Title: No significant difference in N_2O emission, fertilizer-induced N_2O emission factor, and CH_4 absorption between anaerobically digested cattle slurry and chemical fertilizer applied-timothy (*Phleum pratense* L.) sward in central Hokkaido, Japan

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Type of contribution: Full-length paper

Division of the manuscript: 8. Environment

Short running title (less than 40 characters): N_2O and CH_4 emission from a sward in Hokkaido

Abstract

Nitrous oxide (N_2O) and methane (CH_4) fluxes from a fertilized timothy (*Phleum pratense* L.) sward in northern island Japan were measured throughout two years, with a randomized block design field. Objectives of this study are to obtain annual N_2O and CH_4 emission rates and to elucidate the effect of the applied material (control (no nitrogen), anaerobically digested cattle slurry (ADCS), or chemical fertilizer (CF)) and the application season (autumn or spring) on the annual N_2O emission, fertilizer-induced N_2O emission factor (EF), and the annual CH_4 absorption. Ammonium sulfate was applied to CF plots at the same application rate of NH_4-N to ADCS plots. Three way ANOVA was used to examine the significance of factors (the applied material, the application season, the year). ANOVA for the annual N_2O emission rates showed significance of the applied material ($P = 0.042$). The annual N_2O emission rate from control plots (0.398 kg N_2O-N ha^{-1} y^{-1}) was significantly lower than that from ADCS plots (0.708 kg N_2O-N ha^{-1} y^{-1}) and CF plots (0.636 kg N_2O-N ha^{-1} y^{-1}). There was no significant difference in the annual N_2O emission rate between ADCS and CF plots. ANOVA for the EFs showed insignificance of all factors ($P > 0.05$). Total mean ± standard error of mean of EFs (fertilizer-induced N_2O-N emission / total applied N) was 0.0024 ± 0.0007 (kg N_2O-N [kg N]$^{-1}$), which is similar to the reported EF (0.0032 ± 0.0013) for well-drained uplands in Japan. ANOVA for CH_4 absorption rates showed significance of the year ($P = 0.014$). CH_4 absorption rate in the first year (3.28 kg CH_4 ha^{-1} y^{-1}) was higher than that in the second year (2.31 kg CH_4 ha^{-1} y^{-1}), probably due to lower precipitation in the first year. In conclusion, under the same application rate of NH_4-N, the difference in the applied materials (ADCS or CF) and the application season (autumn or spring) led no significant difference in N_2O emission, fertilizer-induced N_2O EF, and CH_4 absorption.

Key words: emission factor, methane, nitrous oxide, slurry, timothy

INTRODUCTION

More efficient use and appropriate management of livestock excreta is required in the context of

nutrient utilization and demand of reducing environmental pollution (e.g., nitrogen (N) leaching, emission of greenhouse and other pollutant gases). Management system of livestock excreta can be classified roughly into two groups, aerobic treatment (e.g., composting, purification in aeration system) and anaerobic treatment (e.g., digestion treatment).

Anaerobic digestion of livestock slurry by biogas plant provides valuable energy source of methane (CH_4) and reduces CH_4 emission of stored slurry (Clemens *et al*. 2006). The product after anaerobic digestion of cattle excreta is the so-called "anaerobically digested cattle slurry (ADCS)", which contains nutrient element essential for plant and crop growth (Matsunaka *et al*. 2002). Moreover, the concentration of bad odor and pathogenic bacteria in ADCS is lower than that in un-digested slurry (Pain *et al*. 1990; Saxena *et al*. 1989). In Japan, biogas plant using stock farming as feedstock has not widely introduced because of high cost of building (Tsukahara *et al*. 2005). However, understanding nutrient effect and environmental pollution followed by ADCS application to upland and grassland is important for promoting biogas plant system.

Matsunaka *et al*. (2003) revealed that the nutrient effect of ADCS as N on the dry matter production of grass was substantially same to the conventional slurry or chemical fertilizer, and that the effect depended on the net applied ammonium nitrogen (NH_4-N), which was the difference between the applied NH_4-N and the nitrogen loss through ammonia (NH_3) volatilization. A lysimeter experiment on timothy sward, conducted in central Hokkaido (northern island) Japan, showed nitrate (NO_3-N) leaching associated with ADCS application in autumn tends to be higher than that in spring, due to snow cover and snow melting, resulting in higher N use efficiency of the ADCS in spring application (Matsunaka *et al*. 2006). It is also well-known that N application and soil environmental factors (e.g., temperature, moisture, pH, oxidation-reduction potential, availability of organic carbon, NH_4-N, and NO_3-N) affect nitrous oxide (N_2O) and CH_4 fluxes from the soil (Sahrawat & Keeney 1986; Le Mer & Roger 2001). From these facts, it is suggested that N_2O and CH_4 fluxes may be affected by the applied material and the application season, even if the same level of NH_4-N was

applied to the grassland soil. These effects have not been reported yet.

In addition, only a few peer-reviewed papers investing both N_2O emission and CH_4 absorption from Japanese grasslands throughout the year have been published (Mori *et al*. 2005, 2008). These studies were conducted in Nasu, Tochigi prefecture located in central Japan and reported annual N_2O emission and CH_4 absorption. However, they did not report fertilizer-induced N_2O emission factor (EF). In Japan, most (92%) grassland for livestock production is located in Hokkaido (Statistics Bureau 2008). Investigations of annual N_2O emission rates and CH_4 absorption rates and EFs in Hokkaido grassland are important for estimation of GHGs emission from Japanese grassland.

In Hokkaido Japan, timothy swards for making silage and/or hay are commonly fertilized in spring (after snow melting) and in early summer (June, after 1st cutting), and harvested in early summer (June) and late summer (August to September). However, in farms producing ADCS, it is necessary to apply ADCS to sward in autumn because the storage tank should be cleared before snow season. The effects of the applied materials and the application seasons on N_2O emission and CH_4 absorption have not been demonstrated. Here, we report the results of two-year field experiment in central Hokkaido, with three materials (control, ADCS, or chemical fertilizer (CF)) and two application seasons (autumn or spring). Objective of this study are to obtain annual N_2O and CH_4 emission rates and to examine the effect of the applied material and the application season on the annual N_2O and CH_4 emission and fertilizer-induced N_2O EF.

MATERIALS AND METHODS

Site description

A field study was carried out on timothy (*Phleum pratense* L.) grassland established in 2002 at the Rakuno Gakuen University in central Hokkaido Japan (latitude 43°04′N, longitude 141°30′E, altitude 61 m). The soil was gray upland soil (Aeric, Typic, Epiaquults). The Ap horizon was 0-20 cm depth. The bulk density and texture were 0.81 Mg m^{-3} and loam (clay 14%, silt 43%, sand 43%),

respectively. The pH(H_2O), total carbon content, total nitrogen content, and CEC were 6.3, 30 g kg^{-1}, 2.5 g kg^{-1}, and 39 $cmol_{(+)}$ kg^{-1}, respectively. The 5-year (2003-2007) averages of precipitation and air temperature were 928 mm y^{-1} and 7.0°C, respectively (A the nearest weather station, 7 km northeast from the site. [Japan Meteorological Agency 2008]).

