Title

Nitrous oxide emissions and nitrogen cycling in a managed grassland in southern Hokkaido, Japan

Running title

N_2O emissions and N cycling in grassland

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Abstract

Nitrous oxide (N_2O) emissions were measured and nitrogen (N) budgets were estimated for two years in the fertilizer, manure, control and bare plots established in a reed canary grass (*Phalaris arundinacea L.*) grassland in Southern Hokkaido, Japan. In the manure plot, beef cattle manure with bark was applied at a rate of 43 - 44 Mg fresh matter (236 - 310 kg N) ha^{-1} $year^{-1}$, and a supplement of chemical fertilizer was also added to equalize the application rate of mineral N to that in the fertilizer plots (164 - 184 kg N ha^{-1} $year^{-1}$). Grass was harvested twice per year. The total mineral N supply was estimated as the sum of the N deposition, chemical fertilizer application and gross mineralization of manure (GMm), soil (GMs), and root-litter (GMl). GMm, GMs and GMl were estimated by dividing the carbon dioxide production derived from the decomposition of soil organic matter, root-litter and manure by each C:N ratio (11.1 for soil, 15.5 for root-litter and 23.5 for manure). The N uptake in aboveground biomass for each growing season was equivalent to or greater than the external mineral N supply, which is composed of N deposition, chemical fertilizer application and GMm. However, there was a positive correlation between the N uptake in aboveground biomass and the total mineral N supply. It was assumed that 58% of the total mineral N supply was taken up by the grass. The N supply rates from soil and root-litter were estimated to be 331 -

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384 kg N ha^{-1} $year^{-1}$ and 94 - 165 kg N ha^{-1} $year^{-1}$, respectively. These results indicated that the GMs and GMl also were significant inputs in the grassland N budget. The cumulative N_2O flux for each season showed a significant positive correlation with mineral N surplus, which was calculated as the difference between the total mineral N supply and N uptake in aboveground biomass. The emission factor of N_2O to mineral N surplus was estimated to be 1.2%. Furthermore, multiple regression analysis suggested that the N_2O emission factor increased with an increase in precipitation. Consequently, soil and root-litter as well as chemical fertilizer and manure were found to be major sources of mineral N supply in the grassland, and an optimum balance between mineral N supply and N uptake is required for reducing N_2O emission.

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Keywords: Grassland; Gross mineralization; Manure; Nitrogen surplus; Nitrous oxide

1. Introduction

Grassland is one of the world's most abundant land cover types accounting for approximately 40.5% of the earth's terrestrial land area, excluding the areas of permanent ice cover (Adams et al. 1990; White et al. 2000). Grassland is an important ecosystem to support the production of herbivorous livestock (Soussana et al. 2007). Application of chemical fertilizer and animal manure to grasslands has increased grass

production, especially in developed countries where grassland-based livestock production is important (Bouwman et al. 2002). However, Nitrogen (N) application to grasslands also poses a risk of N loss to the environment in the form of nitrous oxide (N_2O) emission and nitrate leaching (Bouwman 1996; Jordan et al. 1997).

N_2O is a potent greenhouse gas, which has a global warming potential of 298 times that of carbon dioxide (CO_2) over a 100-year time horizon (IPCC 2007). Furthermore, N_2O destroys the stratospheric ozone layer (Crutzen 1974). N_2O concentration in the atmosphere has increased from about 270 ppbv in the preindustrial era to 319 ppbv in 2005 (IPCC 2007). Global N_2O source have been estimated to be 17.7 Tg N year^{-1} (IPCC, 2007). N_2O emission from fertilized agricultural fields amounted to 2.8 Tg N year^{-1}, which is the biggest anthropogenic N_2O source (IPCC2007). Isotopic measurements made on polar firn air also suggested that the anthropogenic N_2O sources have been dominated by agricultural emissions (Rahn & Wahlen 2000; Rockmann et al. 2003).

N_2O emissions from soils result principally from microbial nitrification and denitrification (Bouwman 1990). These processes require mineral N (NH_4^+ and NO_3^-) as a substrate, and are controlled by soil moisture content, temperature, pH and organic carbon (Bouwman 1990; Maag & Vinther 1996; Mosier 1998; Yamulki et al. 1997). In

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managed grassland, the sources of mineral N are considered to be chemical fertilizer, manure, soil organic matter, plant litter, N fixation and atmospheric deposition (Dam Kofoed 1981; Dubeux et al. 2007). Therefore, estimating the supply rate of mineral N from each source and clarifying the factors controlling N_2O emissions can be helpful in establishing environmental-friendly N management.

In the grasslands of Southern Hokkaido, Japan, which were used for this study, carbon budgets have been measured using an ecological technique (Shimizu et al. 2009). We found that the CO_2 fluxes from soil surface including roots in the fertilizer, manure and control plots were higher in spring (beginning of March - end of June) than in other seasons (beginning of July - end of February) at the same soil temperature, while the relationship between CO_2 flux and soil temperature in the root-excluded bare plot was well explained by a single exponential regression throughout the year. This suggests that there might be an increase in root respiration or heterotrophic respiration of litter produced by fine roots. If the increment in spring was caused by root respiration, the root respiration would not have been counted in the carbon budget. If it was caused by heterotrophic respiration of fine root litter, the heterotrophic respiration was offset by the fine root production because of an unclear yearly change, and had no effect on the estimation of carbon budget. Therefore, we concluded that either root respiration or

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heterotrophic respiration had no effect on the carbon budget in our estimations. However, the distinction between the heterotrophic respiration and root respiration has a great influence on N budgets. Heterotrophic respiration accompanies N mineralization (Schimel 1986); while an increase in root respiration does not enhance the N mineralization. Also, the processes of nitrification and denitrification after N mineralization would have a major influence on N_2O emission (Bouwman, 1990), and the determination of the source of CO_2 emission is an important factor when clarifying the source of N_2O emission. Actually, a positive relationship between CO_2 and N_2O emissions was reported by several field studies (Toma & Hatano 2007; Chu et al. 2007) and a laboratory incubation (Hashidoko et al. 2007).

The objectives of this study were to identify the sources of mineral N, their supply rate in managed grasslands and to clarify the factors controlling N_2O emission from the grassland.

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2. Methods

2.1. Study site

This study was conducted for two years from mid-April 2005 to early May 2007 in managed grasslands located on the Shizunai Experimental Livestock Farm, Field

Science Center for the Northern Biosphere of Hokkaido University in Southern Hokkaido, Japan (42°26'N, 142°29'E). The site is characterized by a humid continental climate with cold winters and cool summers but without apparent wet or dry seasons. The mean annual precipitation is 1365 mm and the mean annual temperature is 7.9 ˚C, with the mean monthly temperature ranging from 20.7 ˚C in August to -3.9 ˚C in January. The site is covered with snow from the end of December to the beginning of March.

The soil is derived from Tarumae (b) volcanic ash, and is classified as Thaptic Melanudands (Soil Survey Staff 2006; Mollic Andosol (IUSS Working Group, WRB 2006)). A layer of three cm thick root-mat was found on the top, and a 21 cm thick Ap-layer was found under the root-mat in a survey conducted in August 2004. The C and N contents in the Ap-layer were 3.7% and 0.33%, respectively, and the C:N ratio was 11.1.

The grassland was established more than 30 years ago and has been continuously used as grassland. The average of chemical fertilizer application rates from 1984 to 2004 were 133 ± 36 kg N ha^{-1} $year^{-1}$. The dominant species of the grassland were reed canary grass (*Phalaris arundinacea L.*) and meadow foxtail (*Alopecurus pratensis L.*), and the percentage of legume was less than 2.9% (Table 1). The harvesting was carried

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out twice a year (21st June and 11th August in 2005 and 27th June and 23rd August in 2006) in accordance with the local practice.

