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Carbon budget and methane and nitrous oxide emissions over the growing season in a *Miscanthus sinensis* grassland in Tomakomai, Hokkaido, Japan

Running title:
Carbon budget and greenhouse gas emissions of a *M. sinensis* grassland

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Abbreviations: C, carbon; CO_2, carbon dioxide; , CH_4 methane; N_2O,nitrous oxide; ANPPP, aboveground net primary production; BNPP, belowground net primary production; GWP, global warming potential; AET, accumulative effective temperature above 10°C

Abstract

Species in the *Miscanthus* genus have been proposed as biofuel crops that have potential to mitigate elevated atmospheric carbon dioxide (CO_2) levels and nitrous oxide (N_2O) and methane (CH_4) emissions. *Miscanthus sinensis* is widespread throughout Japan and has been used for biomass production for centuries. We assessed the carbon (C) budget and N_2O and CH_4 emissions over the growing season for two years in a *M. sinensis*-dominated grassland that was naturally established around 1972 in Tomakomai, Hokkaido, Japan, which is near the northern limit for *M. sinensis* grassland establishment on Andisols. Average C budget was -0.31 Mg C ha^{-1}, which indicates C was released from the grassland ecosystem to the atmosphere. Dominant components in the C budget appeared to be aboveground net primary production of plants (1.94 to 2.80 Mg C ha^{-1}) and heterotrophic respiration (2.27 to 3.11 Mg C ha^{-1}). The measurement of belowground net primary production (BNPP) of plants in the *M. sinensis* grassland was extremely variable, thus only an approximate value could be calculated. Mean C budget calculated with the approximated BNPP value was 1.47 and -0.23 Mg C ha^{-1} for 2008 and 2009, respectively. Given belowground biomass (9.46 to 9.86 Mg C ha^{-1}) was 3.1 to 6.5 times higher than that of aboveground biomass may provide additional evidence suggesting this grassland represents a C sink. Average CH_4 emissions across years of -1.34 kg C ha^{-1} would indicate this grassland acts as an atmospheric CH_4 sink. Furthermore, average N_2O emissions across years were 0.22 kg N ha^{-1}. While the site may contribute N_2O to the atmosphere, this value is lower compared with other grassland types. Global warming potential calculated with the approximated BNPP value was -5.40 and 0.95 Mg CO_2eq ha^{-1} for 2008 and 2009,

respectively, and indicates this grassland could contribute to mitigation of global warming.

Introduction

Using bioenergy instead of fossil fuels could potentially mitigate human-induced global climate change. Although fossil fuels emit carbon (C), the primary source of C for bioenergy is atmospheric carbon dioxide (CO_2). Also, due to the steadily rising global demand for energy and the dwindling global reserve of petroleum (Owen, in press), production of bioenergy from plant biomass has increasingly been considered as a viable alternative source of fuel (Milliken *et al*., 2007; Heaton *et al*., 2008). Perennial grassland plant species, such as switchgrass (*Panicum virgatum*) or *Miscanthus* x *giganteus*, have been evaluated as non-food bioenergy feedstock due to their potential for large biomass production and concomitant reduction of greenhouse gas (GHG) emissions (Davis *et al*., 2009). Using the model predictions from RothC, which is used to calculate projections of soil-C turnover, and *Miscanthus* yields from MISCANFOR, a recently developed *Miscanthus* growth model (Hastings *et al.*, 2009), Dondini *et al*. (2009) reported soil under *M.* x *giganteus* production has potential to store, on average, 2 to 3 Mg C ha^{-1} yr^{-1} over a 20-year period.

The *Miscanthus* genus, which utilizes the energy-efficient C_4 photosynthetic pathway (Naidu *et al*., 2003), is comprised of several species that are considered potential bioenergy crops because of their relative low-nutrient requirements (Lewandowski *et al*., 2003; Heaton *et al*., 2004), high water-use efficiencies (Clifton-Brown *et al*., 2002), and high productivity (Clifton-Brown *et al*., 2001; Jones & Walsh, 2001; Stewart *et al*., 2009). *M.* x *giganteus* contributes to C accumulation (0.59 to 1.12 Mg C ha^{-1} yr^{-1}) in soil (Hansen *et al*., 2004; Clifton-Brown *et al*., 2007), which indicates the potential of this species not only as a bioenergy crop, but also as a C sequester. Application of nitrogen (N) fertilizer often results in reduction of CH_4 absorption and increase in N_2O emission from soil (Schimel *et al*., 1993; Conrad, 1995; Bouwman *et al*., 2001). Given *Miscanthus* can be produced with relatively little N fertilization (Heaton *et al*., 2004), species within this genus represent a viable alternative to other grasses, not only because their production can contribute to reductions in GHG emissions and energy use by requiring less N fertilizer. In addition, as *Miscanthus* is perennial, it does not require annual energy-intensive management such as tillage and seedbed preparation. All these factors can contribute to a relatively low global warming potential (GWP) of *Miscanthus* as a bioenergy crop.