Experimental design and field management

Experimental plots as randomized block design were established in mid-October 2003, with three replications (blocks) and with five treatments (Fig. 1). Each plots had 9.0 m^2 in area (3.0 × 3.0 m^2). Treatments were as follows, 1) CF (as $(NH_4)_2SO_4$) application in autumn (27 October 2003, 25 October 2004); 2) ADCS application in autumn (the same as above); 3) no nitrogen application (Control); 4) CF application in spring (22 April 2004, 28 April 2005); 5) ADCS application in spring (the same as above).

Both ADCS and CF were manually applied onto grassland surface. ADCS was applied at a rate of 6.0 kg m^{-2} (60 Mg ha^{-1}), which is the recommended rate in Hokkaido (Matsunaka *et al*. 1998). The concentration of ammonium nitrogen (NH_4-N) and total N were measured at each application. Ammonium sulfate was applied at the same application rate of NH_4-N in ADCS. Applied NH_4-N ranged from 71 to 102 kg N ha^{-1} (Table 1). For ADCS treatments, four kinds of N application rate were calculated by considering NH_3 volatilization immediately after application (mostly within 48 hours; Matsunaka *et al*. 2008) (Table 1). P (superphosphate) and K (potassium sulfate) were applied to all plots at a rate of 60 kg P_2O_5 ha^{-1} y^{-1} and 180 kg K_2O ha^{-1} y^{-1}, respectively. Two third of those was applied in spring (the end of April) and the rest was applied after first harvest. Harvest was performed two times a year, in June (24 June 2004, 16 June 2005) and in August (26 August 2004, 23 August 2005).

Although there were five treatments in the field, six treatment datasets were obtained by considering two periods (autumn start and spring start) in Control (Fig. 1). As shown in Fig. 1,

Y2004 and Y2005 are the dataset in first and second year, respectively. For autumn application, Y2004 and Y2005 were obtained from 25 October 2003 to 16 October 2004 and from 17 October 2004 to 27 October 2005, respectively. For spring application, Y2004 and Y2005 were obtained from 21 April 2004 to 24 April 2005 and from 25 April 2005 to 28 April 2006, respectively.

Field measurement and laboratory analysis

N_2O and CH_4 fluxes measurement and soil sampling for determination of ammonium (NH_4-N) and nitrate (NO_3-N) content in the soil were conducted from 25 October 2003 to 28 April 2006, including snow season periods. The sampling frequency was higher (at least two times a week) just after CF and ADCS application than in growing season (usually one time a week, at least one time per two weeks). The total number of field sampling was 75. In most sampling date, flux measurement was conducted in the morning and soil sampling in the afternoon.

Closed chamber technique was used to determine N_2O and CH_4 fluxes from the soil surface to the atmosphere. In each plot, a round shape metal collar (0.285 m in inside diameter or 0.0638 m^2 in area) equipped with small ditch on the top side was installed to the soil surface in mid-October 2003. These collars were not removed until the end of April 2006. At each sampling time, a cylindrical PVC chamber (49.0 cm in height) was inserted into the ditch with some water to make airtight, and the chamber was placed for 0.50 hour. The total volume inside the chamber was 0.0392 m^3. The gas samples inside the chamber (about 25 cm^3) were collected into the evacuated glass bottles (15 cm^3) with butyl rubber stopper by using a portable syringe at 0.00 and 0.50 hour after chamber placement. A gas chromatograph equipped with an electron capture detector (GC-14B, Shimadzu, Japan) and a gas chromatograph equipped with a flame ionization detector (GC-14B, Shimadzu, Japan) were used for N_2O and CH_4 concentration, respectively. Flux was calculated using the following equation.

$$F = \rho \times V/A \times (C_{0.50} - C_{0.00})/t \times (273.15/T)$$

where F is the gas flux (μg N_2O-N m^{-2} h^{-1} or μg CH_4 m^{-2} h^{-1}); ρ is the gas density under STP (1.26×10^3 g m^{-3} for N_2O-N, 0.717×10^3 g m^{-3} for CH_4); V and A are the volume (0.0392 m^3) and area (0.0638 m^2) of the chamber, respectively; $C_{0.50}$ and $C_{0.00}$ are the N_2O or CH_4 concentration (μm^3 m^{-3}) at 0.50 and 0.00 hour after chamber placement, respectively; t is the time of chamber placement (0.50 h); T is the average temperature (K) measured inside chamber. Note that CF or ADCS was manually applied to both inside and outside of the chamber collars with the rate as presented above. In the snow seasons, the same collar was temporarily set on the snow surface in each plot and the same procedure was conducted.

The topsoil at the depth of 0–15 cm was collected at each sampling date to determine the soil NH_4-N and NO_3-N content. Field-moist soil samples were extracted in 100 g L^{-1} KCl solution (soil: solution = 1:5), and NH_4-N and NO_3-N concentrations in the extracted solution were determined by a flow injection analyzer (FIA star 5012 analyzer, FOSS, Denmark). Soil NH_4-N and NO_3-N content (mg N kg^{-1}) were calculated by using these concentrations and soil moisture content measured by drying soil at 105°C for more than 48 hours. WFPS (water filled pore space) was calculated using soil moisture content, bulk density (0.81 Mg m^{-3}), and soil particle density (assumed to be 2.65 Mg m^{-3})

At each harvest, timothy grass of 1.0 m^2 area in each plot was cut at 5 cm height from the soil surface, which was collected and weighed. One hundred gram of them was taken and dried at 70°C for more than 48 hours. After weighing the dry matter of it, a portion of it was milled and N content was determined by wet ashing and steam distillation technique. Finally, N uptake (kg N ha^{-1}) by timothy grass was calculated.

Daily precipitation (mm) and daily mean air temperature (°C) of the experimental sward were obtained by the weather station at Rakuno Gakuen University.

Fertilizer-induced N_2O emission factor and statistical analysis

Fertilizer-induced N_2O-N emission factor (EF) was calculated in each block and year. For ADCS treatments, four kinds of N application rate were calculated by considering NH_3 volatilization (Table 1). According to these four values, four types of EFs can be calculated for ADCS plots, as follows.

$$EF_{NH4–NH3} = N_2O\text{-}N / N_{NH4–NH3}$$

$$EF_{NH4} = N_2O\text{-}N / N_{NH4}$$

$$EF_{TN–NH3} = N_2O\text{-}N / N_{TN–NH3}$$

$$EF_{TN} = N_2O\text{-}N / N_{TN}$$

where EF is the fertilizer-induced N_2O emission factor (kg N_2O-N [kg N]$^{-1}$); N_2O-N is the annual fertilizer-induced N_2O emission rate (kg N_2O-N ha^{-1} y^{-1}), which was obtained by subtracting the emission of control plot in each block and year; $N_{NH4–NH3}$ is the amount of the applied NH_4-N minus NH_3 volatilization (kg N ha^{-1} y^{-1}); N_{NH4} is the amount of the applied NH_4-N (kg N ha^{-1} y^{-1}); $N_{TN–NH3}$ is the amount of the applied total N minus NH_3 volatilization (kg N ha^{-1} y^{-1}); N_{TN} is the amount of the applied total N (kg N ha^{-1} y^{-1}). In this paper, these four types of EF were calculated. For CF plots, EF_{NH4} was calculated but all four EFs types are practically assumed to be equal ($EF_{NH4} = EF_{NH4–NH3} = EF_{TN–NH3} = EF_{TN}$), because of no NH_3 volatilization from the CF plots (Matsunaka *et al*. 2008).