2.2. Study period

We defined the crop growing season as the period of time with 7-day moving average of daily air temperature above 5 ˚C and the non-growing (NG) season as the rest. The growing season was 215 days in 2005 (From 10th April 2005 to 10th November 2005) and 218 days in 2006 (From 15th April 2006 to 18th November 2006), and the NG season was 155 days in 2005 and 151 days in 2006. The growing season was divided into three parts; from the beginning of the growing season to the first crop harvest (G1), from the first harvest to the second harvest (G2), and from the second harvest to the end of the growing season (G3). The divided growing season was 72 days in 2005 and 73 days for G1, 51 days in 2005 and 57 days for G2, and 92 days in 2005 and 88 days in 2006 for G3. The non growing season was 155days in 2005 and 151 days in 2006.

2.3. Treatment

Four experimental plots were set up on the study site, one for treatment with chemical fertilizer (fertilizer plot), another with both beef cattle manure and chemical fertilizer (manure plot), a third with no fertilizer or manure (control plot) and the other with no plants (bare plot). Neither manure nor fertilizer was applied to the bare plot.

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The treatments were initiated in the spring of 2005. Eighteen subplots (5 m × 4 m) were established for the fertilizer, manure and control plots with six replications, and six subplots (1 m × 1 m) for the bare plot (Fig. 1). In the bare plot, the plants aboveground and roots were removed by hand, with a shovel and a root-proofing permeable sheet (BKS9812, TOYOBO, Osaka, Japan) was vertically inserted to a depth of 20 cm below the ground surface to inhibit regrowth of roots. The plants grown on the bare plot were removed every couple of weeks.

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Table 2 shows the information on the date of application and the application rates of fertilizer and manure. The fertilizer application rates in the fertilizer plot were at the recommended level for this site on the basis of soil diagnosis, and were 164 kg N ha^{-1} $year^{-1}$ in 2005 and 184 kg N ha^{-1} $year^{-1}$ in 2006 as ammonium sulfate and ammonium phosphate (Table 2). The manure application rates were based on adequate amounts of potassium application to the fields. Beef cattle manure with bedding litter (bark) was applied to the manure plot and the application rates were 44 Mg FM ha^{-1} (236 kg T-N ha^{-1} and 5.8 Mg T-C ha^{-1}) in May 2005 and 43 Mg FM ha^{-1} (310 kg T-N ha^{-1} and 6.0 Mg T-C ha^{-1}) in May 2006 (Table 2). In the manure plot, the nutrient supply rates from manure were estimated by multiplying the application rate by the mineralization rate (Table 3), and the differences between the supply rates in manure and the application

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rates in the fertilizer plot were supplied by chemical fertilizer. The N mineralization rates were estimated based on Uchida's model (Shiga et al. 1985) which was developed in Japan and were 13.2% and 7.0%, respectively in the first and second years after application (Table 3). The mineralization rates of phosphorus and potassium from the manure were estimated based on the handbook of animal waste management and utilization in Hokkaido 2004 (Hokkaido Prefectural Experiment Stations and Hokkaido Animal Research Center 2004). The phosphorus mineralization rate was 20% in the first year and 10% in the second year (Table 3). The potassium mineralization rate was 70% in the first year and 10% in the second year (Table 3).

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2.4. Nitrogen deposition

A rainfall collector (0.9 m length, 0.1 m width) with a tank (20 L) was set up, and the water sample was collected once or twice each month from April to November (Morishita et al. 2004). A bucket (0.3 m diameter, 0.4 m depth) was set up to collect snowfall, and the water sample was collected once a month. Thymol ($C_{10}H_{14}O$) was used as a biocide in the tank or bucket to prevent biological alteration and utilization of the N species (Gillett & Ayers 1991). All the samples were filtered through a membrane filter (0.2 μm) and kept at 4°C before analysis. NH_4^+-N content was determined by the indophenol-blue method (UV mini 1240, Shimadzu, Kyoto, Japan), and NO_3^--N content

was analyzed by ion chromatography (Dionex QIC Analyzer, Dionex Japan, Osaka, Japan). The amount of N deposition was calculated by multiplying the amount of water by NH_4^+-N and NO_3^--N concentrations.

2.5. Gross N mineralization

The soil organic matter, root-litter and manure are supposed to release mineral N during decomposition in the managed grassland, and gross N mineralization (GM) was estimated using the following equation.

$$GM = GMs + GMl + GMm \quad (1)$$

where suffixes "s", "l" and "m" indicate soil organic matter, root-litter and manure, respectively. The GM was estimated by dividing heterotrophic respiration (RH) of soil organic matter, root-litter and manure by each C:N ratio (Luxhoi et al. 2006).

$$GM = RH / C{:}N \text{ ratio} \quad (2)$$

The RH from soil organic matter, root-litter and manure was previously estimated using the static closed chamber method on this study site (Shimizu et al. 2009). These measurements were carried out at the same time with the measurement of N_2O and NO fluxes as described below. RH from the soil organic matter (RHs) was estimated using the exponential regression model for the bare plot driven by the soil temperature at each plot. The RH from manure (RHm) was estimated as the difference in soil respiration,

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which is a CO_2 flux from the soil surface composed of RH and root respiration, between manure and fertilizer plots. The RH from root-litter (RHl) was assumed as the following. Soil respiration in the fertilizer, manure and control plots was higher in spring (beginning of March - end of June) than in the other season (beginning of July - end of February) at the same soil temperature (Shimizu et al. 2009). We hypothesized that the increment of a CO_2 flux in the spring was RHl. To estimate the increment of a CO_2 flux in spring, we calculated the cumulative CO_2 flux in spring using the exponential regression equation of the two seasons driven by the soil temperature in spring, and estimated the difference between the cumulative CO_2 fluxes (Shimizu et al. 2009).

We used a C:N ratio of soil in the Ap-layer (11.1) to estimate GMs. Roots of less than 2 mm in diameter were obtained four times in 2006 (mid April, late June, mid August and late October), and the C:N ratio was analyzed using an N/C analyzer (SUMIGRAPH NC-1000, Sumika Chemical Analysis Service, Ltd., Osaka, Japan). The average value was 15.5, and we used it for the C:N ratio of root-litter to estimate the GMl. The C:N ratio of manure was 25 for 2005, and was assumed to be 22 for 2006, which was the average of two years, because manure applied in 2005 have remained in 2006.

2.6. N uptake in aboveground biomass

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The aboveground biomass, which includes live and dead parts, was measured four times a year; in mid April, late June (before the first crop harvest), mid August (before the second crop harvest) and in late October. The aboveground biomass at the time of the harvest was estimated to be the sum of the harvests and the residue. The harvest was measured by clipping at 5 cm above the ground using the 100 cm × 100 cm quadrates, and the harvest residue and the aboveground biomass in April and October were measured by clipping using the 50 cm × 50 cm quadrates, with six replications conducted in 2005 and eight replications conducted in 2006. All the samples were oven-dried at 70 ˚C for 72 hours and weighed. Each dried sample was analyzed for the total N content with an N/C analyzer (SUMIGRAPH NC-1000, Sumika Chemical Analysis Service, Ltd., Osaka, Japan).

The N uptake in aboveground biomass was estimated as an increment in biomass N during each season. Apparent N recovery (ANR) (kg N (kg N)$^{-1}$) was estimated using the following equation (Kaffka & Kanneganti 1996):

$$ANR = (CN_i - CN_c) / N_i \quad (3)$$

where CN_i and CN_c are the annual N uptake in aboveground biomass in treated and control plots, and N_i is the total amount of fertilizer and manure N applied to the treatment i.