Over the past 20-30 years, considerable interest has been directed toward *M.* x *giganteus* as a source for bioenergy due to some of the advantages previously mentioned. Interestingly, *M.* x *giganteus*, which is a sterile allopolyploid hybrid, was first collected in Japan in 1935 and then cultivated throughout Europe as an ornamental plant (Scally *et al*., 2001). In Japan, natural and semi-natural grasslands, which constitute 4% of the land area, are comprised of several graminoid and forb species, including *Miscanthus sinensis*, which is one of the parent species of *M.* x *giganteus* (Himiyama *et al*., 1995). Nearly 24% of these grasslands are dominated by *M. sinensis* (National Parks Association of Japan, 1996). Extensive research in Japan over the past several decades focused on the use of *M. sinensis* as thatching material for roofs of traditional houses and buildings, organic fertilizer, and livestock feed (Stewart *et al*., 2009).

Past studies also focused on biomass production and vegetation characteristics of *M.*

sinensis grasslands (e.g., Numata, 1975; Midorikawa, 1978). Within the past 10 years, research has been directed towards understanding the effects of these grasslands on climate change (cf. Yazaki *et al*., 2004; Toma *et al*., 2010a). However, there are few studies on C-cycling in *M. sinensis* grasslands (Yazaki *et al*., 2004). There is also no information available concerning the impact of cultivation of *M. sinensis* on CH_4 and N_2O emissions from the soil. In Europe and the U.S., considerable effort has been devoted over the past 10-20 years to understanding C sequestration in *M.* x *giganteus* fields (Hansen *et al*., 2004; Clifton-Brown *et al*., 2007). However, those studies were conducted in relatively short-term research plots (i.e., 16 years or less) and may not represent long-term conditions. The slower rates of turnover of the humic fractions of organic matter in soil suggest the greatest long-term benefits arise from sequestration in more recalcitrant soil-C pools (Clifton-Brown *et al*., 2007). In contrast to relatively short duration of time cultivated fields of *M.* x *giganteus* have been established, *M*. *sinensis*-dominated grasslands in Japan have been actively managed, mostly through burning and mowing, for decades to hundreds of years (Otaki, 1999).

Although *M. sinensis* exhibits high productivity (1.8 to 13 Mg ha^{-1} yr^{-1}, Stewart *et al*., 2009), biomass production depends on environmental conditions such as temperature and general soil properties (Numata & Mitsudera, 1969). However, there is interest in producing bioenergy crops in lands that may be marginal for other more traditional agricultural crops. The ability of *M. sinensis* to thrive in limiting environments may represent an important niche for this species as different environments are considered for bioenergy production. However, it is critically important to increase our fundamental understanding of C budgets and greenhouse gas emissions under such limiting environments.

The unique settings of both long-term management and limiting environmental conditions found in some *M. sinensis* grasslands in northern Japan represent a valuable opportunity to study C sequestration and GHG emission parameters. Although the soil and climatic conditions in Japan where these grasslands naturally occur likely differ from where they could be cultivated in the U.S. and Europe, it should be acknowledged that they provide unique and incomparable information in determining how *Miscanthus* influences soil dynamics, at least until more long-term data can be acquired in soils more suited for agriculture. Also, this information could ultimately be related to long-term production of *Miscanthus* for biomass or biofuel. Thus, our objective was to characterize the C budget and CH_4 and N_2O emissions in a naturally established *M. sinensis* grassland growing on nutrient-poor soils in a cool climatic region.

