.0 °C, respectively, for the Memuro site. The maximum snow cover height was 0.83 m for the Sapporo site and 0.72 m for the Memuro site. The first snowfall occurred on 20 November (324 DOY 2008) at the Sapporo site and on 12 December (346 DOY 2008) at the Memuro site. The precipitation from 1 November (305 DOY 2008) to the date of first snowfall was 45 and 52 mm for the Sapporo and Memuro sites, respectively. Complete snowmelt occurred on 30 March (88 DOY 2009) for the Sapporo site and on 3 April (92 DOY 2009) for the Memuro site. The use of charcoal on the Sapporo and the Memuro SM plots resulted in the acceleration of a complete snow cover melting by 5 and 16 days, respectively, compared to those of the respective CO plots. Soil frost started in early January and late November for the Sapporo and Memuro sites, respectively. The maximum soil frost depth (referred to as D_{max}) was found in February for both sites. The D_{max} values at the CO, SR and SM plots, respectively, were 0.03, 0.19 and 0.03 m for the Sapporo site and 0.17, 0.41 and 0.12 m for the Memuro site. Soils thawed from the bottom and top side, and the frost disappeared completely on 22 March (80 DOY 2009) for the Sapporo site and on 14 April (103 DOY 2009) for the Memuro site.

Spike-like net N_2O emission rates were detected from plots that exhibited greater D_{max} when the daily mean air temperature crossed above 0 °C (Fig. 1). The annual cumulative N_2O emissions (N_2O fluxes) over November 2008 through October 2009 at the CO, SR and SM plots, respectively, were 0.09, 0.32 and 0.60 kg N ha^{-1} for the Sapporo site and 1.12, 2.30 and 0.59 kg N ha^{-1} for the Memuro site. The Memuro-SR, Memuro-CO and Sapporo-SR plots had maximum net N_2O emission rates of 1553, 285 and 201 $\mu g\,N\,m^{-2}\,h^{-1}$, respectively. These rates were higher than the highest emission rate during the summer period for the Sapporo SM plot (98.2 $\mu g\,N\,m^{-2}\,h^{-1}$), Memuro CO (57.8 $\mu g\,N\,m^{-2}\,h^{-1}$), Memuro SM (48.5 $\mu g\,N\,m^{-2}\,h^{-1}$) and Memuro SR (38.0 $\mu g\,N\,m^{-2}\,h^{-1}$) plots. N_2O emissions at the Memuro site were less than 40 $\mu g\,N\,m^{-2}\,h^{-1}$ within the snow-covered period, whereas those at the Sapporo SM and Sapporo CO plots were very low and sometimes a negative rate.

The cumulative N_2O emissions between November 2008 and April 2009, winter-to-spring N_2O fluxes, were −0.10, 0.24 and 0.02 kg N ha^{-1} for the Sapporo site and 0.65, 1.95 and 0.12 kg N ha^{-1} for the Memuro site at the CO, SR and SM plots, respectively (Fig. 2a). The winter-to-spring N_2O fluxes had a significant positive correlation with the $ADH_{0.05}$ ($r = 0.991$, $p < 0.001$), and similar relationships were found in the ADH at the 0.10-m depth ($ADH_{0.10}$, $r = 0.985$, $p < 0.001$) and the D_{max} ($r = 0.951$, $p < 0.001$). The winter-to-spring N_2O fluxes also had a significant positive correlation with the duration of the period when the soil was frozen ($r = 0.782$, $p < 0.05$) and a significant negative correlation with the annual minimum soil temperature (daily mean values: $r = -0.856$, $p < 0.05$) at a depth of 0.05 m. Among these parameters related to soil frost conditions, the ADH, the D_{max} and the soil temperature clearly described the differences between the plots with and without the spike-like N_2O emission, whereas the frozen soil period did not (see circles in Fig. 2a). The ADH had a significant, positive correlation with the D_{max} ($r = 0.981$, $p < 0.001$ for $ADH_{0.05}$;