2.7. N_2O and NO fluxes

N_2O and NO fluxes from the soil to the atmosphere were measured by the static closed chamber method on the fertilizer, manure, control and bare plots (Shimizu et al. 2009). The flux measurements were conducted in 2 - 28 day intervals during the crop growing season and 10 - 30 day intervals during the non growing season and between 8:00 and 11:00 h each measuring day to minimize the effect of diurnal temperature variation. The stainless steel chambers were 40 cm in diameter and 30 cm high in the fertilizer and manure plots, and 20 cm in diameter and 25 cm high in the control and bare plots. The chambers were placed directly into the soil to a depth of about 3 cm, 12 hours before the measurement of each subplot, and contained no aboveground biomass in the fertilizer, manure and control plots. Before closing the chamber, a 250 ml gas sample from the headspace of each chamber was extracted into a Tedlar bag for NO analysis, and a 20 ml gas sample was injected into an evacuated vial (10 ml) for N_2O analysis. This measurement was regarded as time 0 min. After 20 min or 30 min under a closed-chamber condition, 250 ml of the headspace gas sample was extracted from each chamber into a bag, and 20 ml was injected into a vial. From these bag samples, NO gas concentrations were determined in a laboratory within 16 hours using a chemiluminescence nitrogen oxides analyzer (Model 265P, Kimoto Electric, Osaka,

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Japan). N_2O gas concentrations were determined in a laboratory within 1 month using a gas chromatograph (Model GC-14B, Shimadzu, Kyoto, Japan) fitted with an electron capture detector.

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2.8. Environmental variables

Daily precipitation and daily maximum depths of snow were obtained at the closest AMeDAS (Automated Meteorological Data Acquisition System) station by the Japan Meteorological Agency. Air temperature and soil temperature at a 5 cm depth were measured at the same time with the flux measurements using a thermistor thermometer (CT220, CUSTOM, Tokyo, Japan), and soil moisture content at a 0 to 6 cm depth was measured using the Frequency Domain Reflectometry (FDR) method (DIK-311A, Daiki, Saitama, Japan). Soil core samples (14 cm diameter, 13 cm height) were collected in April 2007, and calibration curves were made to calculate water-filled pore space (WFPS) from the FDR device reading (m^3 m^{-3}) and percent total porosity (Linn & Doran, 1984). The percent total porosity was measured using a 100 ml soil core collected in April 2007 and was regarded as constant throughout the study period because of no tillage.

2.9. Statistical analyses

Statistical analyses were performed with Prism ver. 4 (GtaphPad Software Inc., San

Diego, CA, USA) and Excel Statistics version 5.0 (Esumi, Tokyo, Japan). The differences in soil temperature, WFPS and N_2O flux among the plots were analyzed with a repeated-measures one-way analysis of variance (ANOVA) and Bonferroni test. The differences in GMs, GMl, GMm, total mineral N supply, N uptake in aboveground biomass and cumulative N_2O flux among the seasons were analyzed with a repeated-measures analysis of variance (ANOVA) and Bonferroni test. The differences in the annual values between the years and the plots were analyzed with a two-way factorial ANOVA and Bonferroni test.

Uncertainties in the N uptake in aboveground biomass and the cumulative N_2O flux were calculated using the following equation:

Uncertainty (%) = {(two-sided 95% confidence interval) / 2} / Means × 100 (4)

The two-sided 95% confidence interval was calculated by using the following equation:

Two-sided 95% confidence interval = t (d.f., 0.05) × standard error ×2 (5)

where d.f. is the degree of freedom and t (d.f., 0.05) is the t value at 5% significant level with a two-sided alternative.

3. Results

3.1. Environmental variables

Daily precipitation and daily maximum depths of snow are shown in Fig. 2a. Annual precipitations were 1152 mm and 1047 mm from mid April 2005 to the beginning of April 2006 and from mid April 2006 to the beginning of April 2007, respectively. These values are smaller than the 12-year average (1365 ± 215 mm), but drought did not occur.

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The soil temperature at 5 cm depth increased from April, reaching the maximum temperature from July through August, and then decreased gradually (Fig. 2b). The soil temperature was around 0 ˚C from December to March (Fig. 2b). There was no difference in soil temperature between the fertilizer and manure plots, but soil temperature was higher in the control plot than in the fertilizer and manure plots ($P < 0.05$). Soil temperature in the bare plot was the highest of all plots ($P < 0.01$).

The range of WFPS in a 0 to 6 cm depth in 2005 was 67% - 89% in the fertilizer plot, 67% - 90% in the manure plot, 72% - 89% in the control plot and 83% - 94% in the bare plot (Fig. 2c). The WFPS in the fertilizer, manure, control and bare plots decreased to 47%, 48%, 46% and 65%, respectively in mid August 2007, and then increased with an increase in precipitation. There was no difference in the WFPS between the fertilizer and manure plots; however, it was greater in the control plot than in the fertilizer and manure plots ($P < 0.01$). Furthermore, the WFPS in the bare plot

was found to be the greatest of all the plots ($P < 0.01$).

3.2. N deposition

The annual N deposition was 15 kg N ha^{-1} $year^{-1}$ in 2005 and 4 kg N ha^{-1} $year^{-1}$ in 2006 (Table 4).

3.3. Gross N mineralization

There was a significant difference in the GMs among the seasons ($P < 0.01$). The GMs in the G2 and G3 seasons ranged from 115 to 161 kg N ha^{-1} $period^{-1}$, and was greater than those in the other seasons (Table 4). There was no significant difference in the GMs between the G2 and G3 seasons. The GMs in the G1 season ranged from 59 to 67 kg N ha^{-1} $period^{-1}$, and the GMs in the NG season ranged from 31 to 37 kg N ha^{-1} $period^{-1}$ (Table 4). The annual GMs in 2005 and 2006 were 367 and 331 kg N ha^{-1} $year^{-1}$ for the fertilizer plot, 369 and 336 kg N ha^{-1} $year^{-1}$ for the manure plot, 382 and 348 kg N ha^{-1} $year^{-1}$ for the control plot and 384 and 356 kg N ha^{-1} $year^{-1}$ for the bare plot, respectively (Table 4). The annual GMs value was greater in 2006 than in 2007, and was greater in the bare plot than in the fertilizer plot ($P < 0.01$).

The GMl in the G1 season ranged from 68 to 104 kg N ha^{-1} $period^{-1}$, and was greater than that in other seasons ($P < 0.01$) (Table 4). In the G1 season, the GMl for each plot was greater than the GMs (Table 4). The annual GMl values in 2005 and 2006 were 145

and 126 kg N ha^{-1} $year^{-1}$ for the fertilizer plot, 165 and 122 kg N ha^{-1} $year^{-1}$ for the manure plot, and 108 and 94 kg N ha^{-1} $year^{-1}$ for the control plot, respectively (Table 4).

The GMm value was greater in the G2 and G3 seasons than in the NG season (Table 4). The annual GMm values in 2005 and 2006 were 88 and 96 kg N ha^{-1} $year^{-1}$, respectively (Table 4).

3.4. N uptake in aboveground biomass

Table 5 shows the N uptake in aboveground biomass. There was a significant difference among the seasons ($P < 0.01$), and the N uptake in aboveground biomass in the G1 season was the greatest, followed by those in the G2, G3 and NG seasons. The negative values for the NG season, which were a result of the decrease in aboveground biomass, suggested that the mineral N could have been supplied to soil by the dead aboveground biomass through the decomposition process. The annual values of N uptake in aboveground biomass in 2005 and 2006 were 249 and 219 kg N ha^{-1} $year^{-1}$ for the fertilizer plot, 215 and 201 kg N ha^{-1} $year^{-1}$ for the manure plot, and 109 and 134 kg N ha^{-1} $year^{-1}$ for the control plot (Table 5). The annual N uptake in aboveground biomass was greater in the fertilizer and manure plots than in the control plot ($P < 0.01$), and there were no significant differences among the years.