Three way analysis of variance (ANOVA) was used to examine the significance of the factors. The factors are the material (control, ADCS, CF), the season (autumn, spring), and the year (Y2004, Y2005). PC software, SigmaStat (SPSS 1997), was used for ANOVA.

RESULTS

Seasonal changes in N_2O and CH_4 fluxes

Daily precipitation and mean air temperature from October 2003 to April 2006 are shown in Figs. 2

and 3. Maximum daily precipitation (94.5 mm) was recorded on 7 September 2005. The highest (25.8°C) and lowest (–10.9°C) daily mean air temperature were recorded on 9 August 2004 and 23 January 2006, respectively. It was found that the amount of precipitation in the second year (2005, 807 mm) was greater than that in the first year (2004, 564 mm), and that the air temperature fluctuated with almost same pattern (Fig. 4). Mean air temperature of the ADCS application dates was narrowly ranged from 5 to 11°C. The precipitation within 12 hours after ADCS applications was not more than one mm. These results show that using only one value (0.32) for NH_3 volatilization factor (Table 1) was not unsuitable.

Ammonium and nitrate contents in the topsoil showed fluctuation (Fig. 2). Both contents sharply increased and gradually decreased within one month after the applications of ADCS or CF, although the magnitudes of the variation were not the same. These increase and decrease would be caused as the result of the NH_4 addition by the applied materials, nitrification of the added NH_4, and uptake by timothy. Little or no change was observed after harvesting of grass.

Daily mean N_2O fluxes ranged from –7 to +113 μg N_2O-N m^{-2} h^{-1} (Fig. 3). Comparing with control plots, higher N_2O fluxes were observed in ADCS and CF plots, especially after the autumn application. These higher N_2O fluxes after the ADCS or CF application coincided with the fluctuation of NH_4 and NO_3 contents in the topsoil. Little or no change was observed after harvesting or heavy rain.

Daily mean CH_4 fluxes ranged from –164 to +43 μg CH_4 m^{-2} h^{-1} (Fig. 3). Many CH_4 flux values showed negative values, meaning that the methane was absorbed from the atmosphere into the topsoil. There was no noticeable change in CH_4 flux after the ADCS or CF application, harvesting, and heavy rain. It appears that CH_4 fluxes in the first year were lower (higher absorption) than those in the second year.

Nitrogen uptake by timothy grass

The annual dry matter production and N uptake by timothy ranged from 3.75 to 13.2 Mg ha^{-1} y^{-1} and from 59 to 204 kg N ha^{-1} y^{-1}, respectively (Fig. 5). Dry matter production and N uptake until first harvest accounted for more than a half of the annual uptake (data not shown). The result of ANOVA showed significance of the interaction (season × material, $P < 0.01$) for both dry matter production and N uptake. This interaction appears to be caused by low growth in CF with autumn application (Fig. 5), which was probably due to N loss through nitrate leaching followed by rainfall and snow melting before growing season (Matsunaka *et al.*, 2006). However, the reason that relatively high dry matter production and N uptake in ADCS with autumn application is still uncertain. Although the interaction was found, ANOVA also showed significance of main effects (material, season, year, $P < 0.01$) for both dry matter production and N uptake, i.e., ADCS and CF application increased the dry matter and N uptake of the grass. There was no significant difference in mean N uptake between ADCS and CF plots (Fig. 5). Higher dry matter production and N uptake in the first year (2004) than in the second year (2005) would be caused by higher air temperature in spring. Mean air temperature in May 2004 (12.0°C) was higher than that in May 2005 (9.2°C) (Fig. 4).

Annual N_2O emission and CH_4 absorption rate

Annual N_2O emission rates ranged from 0.349 to 0.926 kg N_2O-N ha^{-1} y^{-1} (Fig. 6). The result of ANOVA showed significance of the material ($P = 0.042$). ADCS and CF application increased the annual N_2O emission; the mean annual N_2O emission rates in ADCS (0.708 kg N_2O-N ha^{-1} y^{-1}) and CF plots (0.636 kg N_2O-N ha^{-1} y^{-1}) were significantly higher than those in the control plots (0.398 kg N_2O-N ha^{-1} y^{-1}) (Fig. 6). There was no significant difference in mean annual N_2O emission between ADCS and CF plots. Autumn application plots showed a relatively higher ($P = 0.134$) N_2O emission rates than spring application plots.

Annual CH_4 absorption rates raged from 1.65 to 4.59 kg CH_4 ha^{-1} y^{-1} (Fig. 6). The result of ANOVA showed significant of the year ($P = 0.014$). Mean CH_4 absorption rate in the first year (3.28

kg CH_4 ha^{-1} y^{-1}) was significantly higher than that in the second year (2.31 kg CH_4 ha^{-1} y^{-1}). ADCS and CF application did not affect the annual CH_4 emission rate.

Fertilizer-induced N_2O emission factor

In the calculation of the fertilizer-induced N_2O emission factor, the fertilizer-induced N_2O emission rate should be obtained by subtracting the emission of control plot. As described in the previous section, significant difference in the annual N_2O emission rate between the applied materials was found, indicating that it is possible to calculate the fertilizer-induced N_2O emission factor (EF). Mean EF ranged from 0.0009 to 0.0089 (kg N_2O-N [kg N]$^{-1}$), and large variations were found (Fig. 7). It appears that EFs in spring application plots were lower than those in autumn application. However, the results of ANOVA showed insignificance of all factors ($P > 0.05$), although the P values for the season are relatively low (0.067–0.160). Table 2 shows total mean (± standard error of mean) of four types of EFs. The total mean values ranged 0.0024 to 0.0043.

DISCUSSION

N_2O and CH_4 flux

In previous reports, N_2O flux increased following rainfall after manure application in a grassland located in Tochigi (Mori *et al*. 2008). In addition, increase in N_2O flux after heavy rain and/or harvest was observed in crop fields located in Hokkaido (Kusa *et al*. 2002, 2006; Katayanagi *et al*. 2008; Mu *et al*. 2008). However, little or no change in N_2O flux was observed after harvesting or heavy rain in this study. In another grassland with no fertilizer application located in Tochigi (Mori *et al*. 2005) and an arable cropping system located in Hokkaido (Koga *et al*. 2004), correspondence relationship between heavy rain and increase in N_2O flux was not clear as in the case of our study.

Prediction of N mineralization rate from cattle manure was proposed (e.g., Shiga *et al*. 1985), but that from organic matter in ADCS applied to grassland surface is unknown. However, according to

the results of Saigusa & Watanobe (2006), it is likely that decomposition of organic matter in the ADCS and subsequent mineral N supply to soil compensate the N loss through NH_3 volatilization. However, in the present study, distinctive increase in mineral N (NH_4, NO_3) and N_2O flux in ADCS plots was not observed in the growing seasons (July to September) (Figs. 2 and 3).