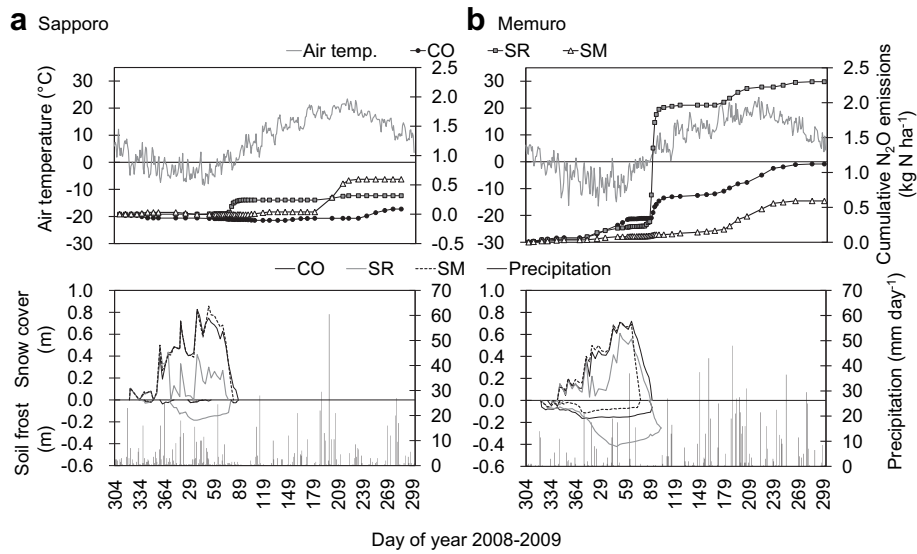**Fig. 1.** Weather conditions, cumulative N_2O emissions, snow cover height and soil frost depth over November 2008 to October 2009 at the Sapporo (a) and Memuro (b) sites. The annual mean air temperature and annual precipitation for the Sapporo site were 8.0 °C and 910 mm, respectively, compared to 6.1 °C and 1003 mm, respectively, for the Memuro site. The snow-covered periods at the CO plots at the Sapporo and Memuro sites were 131 and 110 days, respectively. CO: control, SR: snow cover removal, SM: snowmelt acceleration.

$r = 0.954$, $p < 0.001$ for $ADH_{0.10}$; Fig. 2b). Also, the D_{max} had significant correlations with both the frozen soil period ($r = 0.863$, $p < 0.01$) and the annual minimum soil temperature ($r = -0.963$, $p < 0.01$).

The regression equation between the winter-to-spring N_2O fluxes (Y; kg N ha^{-1}) and $ADH_{0.05}$ was $Y = 0.568 \times 10^{-3}\, ADH_{0.05} - 0.088$ ($R^2 = 0.981$, $n = 6$). Similarly, the regression equation with the D_{max} was $Y = 5.170\, D_{max} - 0.339$ ($R^2 = 0.905$, $n = 6$).
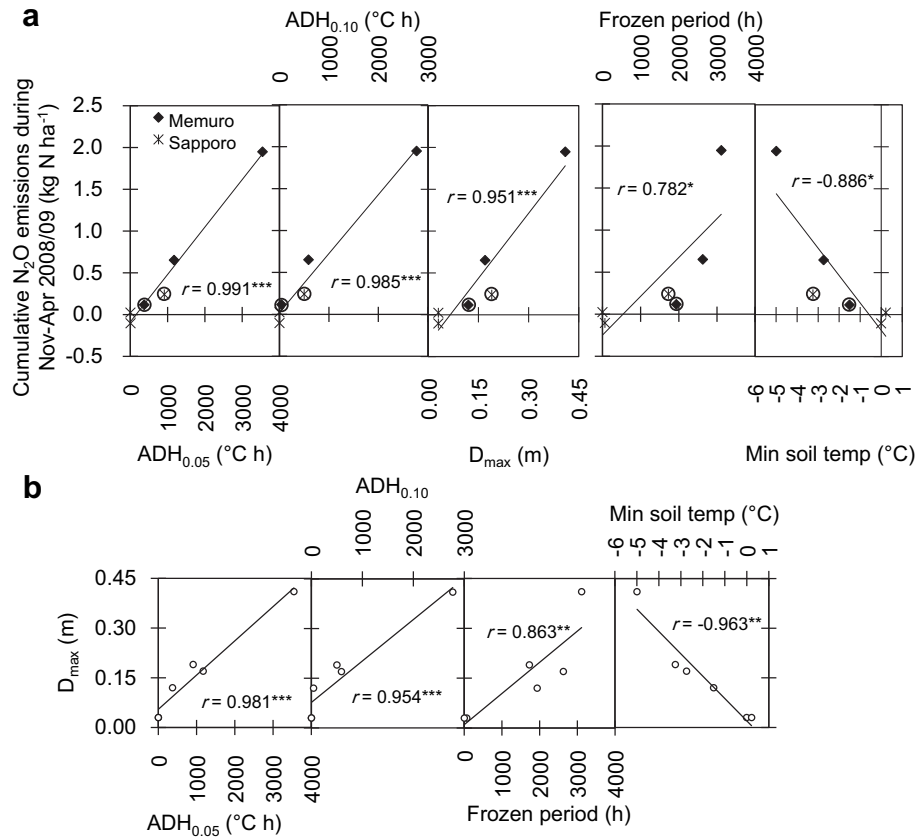