The ANR in 2005 and 2006 was 85% and 46% for the fertilizer plot, and 29% and

15% for the manure plot, respectively.

3.5. N_2O flux

N_2O fluxes in the fertilizer and manure plots increased after the application of fertilizer or manure. These remained at a higher level than in the control plot until the beginning of September (Fig. 2). The N_2O flux in the fertilizer plot increased after fertilization in May and July to 37 and 314 μg N m^{-2} h^{-1} in 2005, and to 152 and 211 μg N m^{-2} h^{-1} in 2006 (Fig. 2). In the manure plot, however, it reached a high peak after the application of manure and fertilizer in May (276 and 1290 μg N m^{-2} h^{-1} in 2005 and 2006, respectively) (Fig. 2). The N_2O flux in the control plot gradually increased with an increase in soil temperature, and reached the maximum values of 50 μg N m^{-2} h^{-1} in August 2005 and 66 μg N m^{-2} h^{-1} in July 2006. The N_2O flux in the bare plot also increased with an increase in soil temperature, but the maximum value (347 μg N m^{-2} h^{-1} in August 2005 and 403 μg N m^{-2} h^{-1} in July 2006) was higher than that in the control plot. The N_2O fluxes in the fertilizer, manure and bare plots were significantly higher than that in the control plot ($P < 0.01$), but there were no significant differences among the fertilizer, manure and bare plots.

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The relationship between the N_2O fluxes and N_2O-N/NO-N ratio is shown in Fig. 3. The N_2O flux increased with an increase in the N_2O-N/NO-N ratio. Bouwman (1990)

summarized the results reported by Anderson and Levine (1986) and Lipschultz et al. (1981), and concluded that the N_2O-N/NO-N ratio was <1 during nitrification and >100 during denitrification. Therefore, N_2O could have been produced mainly by denitrification in our study site.

The cumulative N_2O flux for all seasons is shown in Table 6. The cumulative N_2O flux was higher in the G2 season than all other seasons except in the manure plot in 2006, but there was a significant difference only between the G2 and NG seasons. In each season, the cumulative N_2O flux was compared to the cumulative CO_2 flux (Fig. 4), which was reported by Shimizu et al. (2009). The cumulative N_2O flux in each plot had a positive correlation with the cumulative CO_2 flux (Fertilizer plot; Cumulative N_2O flux = 0.36 × cumulative CO_2 flux – 0.23; $R^2 = 0.48$; $P < 0.05$, Manure plot; Cumulative N_2O flux = 0.38 × cumulative CO_2 flux - 0.17; $R^2 = 0.33$; $P = 0.08$, Control plot; Cumulative N_2O flux = 0.08 × cumulative CO_2 flux - 0.05; $R^2 = 0.76$; $P < 0.01$, Bare plot; Cumulative N_2O flux = 1.30 × cumulative CO_2 flux - 0.39; $R^2 = 0.72$; $P < 0.01$) (Fig. 4).

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The annual N_2O fluxes in 2005 and 2006 were 2.8±0.7 and 3.0±0.8 kg N ha^{-1} $year^{-1}$ for the fertilizer plot, 3.6±1.3 and 4.9±3.0 kg N ha^{-1} $year^{-1}$ for the manure plot, 0.7±0.5 and 0.6±0.3 kg N ha^{-1} $year^{-1}$ for the control plot, and 4.2±3.3 and 3.3±2.1 kg N ha^{-1}

year^{-1} for the bare plot (Table 6). There was no significant difference in the annual N_2O flux among the years, and the annual N_2O flux was significantly higher in the fertilizer, manure and bare plots than in the control plot (Fertilizer plot: $P < 0.05$, Manure plot: $P < 0.01$).

4. Discussion

4.1. Relationship between mineral N supply and N uptake in aboveground biomass

The sources of mineral N supply for plant growth are considered to be N deposition, chemical and manure fertilizer application, biological N_2 fixation, mineralization from soil organic matter (GMs), root-litter (GMl) and manure (GMm). Among these components, N deposition, biological N_2 fixation, chemical fertilizer application and GMm are all external mineral N supply. In this study, the biological N_2 fixation was assumed to be negligible for the estimation of total and external mineral N supplies, because of a low percentage of legumes (Table 1). The plots of the N uptake in aboveground biomass against the external mineral N supply during each growing season showed that the N uptake in aboveground biomass was equivalent to or greater than the external mineral N supply except for the manure plot in the G2 season (Fig. 5a). On the other hand, the plots of the aboveground N uptake against total mineral N supply

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showed that the total mineral N supply exceeded the N uptake in aboveground biomass, and there was a significant linear correlation between them (N uptake in aboveground biomass = 0.58 × total mineral N supply – 47; R^2 = 0.70; P < 0.01, Eq. (6)). (Fig.5b). This relationship suggests that the rate of plant mineral N uptake to the total mineral N supply would be 58%. This value is comparable to the ANR for the fertilizer plot (85% in 2005 and 46% in 2006). The ANR of this study for the fertilizer plot was consistent with the previously reported values for ryegrass (60%; Dam Kofoed 1981) and orchard grass (69%; Kaffka & Kanneganti 1996). The y-interception of the equation (Eq. (6)) was negative value. This can be accounted for by the belowground N uptake and microbial immobilization of N.

The annual GMs was 2.2 to 3.6 times larger than the annual GMl. However, the GMl in the G1 season, which accounted for 61 to 83% of the annual GMl, was 1.1 to 1.7 times larger than the GMs in the season. This indicates that decomposition of fine roots plays an important role in contributing to the mineral N supply especially for the first crop. Some reports suggest significance of fine roots as a source of belowground litter because of their abundance and rapid turnover (Gholz et al. 1986; Gill & Jackson 2000; Gill et al. 2002). Steinaker and Wilson (2005) reported that fine roots accounted for about 90% of the total litter production in natural grassland in Saskatchewan,

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Canada. Parton et al. (2007) and Seastedt et al. (1991) showed that mineral N was proportionally released with decomposition of root litters. Fitzhugh et al. (2001) suggested that an increase in the mortality of fine roots due to soil freezing in boreal forests was likely to be an important source of mobile N, based on the observations using mini-rhizotron. They reported that the mortality of fine roots was concentrated in the winter months as concrete frost permeated the rooting zone (Groffman et al. 2001; Ruess et al. 1998; Tierney et al. 2001). This was the same for our study site too, as the soil is frozen up to about 20 centimeters in the winter (Shimizu et al. 2009), and the mortality of fine roots in the winter could be a main source of belowground litter.

In this study, GM was estimated by dividing heterotrophic respiration by a C:N ratio (Eq. (3)) and it was important specifically as the source of mineral N supply. GMl was a major source of mineral N supply especially in the spring. In the previous report (Shimizu et al. 2009), although the source of the increment of CO_2 flux in the spring was argued as not only root-litter decomposition but also root respiration, the results of this study revealed that root-litter decomposition could possibly be the main source of an increment of CO_2 fluxes in the spring.

4.2. Factors controlling N_2O emission

A significant positive correlation between the cumulative N_2O and CO_2 fluxes in the

bare plot (Fig. 4) indicates that the soil organic matter decomposition contributed to the N_2O flux. This relationship was found also in the three other plots of fertilizer, manure and control. The slope of the regression equation for cumulative N_2O and CO_2 fluxes was largest in the bare plot, because CO_2 fluxes in the other plots include root respiration. However, the slope was higher in the fertilizer and manure plots than in the control plot due to an increase in N_2O fluxes with the application of chemical fertilizer and manure (Bouwman 1996).