Higher N_2O fluxes were observed mainly after the ADCS or CF application, which coincided with the fluctuation of NH_4 and NO_3 contents in the topsoil. Therefore, the increase in the annual N_2O emission rates in ADCS and CF plots compared to control plots could be attributed to N_2O production via nitrification of added NH_4-N in the present study.

Generally, it is considered that organic matter application to the soil increases N_2O emission because the easily degradable carbon enhances de-nitrification. For example, a grassland experiment under the same N application rate in Tochigi Japan showed that annual N_2O emission rates were significantly higher from the manure plots than from the chemical fertilizer plots (Mori *et al*. 2008). In contrast, there was no significant difference in mean annual N_2O emission between ADCS and CF plots (Fig. 6) in this study. In other words, it appears that organic matter (ADCS) application did not increase the N_2O emission under the same NH_4-N application rate. However, we should consider N loss just after ADCS application through NH_3 volatilization. Therefore, comparing EF_{NH4} of CF plots with $EF_{NH4\text{-}NH3}$ of ADCS (Fig. 7) is suitable for checking the organic matter effect. No significance of the material (P = 0.195) was found in ANOVA due to high variation, however, $EF_{NH4\text{-}NH3}$ of ADCS plots tends to be higher than EF_{NH4} of CF plots (Fig. 7). These results suggest that organic matter application onto grassland surface increases N_2O emission to some extent. Effect of organic mater application onto grassland surface on N_2O emission should be analyzed and predicted quantitatively in the future.

Negative CH_4 fluxes were observed, which is considered to be result of CH_4 consumption (oxidation) by methanotrophs in the soil. Generally, it is well known that both methanotrophs and methanogens exist in soils, and that these bacterial activity and CH_4 flux is related to soil water

content and redox condition (Le Mer & Roger 2001). Positive correlation between CH_4 flux and soil water content (Mori *et al*. 2005, 2008) and increase in CH_4 flux after heavy rain (Mori *et al*. 2008) were observed in a grassland in Nasu, Tochigi prefecture located in central Japan. In the present study, noticeable increase in CH_4 flux after heavy rain was not recognized. However, the annual CH_4 absorption rate in the first year was significantly higher than that in the second year, which probably resulted from smaller amount of precipitation and subsequent more aerobic soil condition favorable for methanotrophs activity in the first year. In fact, as shown in Fig. 8, soil WFPS and CH_4 flux tended to be higher in the second year (2005) than in the first year (2004) ($P = 0.08$ for WFPS, $P < 0.01$ for CH_4 flux by *t*-test), and significant positive relation between WFPS and CH_4 flux was found.

Many papers and reviews have reported that CH_4 oxidation potential and CH_4 flux in upland soils are reduced and increased, respectively, by ammonium N fertilizer application (Mosier *et al*. 1991; Le Mer & Roger 2001; Hu *et al*. 2002) and by NH_4-N content in the soil(Mori *et al*. 2005, 2008). However, in the present study, ammonium N fertilizer as ADCS or CF did not reduce the annual CH_4 absorption rate compared to control plots. Possible explanation is that the period with high soil NH_4-N content was very short (about one month after application).

Annual rate of N_2O emission and CH_4 absorption

Annual N_2O emission rates ranged from 0.349 to 0.926 kg N_2O-N ha^{-1} y^{-1} in this study. Compared to N_2O emission rates in Japanese grasslands and pastures reported in peer-reviewed papers, the rates in this study were similar to those in a grassland located in central Hokkaido (0.15 - 1.54 kg N_2O-N ha^{-1} 6-$month^{-1}$, Mu *et al*. 2008) and in a grassland with no fertilizer application located in Tochigi (0.39 - 1.59 kg N_2O-N ha^{-1} y^{-1}, Mori *et al*. 2005); lower than those in a grassland with manure and chemical fertilizer application located in Tochigi (4.7 - 11.0 kg N_2O-N ha^{-1} y^{-1}, Mori *et al*. 2008) and in grasslands and pastures located in southern Hokkaido (1.1 - 42.8 kg N_2O-N ha^{-1} y^{-1}, Katayanagi

et al. 2008).

Total mean of the annual CH_4 absorption rate was 2.80 kg CH_4 ha^{-1} y^{-1} (raged from 1.65 to 4.59). Compared to CH_4 absorption rates in Japanese uplands (grassland, arable field, forest) reported in peer-reviewed papers, the rates in this study were higher than those in an onion field in Hokkaido (–0.78 - 0.36 kg CH_4 ha^{-1} 6-$month^{-1}$, Hu *et al*. 2002), several cropping systems in Hokkaido (0 - 1.1 kg CH_4 ha^{-1} 6-$month^{-1}$, Mu *et al*. 2006), and a grassland fertilized with manure and ammonium sulfate in Tochigi (0.16 - 0.84 kg CH_4 ha^{-1} y^{-1}, Mori *et al*. 2008); similar to those in an arable cropping system in Hokkaido (1.4 - 2.4 kg CH_4 ha^{-1} y^{-1}, Koga *et al*. 2004), no fertilized grassland in Tochigi (1.8 - 2.4 kg CH_4 ha^{-1} y^{-1}, Mori *et al*. 2005), and 10 forests in Hokkaido (1.4 - 6.6 kg CH_4 ha^{-1} 6-$month^{-1}$, Morishita *et al*. 2004); lower than those in 25 forests in Japan (2.7 - 24.8 kg CH_4 ha^{-1} y^{-1}, Ishizuka *et al*. 2009).

These annual N_2O emission and CH_4 absorption rates including our study shows high variation, suggesting that N_2O and CH_4 flux from grassland is regulated by many factors such as plant species, soil type and properties, climate condition, management practices, etc. and their interaction effect. Collaboration between field measurements and model analysis (e.g., Saggar *et al*. 2009) will be required to estimate the amount of N_2O emission and CH_4 absorption in Japanese grasslands in the future.

Fertilizer-induced N_2O emission factor

High variation was observed in the calculated fertilizer-induced N_2O emission factors (EFs), which could be a reason for no significance in all factors. However, EFs in autumn application plots tends to be higher than those in spring applications, which is considered to be a result from relatively higher N_2O emission and the lower N application in autumn application plots than in spring application plots (Table 1). Relatively higher N_2O emission in autumn application plots may be produced by higher N_2O fluxes just after the autumn application. The grass growth (N uptake) rate

following the spring application is usually higher than that following the autumn application (Matsunaka 1987), which would suppress soil NH_4-N or NO_3-N available for N_2O production and flux.

Which type of EF is the most suitable for evaluation of N_2O emission from the ADCS plots? In this study the NH_3 volatilization factor was assumed to be 0.32, but the rate can be changed by field conditions such as temperature and rainfall (Matsunaka *et al*. 2008). In addition, mineralization of organic matter in ADCS occurs, which can be considered in medium- to long-term evaluation, although the mineralization rate is unknown at present. Taking these facts into account, EF_{TN} will be the most explicit, universal, and convenient. Total mean ± standard error of mean of EF_{TN} was 0.0024 ± 0.0007 (kg N_2O-N [kg N]$^{-1}$), which is similar to the reported EF (0.0032 ± 0.0013) for well-drained uplands in Japan (Akiyama *et al*. 2006). Akiyama *et al*. (2006) also reported higher EF of 0.014 for poor-drained uplands in Japan. Toma *et al*. (2007) investigated variation in EF derived from chemical N fertilizer (EF_F) and organic matter (EF_O) in two soils located in Hokkaido. They suggested that EF_F and EF_O were related to mean annual air temperature and mean annual relative humidity, respectively. Along with field measurements and model analysis, considering these factors for EF will contribute to raise the precision of estimation of N_2O emission from grasslands.