Fig. 2. Correlations (a) between cumulative winter-to-spring N_2O emissions and measures of soil freezing and (b) among soil freezing measures. ADH_z: accumulated degree-hour below 0 °C at z m depth of soil, D_{max}: the annual maximum soil frost depth. Frozen period and Min soil temp (the annual minimum daily mean soil temperature) are shown for the depth at 0.05 m. Circles in figure (a) indicate the snowmelt acceleration (SM) plot for the Memuro site and the snow cover removal (SR) plot for the Sapporo site.

3.2. Soil temperature profile and soil gas concentrations

During the snowmelt period, the soil temperature profile was very different between the plots (upper panels in Figs. 3 and 4). In all plots, soil temperature at a 0.30-m depth was higher than that at

0.10- and 0.05-m depths. Only the Memuro SR plot measurement was still below 0.0 °C during the snowmelt period, with intermittent increases from −0.5 °C on March 4 (62 DOY 2009) until complete snowmelt. Infiltration of rain or snowmelt water made sharp decreases in the soil temperature at 0.30 m depth at the CO
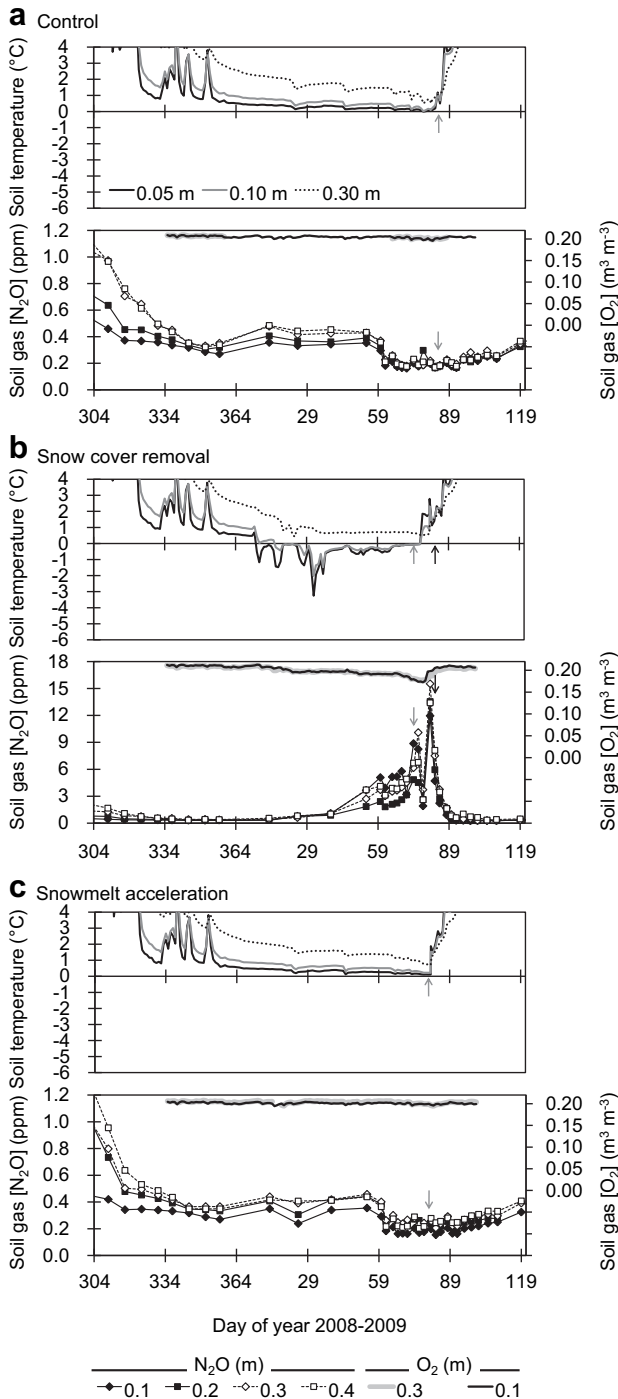