Fig. 6a shows the relationship between the cumulative N_2O flux and the total mineral N supply for each season, which was estimated as the sum of N deposition, the chemical fertilizer application, GMm, GMs and GMl. There was a significant positive correlation between the cumulative N_2O flux and the total mineral N supply (Cumulative N_2O flux = 0.0059 × (total mineral N supply) – 0.103; $R^2 = 0.37$; $P < 0.01$, Eq. (7)). However, the rate of the cumulative N_2O flux to the total mineral N supply was higher in the bare plot than in the other three plots including plant growth (Fig. 6a). This is because of the absence of plant N uptake in the bare plot while the N uptake in aboveground biomass accounted for 58% of the total mineral N supply in the other three plots (Fig. 5b, Eq. (6)). When the cumulative N_2O flux for each season was plotted against the mineral N surplus, which was estimated as the difference between the total

mineral N supply and the aboveground N uptake (Fig. 6b), a much better significant positive correlation was found (Cumulative N_2O flux = 0.0120 × (mineral N surplus) – 0.537; R^2 = 0.50; P < 0.01, Eq. (8)). The slope of the regression equation showed that the N_2O emission factor against the mineral N surplus was 1.20%, and a negative value of the y-interception showed immobilization of mineral N (Schimel et al. 1985). Kaiser and Ruser (2000) reported that the annual N_2O emission measured in the arable land soils in Germany had an even stronger correlation with the N balance estimated as the difference between the N fertilization and the crop N uptake than with N fertilization. Katayanagi et al. (2008) also found that N_2O emissions conducted on the same field in this study including cornfields had a positive correlation with the N surplus which was estimated as the difference of all total input (application of fertilizer, manure and slurry, and livestock excreta during grazing) and total output (yield and grazed grass). However, those reports did not take into consideration the contribution of N mineralization to N_2O emission. Kaiser and Ruser (2000) suggested the contribution of gross N mineralization to N_2O emissions from the result, that unfertilized soil produced an N yield of 60 kg N ha^{-1} $year^{-1}$ and an N_2O emission of 2 kg N ha^{-1} $year^{-1}$. In this study, the sum of GMs and GMl in the fertilizer plot in 2005 and 2006 were 512 and 457 kg N ha^{-1} $year^{-1}$, respectively. These values are significantly larger than the application rate of chemical

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fertilizer (164 and 183 kg N ha^{-1} $year^{-1}$). Mu et al. (2008) reported that the sum of chemical N fertilizer and GMs correlated with the cumulative N_2O fluxes better than only the chemical N fertilizer. Thus, not only amounts of N input and crop N uptake, but also the amount of gross N mineralization is indispensable in predicting the amount of N_2O emission.

Despite the significant positive relationship between the cumulative N_2O flux and mineral N surplus, there was variability in the relationship (Fig. 6a). Therefore, we conducted multiple regression analysis to explain the variability. We selected the N_2O emission factor against the mineral N surplus for each season as an objective variable, and mean precipitation, mean daily air temperature and mineral surplus N as explanatory variables. The results showed that the emission factor against mineral N surplus was significantly correlated with the mean precipitation (Cumulative N_2O / mineral N surplus × 100 = 0.195 × mean precipitation - 0.055; $R^2 = 0.21$; $P < 0.01$) (Fig. 7). This suggests that an N_2O emission from surplus mineral N increases with an increase in precipitation. In this study, the predominant source of N_2O appeared to be denitrification (Fig. 3), and high precipitation should have enhanced denitrification. Mori et al. (2008) also reported that high precipitation enhanced N_2O production from denitrification. These results indicated that an optimum N input aiming at reducing the

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N surplus is required to minimize the environmental risk associated with N_2O emission.

5. Conclusion

Together with chemical fertilizer and manure, mineralization from soil organic matter and root-litter were the major sources for plant growth and N_2O emission in the managed grassland. Mineral N surplus, which was estimated as the difference between the total mineral N supply and N uptake in aboveground biomass, was a better indicator of N_2O emission than the total mineral N supply. The N_2O emission factor against the mineral N surplus was estimated to be 1.20%, and the emission factor in each season increased with an increase in precipitation. These results also indicated that the reduction in mineral N surplus would be able to mitigate N_2O emission from the grassland.

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Figure captions

Fig. 1. Location of the study site (a) and the layout of the experimental plots (b)

Fig. 2. Seasonal variations in meteorological variables and the N_2O and NO flux; Daily precipitation and daily maximum snow depth (a), soil temperature at a 5 cm depth (b), water-filled pore space (WFPS) (c), N_2O flux (d) and NO flux (e). Data of soil temperature, WFPS, N_2O flux and NO flux represent means ± S.D. (n = 6). The arrows indicate the timing of fertilizer or manure application.

Fig. 3. The relationship between N_2O fluxes and the N_2O-N/NO-N rate.

Fig. 4. The cumulative N_2O flux for each seasons (G1, G2, G3 and non growing season (NG)) compared to the cumulative CO_2 flux. G1 is the period from the beginning of the growing season to the first crop harvest, G2 is the period from the first harvest to the second harvest and G3 is the period from the second harvest to the end of the growing season. Data of the cumulative N_2O flux represent means ± (uncertainties / 100 × means). The cumulative CO_2 flux was referred to Shimizu et al. (2009).

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Fig. 5. N uptake in aboveground biomass during each growing season (G1, G2, G3) compared to the external mineral N supply (a) and total mineral N supply (b). The external N

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supply is composed of N deposition, chemical fertilizer application and the gross N mineralization of applied manure. The total mineral N supply is composed of the external N supply, gross N mineralization of soil and gross mineralization of root-litter. G1 is the period from the beginning of the growing season to the first crop harvest, G2 is the period from the first harvest to the second harvest and G3 is the period from the second harvest to the end of the growing season. The data of N uptake in aboveground biomass represent means ± (uncertainties / 100 × means). The dashed-line indicates a regression model for all values, N uptake in aboveground biomass = 0.58 × total mineral N supply – 47; $R^2 = 0.68$; $P < 0.01$.

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Fig. 6. The cumulative N_2O flux for each season (G1, G2, G3 and non growing season (NG)) compared to the total mineral N supply (a) and the mineral N surplus (b). The total mineral N supply is composed of the external N supply (N deposition, chemical fertilizer application and gross N mineralization of applied manure), gross N mineralization of soil and gross mineralization of root-litter. The mineral N surplus was estimated as the difference between the mineral N pool and N uptake in aboveground biomass. G1 is the period from the beginning of the growing season to the first crop harvest, G2 is the period from the first harvest to the second harvest and G3 is the period from the second harvest to the end of the growing season. The data of cumulative N_2O flux represent means ± (uncertainties / 100 × means). The regression model shown in Fig. 6a is for all values, Cumulative N_2O flux =

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0.0059 × (total mineral N supply) – 0.103; R^2 = 0.37; P < 0.01. The regression model shown in Fig. 6b is for all values, Cumulative N_2O flux = 0.0120 × (mineral N surplus) – 0.537; R^2 = 0.50; P < 0.01.

Fig. 7. The N_2O emission factor to mineral N surplus for each season (G1, G2, G3 and non growing season (NG)) compared to mean precipitation. The mineral N surplus was estimated as the difference between the mineral N pool and N uptake in aboveground biomass. G1 is the period from the beginning of the growing season to the first crop harvest, G2 is the period from the first harvest to the second harvest and G3 is the period from the second harvest to the end of the growing season. The data of N_2O emission factor to mineral N surplus represent means ± (uncertainties / 100 × means). The regression line is for all values, Cumulative N_2O / mineral N surplus × 100 = 0.195 × mean precipitation - 0.055; R^2 = 0.21; P < 0.01.

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Tables

Table 1

The botanical composition of grassland (dry matter weight percentage) on the study site.