Conclusions

Under the same level application of NH_4-N, the difference in the applied materials (ADCS or CF) and the application season (autumn or spring) led no significant difference in N_2O emission, fertilizer-induced N_2O EF, and CH_4 absorption.

ACNOWLEDGMENTS

This work was partially supported by New Energy and Industrial Technology Development Organization (NEDO) as a part of a project "Development of Technology to Assess and Verify Life

Cycle CO_2 Emissions; Development of demonstrate the CO_2 emission output on lifecycle of products and others; Application of LCA methodology to local policies: Case studies on biogas plants in Betsukai-Chou, Hokkaido" (FY2003-2005).

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Figure List

Figure 1. Outline of experimental field, measurement, and dataset.

Figure 2. Seasonal changes in daily precipitation (bars) and mean air temperature (line) (A), NH_4 and NO_3 contents in the topsoil (0-15 cm) (B and C). Small letters and dotted lines denote management events, i.e., a, autumn application of anaerobically digested cattle slurry

(ADCS) or chemical fertilizer (CF); b, spring application of ADCS or CF; c, first harvest in June; d, second harvest in August. Ctrl, plots with no application; CF Autumn, plots with CF application in autumn; ADCS Autumn, plots with ADCS application in autumn; CF Spring, plots with CF application in spring; ADCS Spring, plots with ADCS application in spring. Circles and error bars show mean and standard deviation (n = 3), respectively.

Figure 3. Seasonal changes in daily precipitation (bars) and mean air temperature (line) (A), N_2O and CH_4 fluxes from the soil surface to the atmosphere (B and C). Format is the same as that of Fig. 2.

Figure 4. Monthly meteorological data (rain and mean air temperature) in the growing seasons.

Figure 5. Annual dry matter production and N uptake by timothy grass. Bars and error bars show mean and standard deviation (n = 3), respectively. The same superscript of the mean value shows no significance difference by Fisher LSD method ($P > 0.05$).

Figure 6. Annual N_2O emission rate (A) and CH_4 absorption rate (B). Bars and error bars show mean and standard deviation (n = 3), respectively. The same superscript of the mean value shows no significance difference by Fisher LSD method ($P > 0.05$).

Figure 7. Fertilizer-induced N_2O-N emission factor. Bars and error bars show mean and standard deviation (n = 3), respectively.

Figure 8. Relationship between soil WFPS and CH_4 flux in the growing season (April to November) in 2004 and 2005. In the box plots, the boundary of the box closest to zero

indicates the 25th percentile, a line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 90th and 10th percentiles.

Table List

Table 1. Application of ADCS (anaerobically digested cattle slurry) and CF (chemical fertilizer, $(NH_4)_2SO_4$).

Table 2. Summary of the fertilized-induced N_2O emission factor.

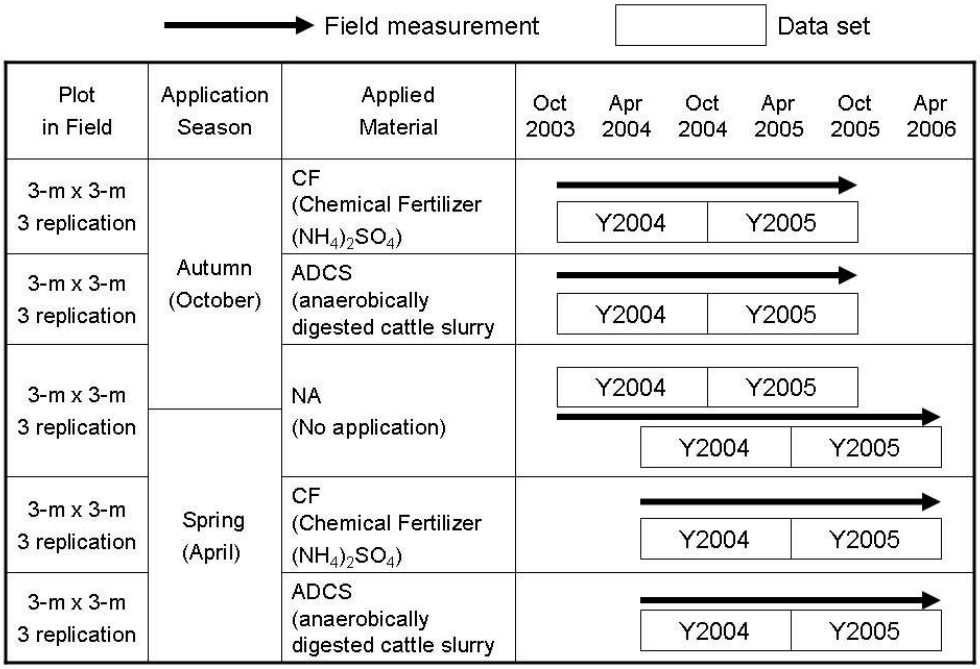

Fig. 1. Outline of experimental field, measurement, and dataset.
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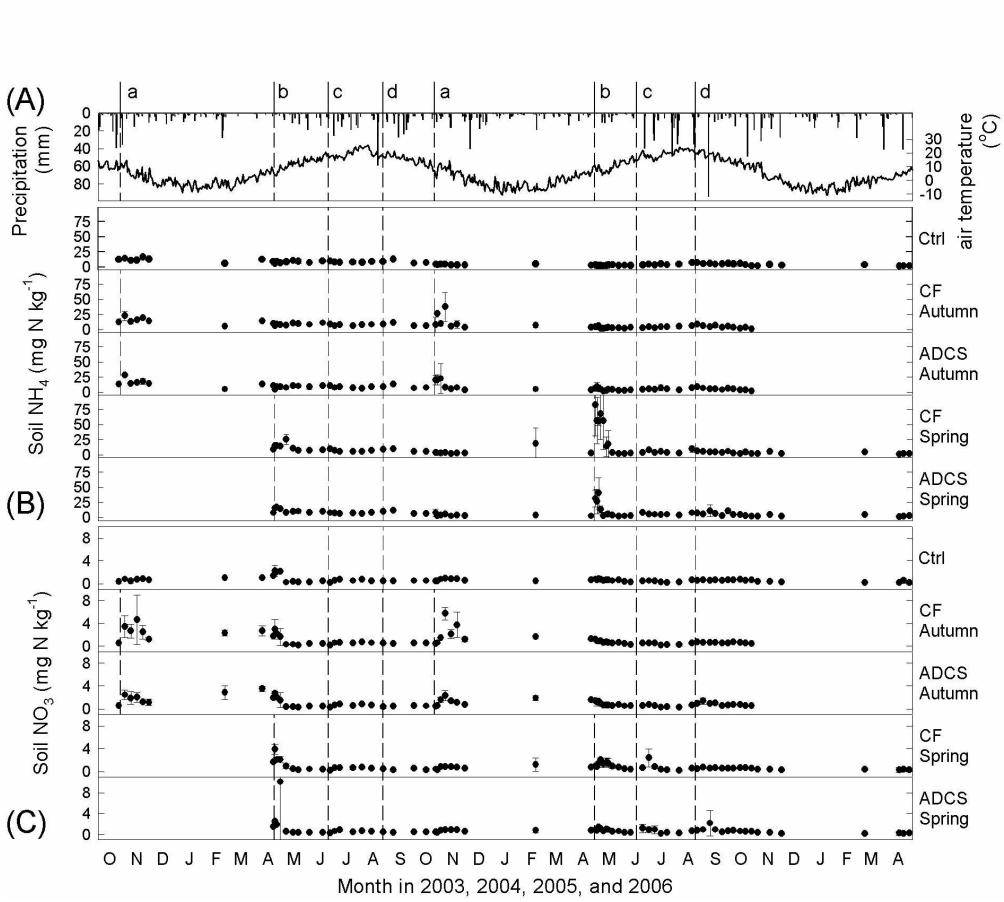