Materials and Methods

Site description

A *Miscanthus sinensis*-dominated grassland located in central Hokkaido, Japan (42°40’N, 141°45’E, 2 m above sea level), in which *M. sinensis* vegetation has naturally grown for more than 30 years on sandy, siliceous, mesic typic endoaquands (USDA, 2010), was selected near the northern natural limit of *M. sinensis*-type grassland (139°47’N to 148°45’N) (Numata 1969) as the study site (0.1 ha). The study was conducted during 2008 and 2009. Selected physical and chemical properties of the soil are presented in Table 1. The top 16-cm layer (horizons Ah1 to C) was deposited in 1739 by a volcanic eruption of Mt. Tarumae (Soya, 1972). The 16 to 40-cm soil depth increment (horizons 2Ab to 2C2) was deposited in 1667, also by an eruption of Mt. Tarumae (Soya, 1972). Except for the top layer, which consisted of sandy loam soil, most of the profile was sand (Table 1). Bulk density of the Ah1 horizon (0 to 5 cm) was 0.2 g cm^{-3}, which increased with depth from 0.68 to 1.05 g cm^{-3}. The top 30-cm layer had 55.7 Mg C ha^{-1} and 3.9 Mg N ha^{-1}. Bray(2) P in the top horizon was low (5.1 mg P kg^{-1}) compared to deeper horizons (24.7-50.6 mg P kg^{-1}). Exchangeable K in the top three horizons was 165-189 mg K kg^{-1}. The site had a 30-year mean annual precipitation of 1,190 mm and air temperature of 7.6°C (Table 2). The 30-year mean temperature of the growing season, which is generally from 1 May to 1 November, where daily mean temperatures are ≥10°C, was 14.7°C. Accumulated effective temperature (AET), which is the sum of mean daily temperatures over 10°C, was calculated following the method of Stewart *et al*. (2009).

Plant species in the study site included *Miscanthus sinensis* (25% canopy coverage), woody plant species (*Myrica gale* (13%) ,*Spiraea salicifolia* (3.5%), and *Lonicera caerulea* (1.0%)), fern species (*Thelypteris palustris* (33%) and *Osmunda cinnamomea* (3.2%)), and herbaceous species (*Aster tripolium* (14%), *Sanguisorba tenuifolia* (3.4%), *Rubia jesoensis* (1.3%), and *Lycopus maackianus* (0.9%)). Canopy coverage of each species was calculated following the method of JSGS (2004) in August 2009. The site was originally a wetland, but was drained with canals beginning in 1972, and subsequently had no additional management. After draining, it is assumed *M. sinensis* naturally established at the study site.

Treatments

Four 1 m x 1 m areas within the study site were randomly assigned as vegetation (V) plots for measurement of CH_4 and N_2O emissions. Three bare-plot treatments (B1, B2, and B3), were randomly assigned to three respective 1 m x 1 m plots for measurement of CO_2 emission. After a glyphosate [potassium *N*-(phosphonomethyl)glycinate] herbicide application, which was applied 50 cm beyond the perimeter of the plots, on 9 June 2008, aboveground dead vegetation in each herbicide-treated area was cut and removed. Root-resistant, water-permeable sheets (Toyobo BKS9812) were installed at a 20-cm soil depth around each of the bare-plot treatments to prevent root growth into the plots. After this initial procedure, the B1 plot remained undisturbed. In the B2 plot, the top 20 cm of soil was removed, sieved with 2-mm mesh to remove all belowground plant material, and then returned to the excavated area. In the B3 plot, the same amount of disturbance as in B2 was produced by sieving, but all soil and plant materials were returned to the excavated area.

Measurement of aboveground and belowground biomass

Aboveground and belowground biomass of *M. sinensis* and other plant species were collected from eight randomly selected 1-m^2 plots within the study site on 18 May, 1 September and 4 November 2008. Aboveground biomass was collected on 11 May, 1 September and 28 October 2009, whereas belowground biomass was collected only on 11 May 2009. After aboveground biomass of *M. sinensis* and other species were collected, living and dead plant tissues of *M. sinensis* were separated based on differences in color in September, whereas aboveground biomass of other plants included both living and dead plant material. Belowground biomass was calculated from materials collected from sieving (4-mm mesh) the top 20 cm of soil (Hayashi *et al*., 1981; Shimizu *et al*., 2009). Rhizomes of *M. sinensis* were separated from roots. Given that roots of *M. sinensis* could not be distinguished accurately from other roots, no separation was attempted. All plant samples were oven-dried at 70°C for 48 hours to constant weight and were then weighed, ground, and analyzed for C with an elemental analyzer (Vario EL III, Elemental, Hanau, Germany).