Fig. 3. Soil temperature profiles and soil gas N₂O and O₂ concentrations at different snow cover management plots over the snowmelt period at the Sapporo site. Gray and black arrows denote the date when the snowpack was at a 0.1-m height before complete melting and an N₂O spike appeared, respectively. For soil temperature and soil gas O₂ concentration, daily mean values are shown. Note the different ranges of soil gas N₂O concentration among plots. In the panel (a), part of soil gas O₂ concentration values at a 0.30-m depth are missing probably due to disconnection.
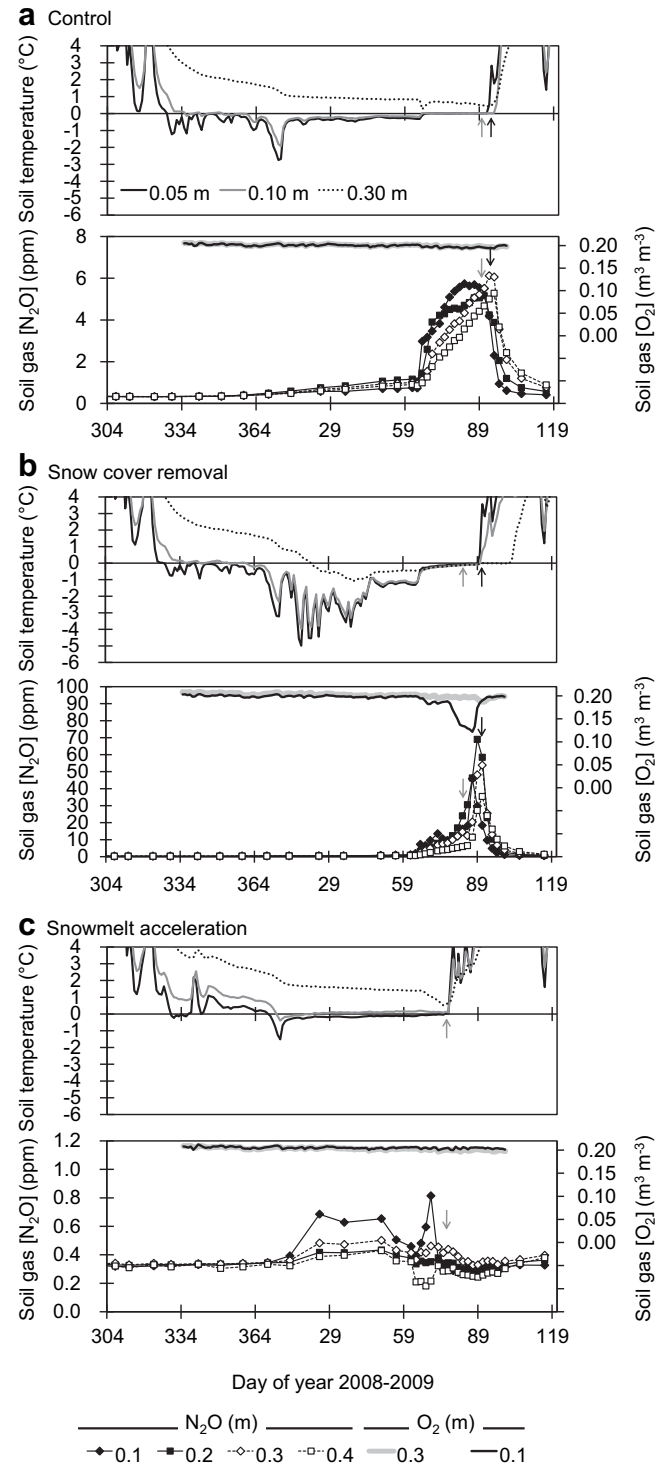