	Gramineae			Leguminosae	Broad-leaved weed
	Reed canarygrass	Meadow foxtail	Others	White clover	
Fertilizer plot	77.9	14.4	7.7	0.0	0.0
Manure plot	49.8	48.7	1.2	0.0	0.4
Control plot	54.8	41.9	0.3	2.9	0.1

Table 2

The applied date and the application rates of chemical fertilizer and manure.

Treatment	Date	Fertilizer type	Application rates (kg ha^{-1})			
			T-C	T-N	P_2O_5	K_2O
Fertilizer	May 2005	Chemical fertilizer[a]	0	103	23	168
	July 2005	Chemical fertilizer[a]	0	61	23	97
	May 2006	Chemical fertilizer[a]	0	124	50	177
	July 2006	Chemical fertilizer[a]	0	59	18	97
Manure	May 2005	Manure[b]	5833	236	191	266
	July 2005	Chemical fertilizer[a]	0	133	7	70
	May 2006	Manure[b]	5958	310	212	167
	May 2006	Chemical fertilizer[a]	0	71	0	33
	July 2006	Chemical fertilizer[a]	0	59	6	97

[a] Chemical fertilizer is comprised of ammonium sulfate, ammonium phosphate, potassium sulfate and potassium magnesium sulfate.

[b] Beef cattle manure with bedding litter (bark) is applied in manure plot.

Table 3

Mineralization rates from the cattle manure.

Year	Mineralization rate[a] from						Estimated supply rate ($kg\ ha^{-1}$)		
	Manure applied in 2005			Manure applied in 2006					
	T-N	P_2O_5	K_2O	T-N	P_2O_5	K_2O	T-N	P_2O_5	K_2O
2005	13%	20%	70%	-	-	-	31	38	186
2006	7%	10%	10%	13%	20%	70%	57	62	144

[a] The N mineralization rates were estimated based on the Uchida's model (Shiga et al. 1985) which was developed in Japan and were 13.2% and 7.0% in the first and second years after application, respectively. The P and K mineralization rates from the manure were estimated based on the handbook of animal waste management and utilization in Hokkaido 2004 (Hokkaido Prefectural Experiment Stations and Hokkaido Animal Research Center 2004).

Table 4

N deposition, gross N mineralization of soil (GMs), root-litter (GMl) and manure (GMm), and mineral N pool during each growing season (G1, G2 and G3) and non-growing season (NG) (kg N ha^{-1} $period^{-1}$). G1 is the period from the beginning of the growing season to the first crop harvest, G2 is the period from the first harvest to the second harvest and G3 is the period from the second harvest to the end of the growing season.

	2005				2006				2005	2006
	G1	G2	G3	NG	G1	G2	G3	NG		
N deposition	12	2	1	1	1	1	2	1	15	4
Gross N mineralization of soil										
Fertilizer plot	60	115	155	37	62	122	115	32	367	331
Manure plot	62	115	155	36	59	129	116	32	369	336
Control plot	64	120	161	37	61	132	121	34	382	348
Bare plot	67	132	150	34	67	153	104	31	384	356
Gross N mineralization of root-litter										
Fertilizer plot	88	44	0	13	104	10	0	13	145	126
Manure plot	103	51	0	11	101	11	0	10	165	122
Control plot	68	27	0	13	75	6	0	13	108	94
Gross N mineralization of manure	13	30	39	6	13	43	34	6	88	96

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Table 5

N uptake in aboveground biomass during each growing season (G1, G2 and G3) and non-growing season (NG) (kg N ha^{-1} $period^{-1}$). G1 is the period from the beginning of the growing season to the first crop harvest, G2 is the period from the first harvest to the second harvest and G3 is the period from the second harvest to the end of the growing season.

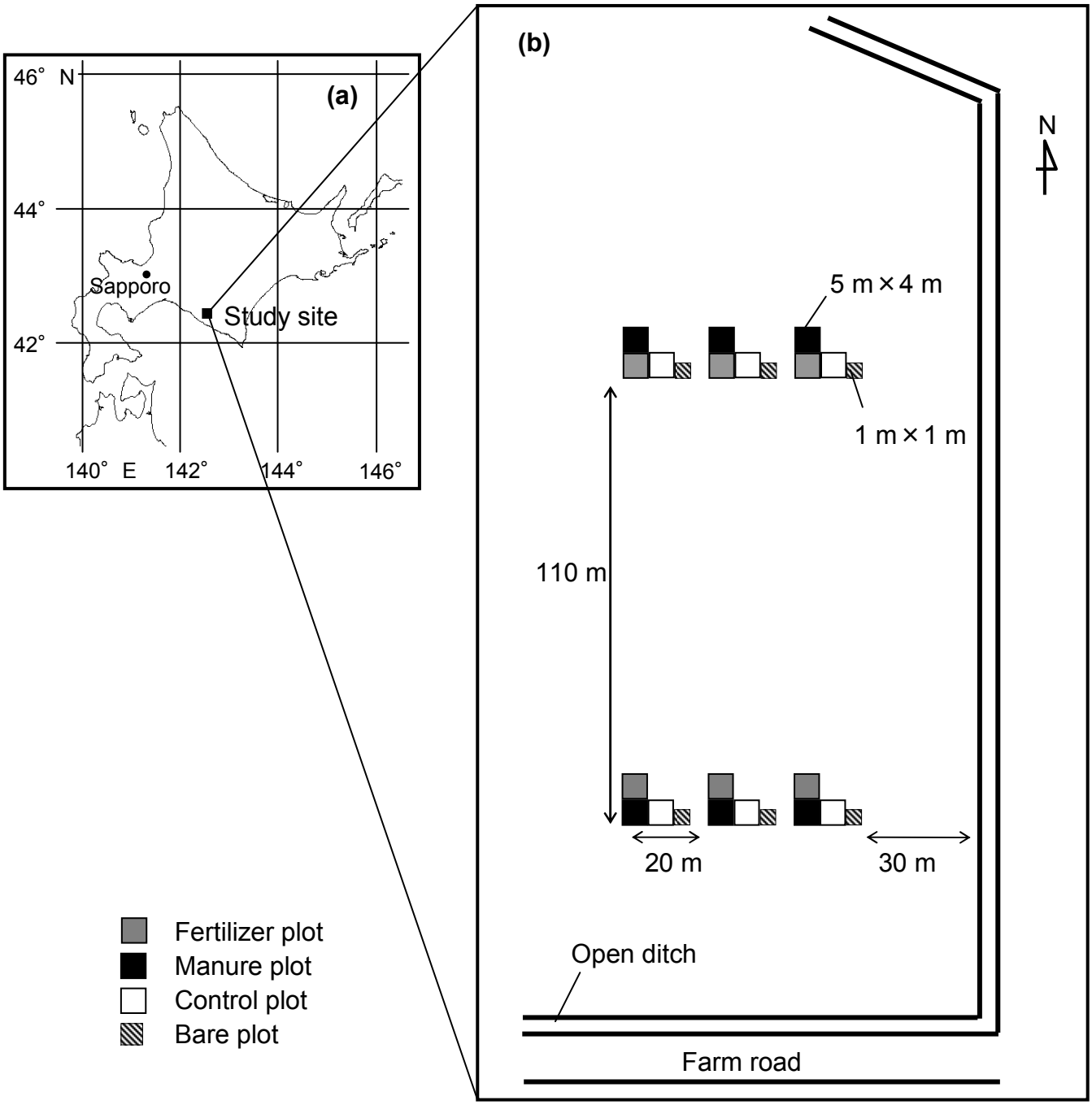
	2005				2006				2005	2006
	G1	G2	G3	NG	G1	G2	G3	NG		
Fertilizer plot	138	94	20	-2	137	70	22	-9	249	219
Manure plot	57	133	36	-10	122	52	30	-3	215	201
Control plot	42	55	24	-11	85	41	10	-1	109	134

Table 6

The cumulative N_2O flux during each growing season (G1, G2 and G3) and non-growing season (NG) (kg N ha^{-1} $period^{-1}$). G1 is the period from the beginning of the growing season to the first crop harvest, G2 is the period from the first harvest to the second harvest and G3 is the period from the second harvest to the end of the growing season.