Calculation of aboveground and belowground net primary production

Throughout Japan, *M. sinensis* initiates growth in spring and senescence prior to the onset of winter (Stewart *et al*., 2009). Considering this seasonal phenology of *M. sinensis*, aboveground net primary production (ANPP) of *M. sinensis* was estimated by its biomass C at the end of the growing season. Since dead biomass of the previous growing season was not removed at the study site in Tomakomai, some equations were used to not include these data in the calculation of ANPP of the current growing season. Aboveground net primary production of *M. sinensis* was calculated as follows:

$$\mathrm{ANPP}\ (\mathrm{Mg\ C\ ha^{-1}}) = \mathrm{MBC_{new}} - \mathrm{MBC_{old}}$$

where $\mathrm{MBC_{new}}$ is *M. sinensis* biomass C at the end of the growing season (November), and $\mathrm{MBC_{old}}$ is *M. sinensis* biomass C in standing biomass from the previous growing season at the end of the current growing season. We assumed standing *M. sinensis* biomass from the previous season decreased at a constant rate during the current growing season (Koike *et al*., 1975). Hence, $\mathrm{MBC_{old}}$ was estimated by the decrease of dead *M. sinensis* biomass C from May to September as follows:

$$\mathrm{MBC_{old}} = [(\mathrm{MBC_{Sep}} - \mathrm{MBC_{May}}) / \mathit{\Delta d}] \times \mathit{\Delta d}' + \mathrm{MBC_{May}}$$

where MBC_{May} and MBC_{Sep} are dead *M. sinensis* biomass C in May and September, respectively, Δd is days between May and September and $\Delta d'$ is days between May and November.

Peak biomass production of other plant species, which included both annual and perennial plants, occurred during the summer. Considering no dead standing biomass was present from the previous year, ANPP of other plant species for each growing season was calculated by the following equation:

$$\text{ANPP (Mg C ha}^{-1}) = \text{OPBC}_{\text{Sep.}} - \text{OPBC}_{\text{May}}$$

where $OPBC_{Sep.}$ is biomass C of other plant species in September and $OPBC_{May}$ is biomass C of other plant species in May.

Belowground net primary production (BNPP) was also calculated from the seasonal difference in biomass C of rhizome or roots of *M. sinensis* and other plant species. However, the time interval to determine BNPP was different for roots and rhizomes, given peak growth occurs at different times. Matumura (1998a) reported rhizome biomass decreased before panicle formation because initial *M. sinensis* growth and development occurred from resources stored in rhizomes. After panicle formation, *M. sinensis* generally replenishes depleted energy reserves (Matumura, 1998a). Since peak growth of rhizomes occurs after panicle formation and before dormancy, BNPP of *Miscanthus* rhizomes ($BNPP_{Rhizome}$) was determined for the period between September and November.

$$\text{BNPP}_{\text{Rhizome}} = \text{RhiC}_{\text{Nov}} - \text{RhiC}_{\text{Sep}}$$

where $RhiC_{Sep.}$ and $RhiC_{Nov.}$ are biomass C of rhizomes in September and November, respectively. However, root development is rapid in the growing season to secure adequate access to water and nutrients for the rapidly growing shoot, but root growth diminishes once plants reach reproductive stages (Eissenstat & Yanai, 2002). Thus, BNPP of roots ($BNPP_{Root}$) was determined during the peak period of growth between May and September.

$$BNPP_{Root} = RC_{Sep} – RC_{may}$$

where RC_{may} and $RhiC_{Sep.}$ are biomass C of rhizome in May and September, respectively. Total BNPP was calculated as follows:

$$BNPP = BNPP_{Rhizome} + BNPP_{Root.}$$

Measurement of CO_2, CH_4, and N_2O fluxes

Fluxes of CH_4 and N_2O were measured in the V plots with a closed-chamber method described by Toma and Hatano (2007). Also, CO_2 flux was measured in the B1, B2, and B3 plots. Stainless steel bases were installed on 21 April 2008 in the V plots and in 31 July 2008 in the three bare plots. Living plant material was not included in the bases. Gas samples for calculating gas fluxes were collected four to five times per month from April to November in both years. Gas fluxes were measured from 10:00 to 14:00. Gas samples (volume = 250 mL) were collected into a tedlar bag (volume = 500 mL) for CO_2 determination 0 and 6 minutes from the time the chambers were deployed (Nakano *et al*., 2004). Carbon dioxide concentrations were measured with a CO_2 analyzer (ZFP-9, Fuji Electric Systems, Tokyo, Japan). Vacuumed 10-mL vials sealed with butyl rubber stoppers (SVF-10, Nichiden-Rika, Kobe, Japan) were used to collect 20-mL CH_4 and N_2O gas samples at 0, 15, and 30 minutes after chamber deployment. Methane and N_2O concentrations were determined with a gas chromatograph equipped with a flameionization detector (GC-8A, Shimadzu, Kyoto, Japan) and an electron capture detector (GC-14B, Shimadzu, Kyoto, Japan), respectively.

Fluxes of CO_2, CH_4, and N_2O were calculated with the following equation:

$$F = \rho \times V/A \times \Delta c/\Delta t \times 273/(273 + T) \times \alpha$$

where F is the flux (mg m^{-2} h^{-1}); ρ is the gas density (1.977×10^6 mg m^{-3} for CO_2, 0.717×10^6 mg m^{-3} for CH_4, and 1.978×10^6 mg m^{-3} for N_2O); V is the volume of the chamber (m^3); A is the cross-sectional area of the chamber (m^2); $\Delta c/\Delta t$ is the ratio of change in the gas concentration (c) inside the chamber per unit time (t) during the sampling period (m^3 m^{-3} h^{-1}); T is the air temperature (°C), and α is a conversion factor for CO_2 to C (= 12/44), CH_4 to C (= 12/16), or N_2O to N (= 28/44).

Calculation of heterotrophic respiration, and cumulative methane and nitrous oxide emissions

Under natural grassland conditions, CO_2 emission from soil surface, which is considered soil respiration (Rs), is the combination of heterotrophic respiration (Rh) and root respiration (Hanson *et al*., 2000; Subke *et al*., 2006). In addition, disturbance of soil structure (e.g. when roots, rhizomes, etc. are removed from soil) often induces CO_2 emission from soil (e.g. Curtin *et al*., 2000; Reicosky *et al*., 1997). The bare-plot treatments were established to accurately estimate Rh, which is often difficult to measure separately from Rs under field conditions (Hanson *et al*., 2000; Subke *et al*., 2006), in the V plots. Components of soil CO_2 flux from each of the bare-plot treatments were determined as follows:

B1: CO_2 flux = Rh + decomposition of dead belowground plant material

B2: CO_2 flux = Rh + CO_2 emission induced by soil disturbance

B3: CO_2 flux = Rh + decomposition of dead belowground plant material + CO_2 emission induced by soil disturbance

Thus, Rh was estimated the following equation.

$$Rh = CO_2 \text{ flux in B1} - (CO_2 \text{ flux in B3} - CO_2 \text{ flux in B2})$$

CO_2 flux from soil strongly depends on temperature (Boone *et al*., 1998). Thus, cumulative Rh will be underestimated if daily variation of soil temperature is not considered. Considering that soil temperature in the V plots may have been lower than in the bare-plot treatments due to the removal of aboveground biomass, Rh in the V plots was estimated using hourly soil temperature data measured at 5-cm depth in the V plots and a regression model between soil temperature at 5-cm depth and CO_2 flux in the bare-plot treatments (Shimizu *et al*., 2009). The regression model, which is a correction function for CO_2 flux (Shimizu *et al*., 2009), is the following:

$$f_{(T)} = a*\exp(b*T)$$

where $f_{(T)}$ is the CO_2 flux (mg C m^{-2} hr^{-1}) and T is soil temperature at 5-cm depth (°C). Thus, the Rh at t °C in V plots ($f_{Rh(t)}$) was estimated using the regression model $f_{(T)}$ for each bare plot (B1 ($f_{B1(t)}$), B2 ($f_{B2(t)}$) and B3 ($f_{B3(t)}$)).

$$f_{Rh(t)} = f_{B1(t)} - (f_{B2(t)} - f_{B3(t)})$$

where t is the soil temperature at 5-cm depth (°C) in V plots. Hourly data of soil temperature was measured with a thermocouple (n = 12) connected to a data logger (FreeSlot-68KD, M.C.S, Hokkaido, Japan) beginning on 30 March 2008 until the experiment was over. The regression model was created by using CO_2 flux and soil temperature derived from manual measurements (n = 5) at 5-cm depth with a digital thermometer (CT-413WR, CUSTOM, Tokyo, Japan) near the soil chamber at the time of gas measurement in the bare-plot treatments. Cumulative Rh, CH_4 and N_2O emissions were calculated by linear integration of flux measurements during the growing season of both years (Toma & Hatano, 2007; Toma *et al*., 2010b).