Fig. 4. Changes in soil temperature profiles and soil gas N₂O and O₂ concentrations at different snow cover management plots over the snowmelt period at the Memuro site. Gray and black arrows denote the date when the snowpack was at a 0.1-m height before complete melting and an N₂O spike appeared, respectively. For soil temperature and soil gas O₂ concentration, daily mean values are shown. Note the different ranges of soil gas N₂O concentration among plots.

and SM plots but made a rapid increase in the soil temperature at 0.05 m for the Memuro SR, Sapporo SR and Memuro CO plots. Even with these temperature rises at the Memuro and Sapporo SR plots, the soil temperature at 0.10 m was still below 0.0 °C, whereas it remained slightly above 0.0 °C at the Memuro CO plot. The N_2O spike occurred when the surface soil temperature crossed above the freezing point after complete snowmelt.

A clear peak of soil gas N_2O concentration was found in the snowmelt period at the plots with greater D_{max} from autumn to spring (bottom panels in Figs. 3 and 4). The soil gas N_2O concentration increased at all measured depths, irrespective of whether or not the layer had reached below 0.0 °C, but the peak value appeared from shallower to deeper soil layers with time. For the shallowest (0.10 m depth) soil gas N_2O concentration, 46, 8.9 and 5.7 ppm were measured in the Memuro SR, Sapporo SR and Memuro CO plots, respectively. Concurrently, the soil frost went through 0.33, 0.12 and 0.12 m, and the soil gas N_2O concentration beneath the frozen layer reached peak values of 35, 6.7 and 4.7 ppm at the respective plots. It was clear that at the Memuro SR plot, the increase in soil gas N_2O concentration corresponded to the decrease in soil gas O_2 concentration at 0.10 m depth. The lowest hourly mean O_2 concentration was 0.12, 0.17 and 0.19 $m^3 m^{-3}$ at the Memuro SR, Sapporo SR and Memuro CO plots, respectively, at a depth of 0.10 m. The soil gas O_2 concentration at 0.30 m depth showed nearly the same time series as at 0.10 m at Sapporo the SR plot, unlike at the Memuro SR plot (Figs. 3b and 4b). The N_2O spike occurred when the soil gas O_2 concentration rapidly increased to the ambient atmospheric level, whereas the soil gas N_2O concentration decreased toward the ambient level. On the other hand, plots with weak soil frost development (the Memuro SM, Sapporo SM, Sapporo CO) did not have a remarkable increase in soil gas N_2O concentration between autumn and spring (Figs. 3a,c and 4c). The soil gas N_2O concentration decreased below the ambient atmospheric level (0.32 ppm) during the snowmelt period, whereas the soil gas O_2 concentration was nearly constant at the ambient atmospheric level (0.21 $m^3 m^{-3}$) during the relevant period.

4. Discussion

4.1. N_2O production in the soil and emissions from the soil during the winter-to-spring period

Compared to the 30-year norms at the two experimental sites, the average air temperature during the observation period (2008–2009) was nearly the same but the annual precipitation was less (Fig. 1). The Memuro site had much colder winter conditions with a later start to the first snowfall than the Sapporo site. The winter conditions resulted in greater soil frost formation in the Memuro site compared to the Sapporo site, and the snow cover removal treatment promoted the development of soil frost at both sites. The earlier complete snowmelt at the Sapporo site was due to warmer conditions than at the Memuro site. On the other hand, the greater effect of the charcoal application on snowmelt rate at the Memuro site was probably due to shallower maximum snow cover height and more continuously sunny days, compared to the Sapporo site. The annual N_2O fluxes measured at three experimental plots (mean ± standard deviation) were $0.33 ± 0.25$ kg N ha^{-1} for the Sapporo site and $1.34 ± 0.87$ kg N ha^{-1} for the Memuro site.