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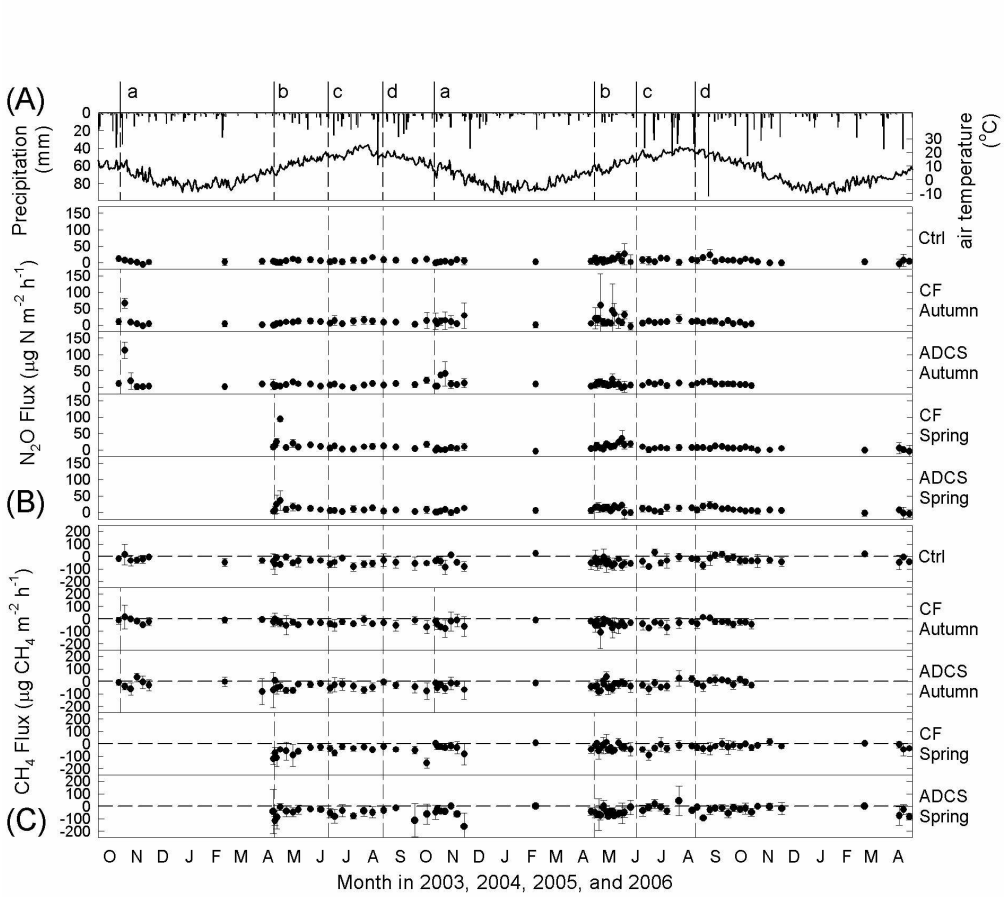

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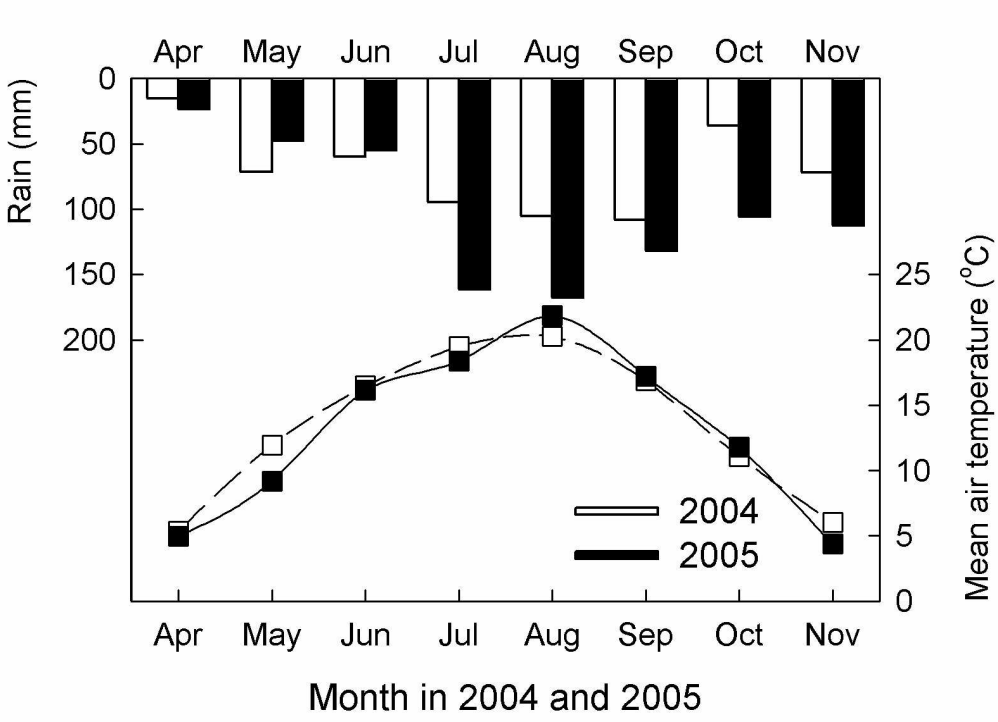