	2005				2006				2005	2006
	G1	G2	G3	NG	G1	G2	G3	NG		
Fertilizer plot	0.2	1.8	0.8	0.1	1.0	1.3	0.6	0.2	2.8	3.0
	(0.1)	(0.4)	(0.3)	(0.0)	(1.0)	(0.5)	(0.3)	(0.1)	(0.7)	(0.8)
Manure plot	0.5	1.9	1.2	0.0	2.5	1.9	0.5	0.1	3.6	4.9
	(0.3)	(0.9)	(0.7)	(0.0)	(2.1)	(1.6)	(0.3)	(0.1)	(1.3)	(3.0)
Control plot	0.1	0.2	0.3	0.0	0.1	0.3	0.2	0.1	0.7	0.6
	(0.0)	(0.1)	(0.4)	(0.0)	(0.1)	(0.3)	(0.1)	(0.1)	(0.5)	(0.3)
Bare plot	0.2	1.4	2.5	0.1	0.8	1.4	0.7	0.4	4.2	3.3
	(0.1)	(0.8)	(2.9)	(0.0)	(0.6)	(1.8)	(0.6)	(0.5)	(3.3)	(2.1)

Data represent means ± (uncertainties / 100 × means).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(b)
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Open ditch
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Fertilizer plot
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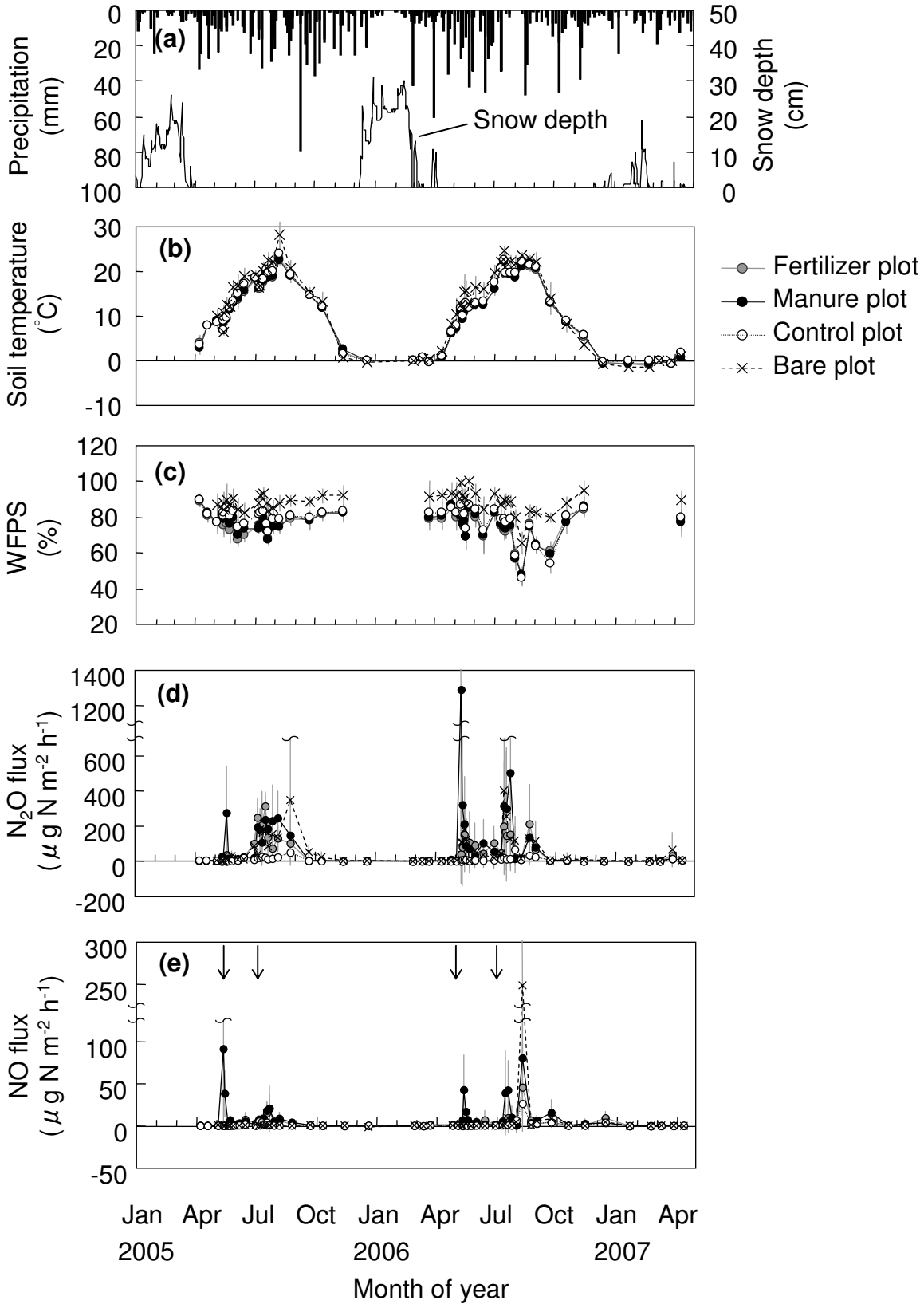
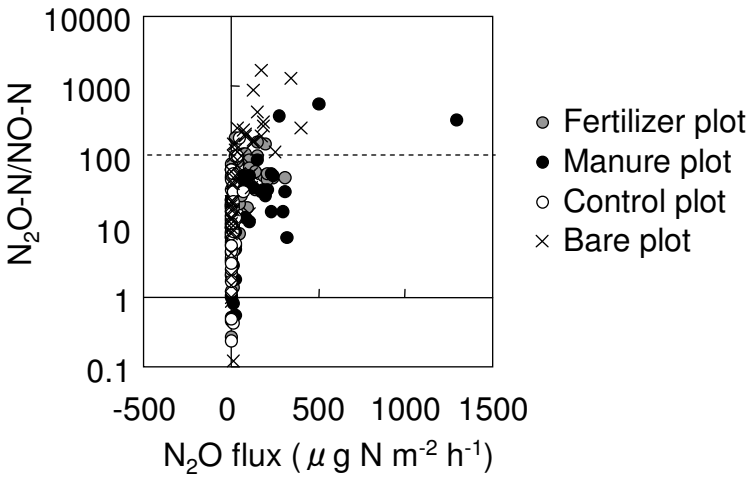
Control plot
Bare plot