The spike-like high N_2O emission rates that were found in this study in early spring ranged from 201 to 1553 µg N m^{-2} h^{-1}, which were similar or higher than the reported values for agricultural land (including mineral or organic soils, cultivated or fallow fields) by Röver et al. (1998), Koga et al. (2004), Syväsalo et al. (2004), Maljanen et al. (2007) and Wagner-Riddle et al. (2007) and lower than those by Flessa et al. (1995), Regina et al. (2004) and Maljanen

et al. (2009). At the period of time, we found an increasing soil gas N_2O concentration (5.7–46 ppm) together with decreasing O_2 (90–57% of the atmospheric level) at a 0.10-m depth (Figs. 3 and 4). The range of the soil gas N_2O concentration was similar to the report by Maljanen et al. (2007) and lower than those by Regina et al. (2004), Syväsalo et al. (2004) and Wagner-Riddle et al. (2008). However, there are no reports related to the time series of soil gas O_2 concentration under soil freeze-thaw conditions.

An increasing soil gas N_2O concentration may be regarded as evidence of N_2O production in the soil by either biotic or abiotic process; a decreasing soil gas O_2 concentration at the corresponding depth may support for the source of N_2O as in situ denitrification. But the presence of time lag between an increasing soil gas N_2O concentration and surface N_2O flux makes it difficult to predict accurate timing for measuring N_2O emission rates, as suggested by Maljanen et al. (2009). The time lag can be due to the spatial variability of snowmelt conditions, which create a difficulty in discerning the relationship between N_2O dynamics, snowpack decrease, thawing of soil frost and soil temperature (Figs. 1, 3 and 4). Or soil water dynamics, such as a disappearance of the surface ponding derived from snowmelt water and rainfall, could trigger the N_2O spike in the spring when N_2O is produced and accumulated in soil. Alternatively, our results indicate that monitoring the soil gas O_2 concentration may be useful in predicting both the timing of N_2O production and spikes under soil freeze-thaw conditions. Under the freeze-thaw conditions, it is difficult to estimate water-filled pore-spaces, which are a well-documented proxy for oxic or anoxic conditions and processes related to N_2O production in the soil (Well et al., 2006). Monitoring the soil gas O_2 concentration can also be a reasonable way to interpret the lowering soil N_2O concentration below the atmospheric ambient level that was found during the snowmelt period at the plots with few soil frost developments (the Sapporo SM, Sapporo CO, Memuro SM; Figs. 3a, c and 4c). In these plots, N_2O reduction to N_2 may not have occurred principally because soil gas O_2 concentration did not decrease like the SR plots at both sites (Figs. 3b and 4b). Rather, it is possible that melt water that dissolves soil gas N_2O and transfers to deeper part of soil layer. To determine the fate of N_2O accumulated in soils from late winter to early spring periods, more studies are necessary regarding the physics and chemistry of melt water and seasonally frozen soil (Maljanen et al., 2009; Jefferies et al., 2010).

4.2. Characterization of N_2O fluxes from winter-to-spring period and its implication for agriculture in cold regions

The winter-to-spring N_2O fluxes represented 58%, 76% and 85% of the annual fluxes in Memuro CO, Sapporo SR and Memuro SR plots, respectively, indicating that we cannot exclude the winter when determining the annual N_2O fluxes in regions where the air temperature falls below 0 °C and the soil undergoes seasonal freezing. Modeling N_2O production in soil and the emission from soil under freezing and thawing conditions has proven to be difficult. Field measurements can only describe large N_2O fluxes in the relevant period. However, the measurement of the N_2O emission rate in the field during the winter-to-spring period using the closed-chamber technique has several issues still under debate in getting reliable data, such as a number of replications, frequency and period of chamber deployment, and gas-tightness between the chamber and the soil. The presence of snowpack on the ground surface itself makes it difficult to access and maintain the observation field (Campbell et al., 2005). This study proposes that measuring soil gas O_2 concentration can improve our understanding of the process that leads to N_2O production in soil below subzero temperature, as suggested by the modeling-based studies by the "ecosys" (Grant and Pattey, 1999) and the modified "DNDC"

(Norman et al., 2008; de Bruijn et al., 2009). Therefore, the elucidation of factors controlling soil gas O_2 concentration should be critical in describing N_2O dynamics in soil. Furthermore, soil freezing intensity can be used as an empirical evaluation of winter-to-spring N_2O flux in the cold regions.