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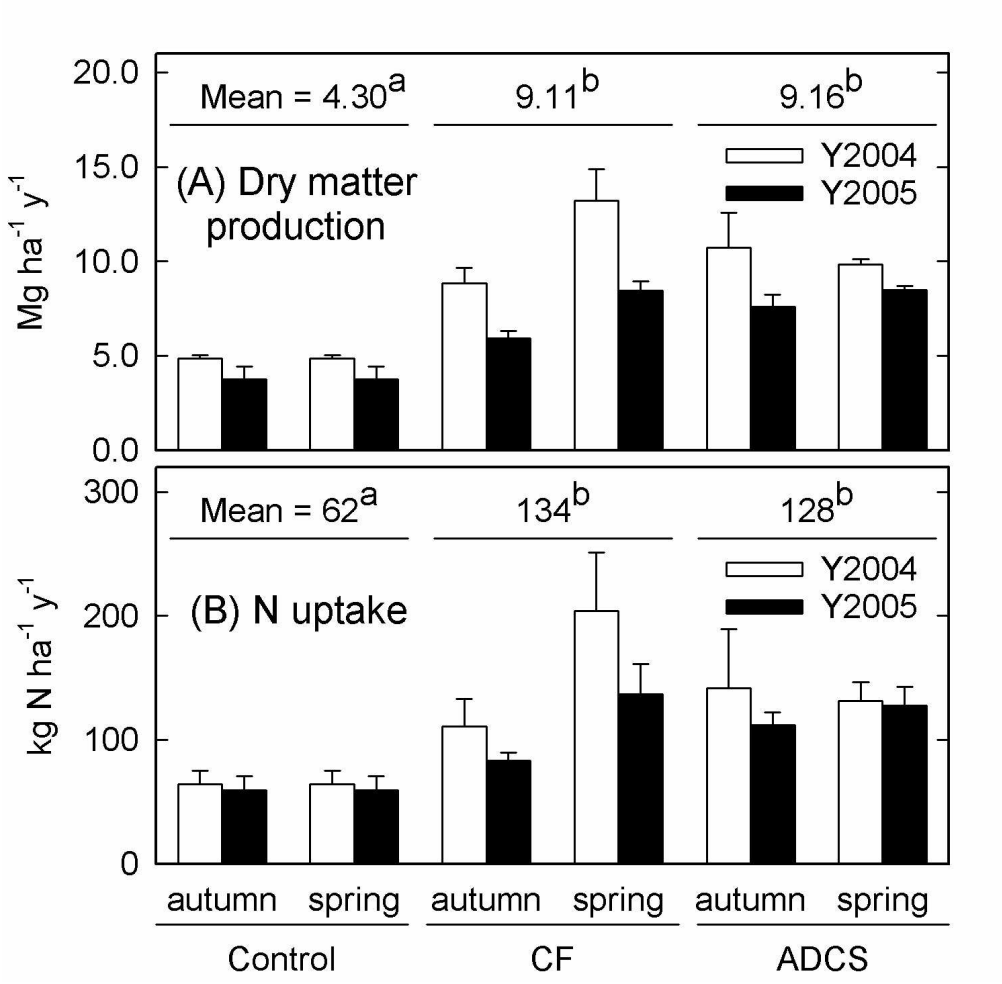

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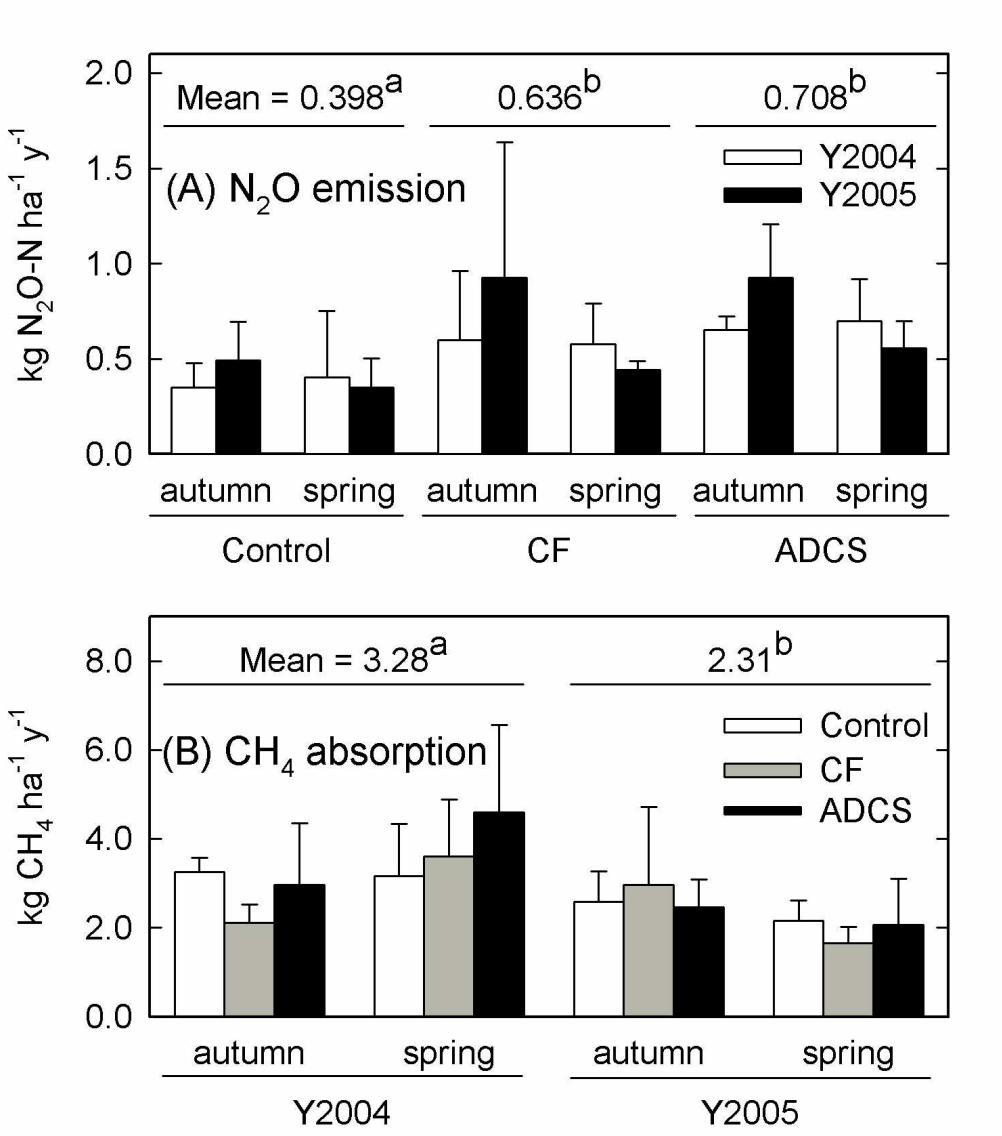