(a)
Precipitation (mm)
Snow depth (cm)
Snow depth
(b)
Soil temperature (°C)
Fertilizer plot
Manure plot
Control plot
Bare plot
(c)
WFPS (%)
(d)
N2O flux (μg N m-2 h-1)
(e)
NO flux (μg N m-2 h-1)
Jan Apr Jul Oct Jan Apr Jul Oct Jan Apr
2005 2006 2007
Month of year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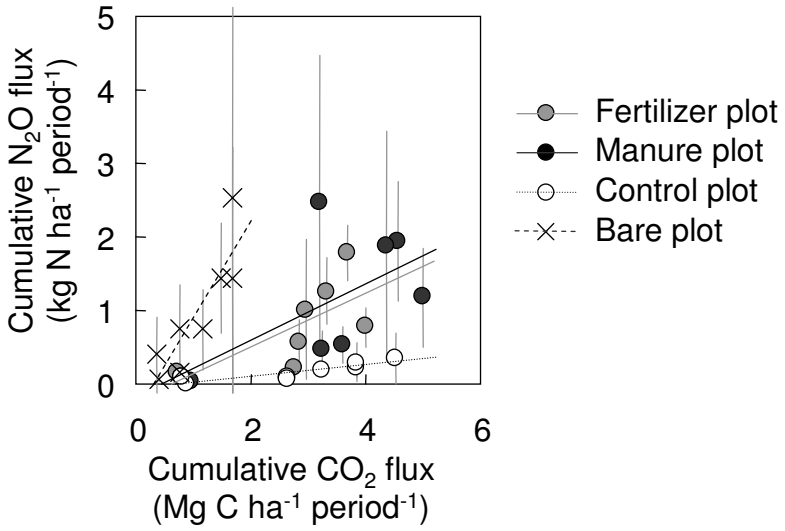
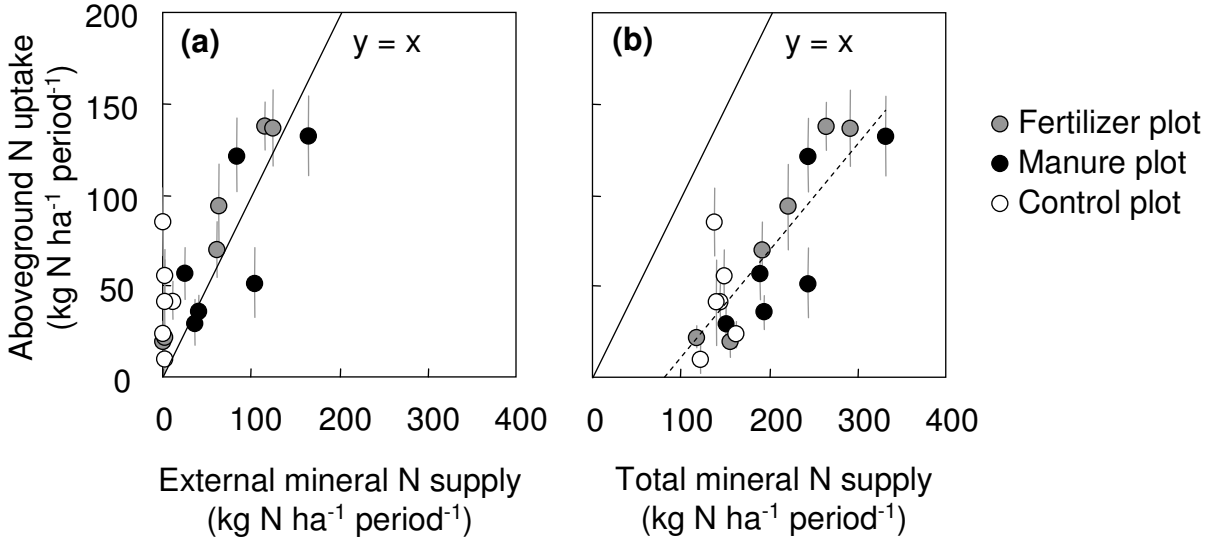
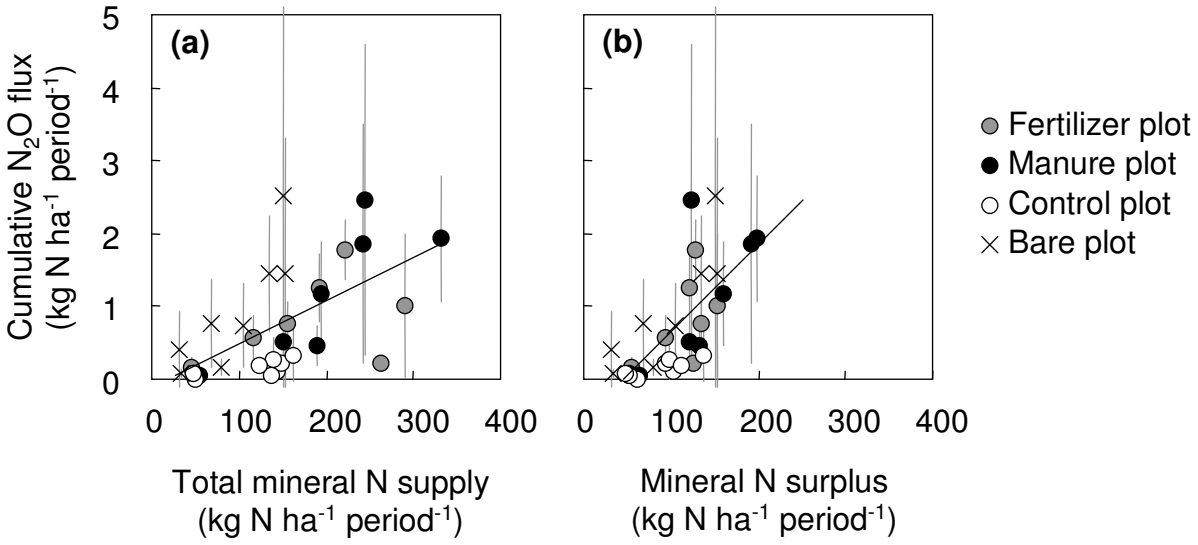
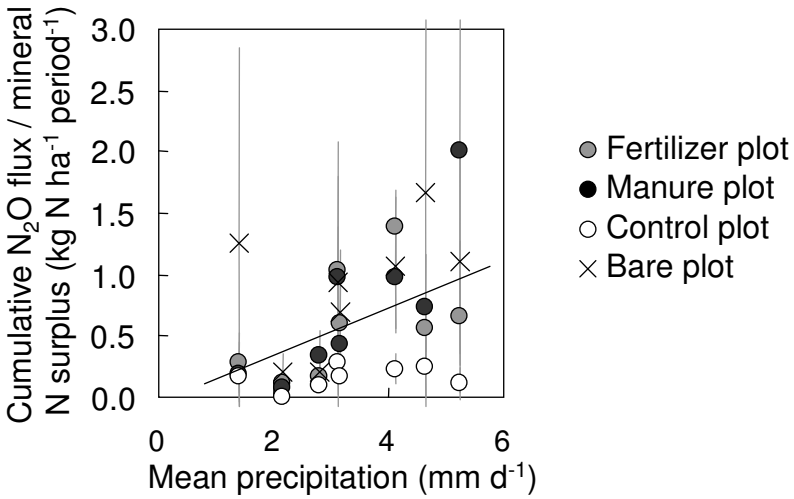
N2O-N/NO-N
N2O flux (μg N m-2 h-1)
Fertilizer plot
Manure plot
Control plot
Bare plot


Cumulative N_2O flux
(kg N ha^{-1} period^{-1})
0
1
2
3
4
5
0
2
4
6
Cumulative CO_2 flux
(Mg C ha^{-1} period^{-1})
Fertilizer plot
Manure plot
Control plot
Bare plot


(a)
y = x
(b)
y = x
Aboveground N uptake
(kg N ha-1 period-1)
0
50
100
150
200
0
100
200
300
400
External mineral N supply
(kg N ha-1 period-1)
Total mineral N supply
(kg N ha-1 period-1)
Fertilizer plot
Manure plot
Control plot


(a)
(b)
Cumulative N_2O flux
(kg N ha^{-1} period^{-1})
0
1
2
3
4
5
0
100
200
300
400
Total mineral N supply
(kg N ha^{-1} period^{-1})
Mineral N surplus
(kg N ha^{-1} period^{-1})
Fertilizer plot
Manure plot
Control plot
Bare plot


Cumulative N_2O flux / mineral N surplus (kg N ha^{-1} period^{-1})
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
2
4
6
Mean precipitation (mm d^{-1})
Fertilizer plot
Manure plot
Control plot
Bare plot