Positive correlation between the winter-to-spring N_2O fluxes and the soil frost intensity (ADH) at 0.05-m depth (Fig. 2a) confirmed the report by Wagner-Riddle et al. (2007). The slope value in this study (0.568×10^{-3}) was of the same order as the value (0.216×10^{-3}) reported by Wagner-Riddle et al. (2007), which was obtained from a multi-year study of different cultivation conditions with various ranges of mineral N contents in the soil. The agreement between the two studies suggests that the relationship between winter-to-spring N_2O flux and soil frost intensity can be robust in a given arable field. However, Maljanen et al. (2009) reported a different intensity of N_2O fluxes among agricultural mineral and organic soils under the snow cover removal experiment, suggesting strong dependency on the nitrate content of the soil and the soil type, which is probably associated with the solubility (availability) of the organic carbon of the soil under freeze-thaw conditions.

The D_{max}, the duration of frozen soil period and the minimum daily mean soil temperature had significant relationships with the winter-to-spring N_2O fluxes (Fig. 2a). Teepe et al. (2004) suggested the importance of the frozen soil period based on a laboratory experiment, and our result obtained from field experiments confirmed that the continuous soil cooling is critical to stimulate the N_2O production of soil. This production is probably linked with the soil incubation created by the decreasing thickness of the unfrozen water film with cooler temperatures (Rivkina et al., 2000; Watanabe and Wake, 2009) and the increasing amount of soluble organic matter released (Vestgarden and Austnes, 2008). Assuming that denitrification is the major process responsible for N_2O production under soil freeze-thaw conditions as suggested by Wagner-Riddle et al. (2008), a decreasing microbial biomass (Jefferies et al., 2010) and stimulation of microbial activity — known as the partial sterilization effect (Yanai et al., 2007) — might be taken into account for understanding the stimulation of soil N_2O production under cold conditions.

The D_{max} and the lowest soil temperature can be used as predictive parameters for winter-to-spring N_2O flux because these data can be obtained before spring arrives, unlike the ADH and duration of the frozen soil period. For the empirical modeling of the winter-to-spring N_2O flux, the $ADH_{0.05}$ seems to be the best parameter in this study, but in terms of prediction, our results provide threshold values for soil frost development (at least 0.15 m deep) or daily mean soil temperature at a depth of 0.05 m (below $-2.0\ ^\circ C$) beyond which large fluxes of N_2O can be expected (Fig. 2a). The reliability of these threshold values are still subject to discussion because the literature does not establish any other clear descriptions of related datasets.

Snowmelt acceleration did not affect soil frost formation in early spring; therefore, there may be a minor effect on stimulating N_2O production of seasonally frozen soil, at least from winter to spring (Figs. 1, 3 and 4). Charcoal (biochar), a promising material for enhancing the rate of snowmelt and thus extending the agricultural production season, also holds the potential to sequester carbon from the atmosphere to soil due to its slow degradation (very stable) property (Nguyen et al., 2009). In this study, a single application of 2 Mg ha^{-1} of the charcoal as an agricultural snowmelt agent resulted in approximately 1.5 Mg C ha^{-1} of input to the soil during the observation period (one year; see Materials and methods), which was nearly equivalent to the annual carbon dioxide emissions in our study site (unpublished data). Hence, N_2O fluxes and the acceleration of snowmelt due to the use of charcoal are likely key issues with respect to the potential for global warming in agricultural lands in cold regions.

5. Conclusions

Based on a one-year plot-scale snow cover manipulation experiment in northern Japan, we established that soil freezing, rather than the melting of snow cover, primarily enhanced soil N_2O production. Because a decreasing soil gas O_2 concentration occurred in the shallow layer and clearly corresponded with an increasing soil gas N_2O concentration, denitrification in the thawing upper layer was likely the major process responsible for N_2O production. We assert that in regions where the maximum soil frost depth exceeds 0.15 m or the daily mean soil temperature at a 0.05-m depth falls below $-2.0\ ^\circ C$, N_2O fluxes might not be negligible, as once thought. To evaluate the annual N_2O fluxes, field monitoring of N_2O emission rates immediately prior to the end of snowmelt might be essential for regions where soils undergo seasonal freezing.

Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.soilbio.2010.06.009.

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