Calculating carbon budget and global warming potential

Carbon budget was calculated as follows:

$$\text{C budget} = \text{ANPP} + \text{BNPP} - \text{Rh} - CH_4 \text{ emission}$$

where ANPP and BNPP are the sums of both *M. sinensis* and other plant species.

Based on estimates that CH_4 and N_2O GWP are 23 and 296 times higher than CO_2, respectively, GWP was calculated by the following equation in CO_2-equivalents (IPCC, 2001)

$$\text{GWP} = CH_4\text{-C emission} \times 16/12 \times 23 + N_2O\text{-N emission} \times 44/28 \times 296 - \text{C budget} \times 44/12$$

Negative GWP values indicate mitigation of global warming.

Ancillary measurements

A composite of three core soil samples from each plot was collected from the 0-10-cm and 10-20-cm depth increments at each gas sampling measurement. Soil samples were analyzed for NH_4^+ and NO_3^- (Toma *et al*., 2010b). Soil samples were extracted with deionized water (1:5) and 2M KCl (1:10) for determining NO_3^- and NH_4^+ concentrations, respectively, in the soil. Samples for soil water-filled pore space (WFPS) and volumetric water content calculations were also collected from each plot at the time of gas sampling.

Soil porosity and bulk density was determined from samples collected on 8 August 2008 (porosity = 0.821 cm^3 cm^{-3} for 0-10-cm depth and 0.761 cm^3 cm^{-3} for 10-20-cm depth, bulk density = 0.274 g cm^{-3} for 0-10-cm depth and 0.515 for 10-20-cm depth). Air temperature and precipitation data were collected from a weather station in the Tomakomai area.

Statistical analysis

All statistical analyses were performed using “R” (version 2.10.1, R Development Core Team, 2005). Differences in belowground total C biomass across the three measurement times (18 May, 1 September and 4 November) in 2008 and across the two years (2008 and 2009) in May were analyzed by analysis of variance. Regression analysis between soil temperature and CO_2 flux was performed by the exponential least squares method. Differences in the regression curves between 2008 and 2009 were also analyzed. The one-sided 95% confidence interval was calculated as follows:

One-sided 95% confidence interval = (two-sided 95% confidence interval)/2

The two-sided 95% confidence interval of Rh was estimated from the regression equation of soil temperature and CO_2 flux. The two-sided 95% confidence interval of ANPP, CH_4 and N_2O emissions were determined as follows:

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

where d.f. is the degree of freedom (d.f. = 3 for CH_4 and N_2O emissions, d.f. = 7 for ANPP), and t (d.f., 0.05) is the t value at 5% significant level with a two-sided alternative.

Results

Although mean annual temperatures in 2008 and 2009 were 0.3 and 0.4°C higher than the 30-year average, respectively, mean temperature for the growing season was near average in 2008 and 0.9°C lower than average in 2009 (Table 2). Precipitation in 2008 was 115 mm lower than the 30-year average, whereas in 2009, precipitation was close to average (Table 2). Cumulative precipitation in July and August in 2008 and 2009 represented 127% (458 mm) and 130% (463 mm) of the 30-year mean precipitation in July and August, respectively. Accumulative effective temperature was 90°C and 67°C higher than the 30-year average in 2008 and 2009, respectively (Table 2). Panicle formation of *M. sinensis* started on 18 August 2008 and 5 August 2009. Accumulative effective temperature from 1 May, which was when growth began in both years, to panicle formation was 1,471°C and 1,275°C in 2008 and 2009, respectively (Table 2).