Fig. 6. Annual N2O emission rate (A) and CH4 absorption rate (B). Bars and error bars show mean and standard deviation (n = 3), respectively. The same superscript of the mean value shows no significance difference by Fisher LSD method (P > 0.05).
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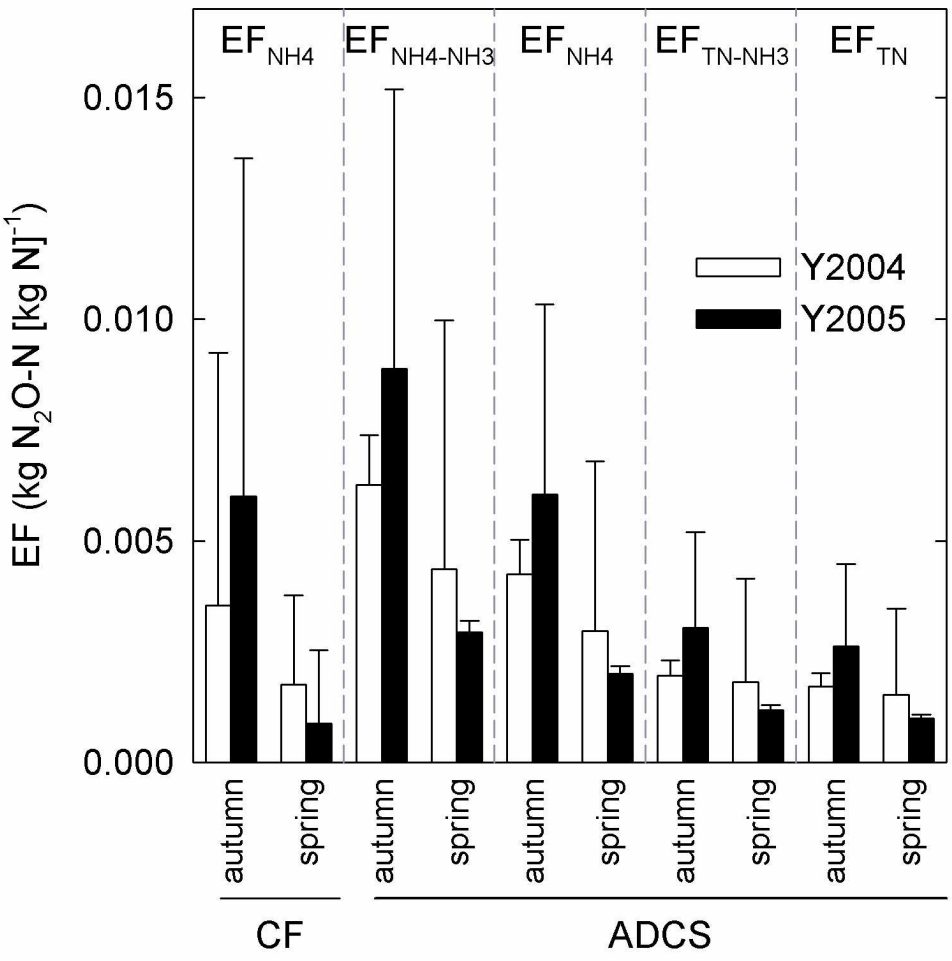

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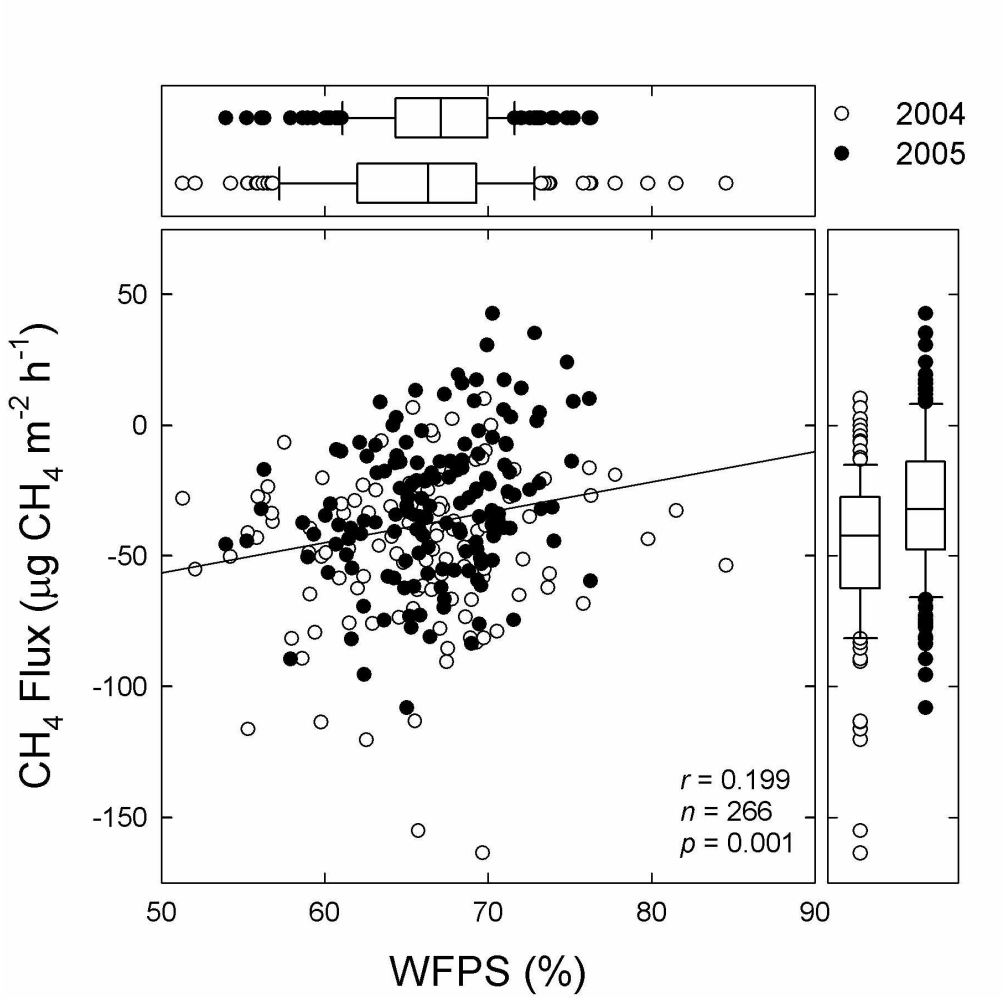

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306x305mm (150 x 150 DPI)

Table 1. Application of ADCS (anaerobically digested cattle slurry) and CF (chemical fertilizer, $(NH_4)_2SO_4$)

Year and application season	ADCS								CF
	Application rate	N concentration †			N application rate ¶				N application rate
		NH_4-N	Total-N	Org-N	NH_4–NH_3	NH_4	TN–NH_3	TN	NH_4
	Mg ha^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	kg N ha^{-1}	kg N ha^{-1}	kg N ha^{-1}	kg N ha^{-1}	kg N ha^{-1}
Y2004									
Autumn in 2003	60	1.18	2.94	1.76	48	71	154	176	71
Spring in 2004	60	1.67	3.27	1.60	68	100	164	196	100
Y2005									
Autumn in 2004	60	1.20	2.77	1.57	49	72	143	166	72
Spring in 2005	60	1.70	3.41	1.71	69	102	172	205	102

† Nitrate (NO_3-N) was not detected.

¶ NH_3 volatilization factor was assumed to be 0.32 (Matsunaka *et al*. 2008). NH_4–NH_3 is the amount of the applied NH_4-N minus NH_3 volatilization; NH_4 is the amount of the applied NH_4-N; TN–NH_3 the amount of the applied total N minus NH_3 volatilization; TN is the amount of the applied total N.

Table 2. Summary of the fertilized-induced N_2O emission factor.

Type of EF		Mean ± standard error of mean
CF	ADCS	
EF_{NH4}	$EF_{NH4–NH3}$	0.0043 ± 0.0009 (n = 24)
EF_{NH4}	EF_{NH4}	0.0034 ± 0.0008 (n = 24)
EF_{NH4}	$EF_{TN–NH3}$	0.0025 ± 0.0007 (n = 24)
EF_{NH4}	EF_{TN}	0.0024 ± 0.0007 (n = 24)

Unit: kg N_2O-N [kg N]$^{-1}$