Variation in soil temperature at 5-cm depth was similar to that of air temperature (Figs. 1, 2). Water-filled pore space was generally below 40% for both surface (0-10 cm depth) and subsurface (10 to 20 cm depth) soil layers across the growing season in 2008. However, in 2009, only the surface layer was generally below 40% WFPS (Figs. 2c, d). The subsurface layer in 2009 was normally around 40% WFPS with higher levels between July and August due to large precipitation events (Fig. 1). Soil NH_4^+ concentrations were fairly low and constant across 2008 at the surface and subsurface layers of the soil (Fig. 2e). Similarly, NO_3^- concentrations in the subsurface were low and constant in 2008, whereas the surface layer had low concentrations through June with a modest increase thereafter (Fig. 2g). In 2009, NH_4^+ levels were higher than in 2008 especially for the surface soil layer (Fig. 2f). Unlike 2008, surface layer NO_3^-

concentrations in 2009 were generally higher before June (Fig. 2h).

Amounts of aboveground biomass-C of plant materials in both years and belowground biomass C in 2008 are provided in Table 3 and Table 4, respectively. Although belowground biomass was 3.1 to 6.5 times higher than aboveground biomass, there were no significant differences in biomass-C of rhizome or roots of *M. sinensis* and other plant species across sampling times (rhizome: $P = 0.48$, roots: $P = 0.59$). Rhizome-C did not significantly differ between September and November ($P = 0.72$) nor for root-C between May and September ($P = 0.57$). In addition, belowground biomass-C did not significantly differ between May 2008 and May 2009 (rhizome, $P = 0.64$; roots, $P = 0.13$).

There was significant correlation between soil temperature and CO_2 flux in the bare-plot treatments (Figs. 3a-c). In the B1 plot, exponential regression models significantly differed between years (Fig. 3a). Estimated values of Rh increased during the summer (Figs. 4a, b). Average Rh in 2009 was 1.37 times higher than in 2008 (Table 5). Decomposition of dead belowground plant material and CO_2 flux induced by soil disturbance in 2008 and 2009 were varied from 0.29 to 0.55 and -0.56 to -0.90 Mg C ha^{-1} growing season^{-1}.

Methane flux was zero or negative except during August (19.4 µg C m^{-2} h^{-1}) and September (25.2 µg C m^{-2} h^{-1}) in 2008 (Figs. 5a, b). Average CH_4 emission across years calculated from the values in Table 6 was -1.34 kg C ha^{-1} growing season^{-1}. During the growing season, N_2O flux tended to peak in August (13.8 and 14.5 µg N m^{-2} h^{-1} in 2008 and 2009, respectively) (Fig. 5c, d). In 2009, high N_2O fluxes were also observed in April (47.5 and 52.3 µg N m^{-2} h^{-1}) (Fig. 5d). Average N_2O emission across years calculated from the values in Table 6 was 0.22 kg N ha^{-1} growing $season^{-1}$. There was significant, but weak positive correlation between CH_4 flux and WFPS in the top 10 cm of soil throughout the study period (n = 179, R^2 = 0.03, $P < 0.05$).

Averaged across years, total values of ANPP of *M. sinensis* and other plant species calculated from values in Table 7 were 1.04 and 1.34 Mg C ha^{-1} growing $season^{-1}$, respectively. Differences in biomass-C of rhizomes between the periods May to September and September to November in 2008 calculated from values in Table 4 were -0.39 and 0.15 Mg C ha^{-1}, respectively. However, biomass-C values of roots of *M. sinensis* and other plant species for the same time periods were 0.79 and -0.45 Mg C ha^{-1}, respectively. Given the distinct peak growth periods of these different belowground organs, BNPP values of rhizome, root, and the sum of both were calculated to be 0.15, 0.79, and 0.94 Mg C ha^{-1} growing $season^{-1}$, respectively. Unfortunately, due to extreme variability in BNPP measurements, these BNPP values were considered only an approximation due to lack of statistical differences in belowground biomass of rhizomes of *M. sinensis* and roots of *M. sinensis* and other plant species among the three sampling times. Thus, the C budget presented in Table 7 was a partial calculation that did not include BNPP. Although the C budget in 2008 was positive (0.53 Mg C ha^{-1} growing $season^{-1}$), in 2009, it was negative (-1.17 Mg C ha^{-1} growing $season^{-1}$) (Table 7). The average of the C budget across years was -0.31 Mg C ha^{-1} growing $season^{-1}$. Net GWP values in 2008, 2009, and the average across years were -1.95, 4.40, and 1.22 Mg CO_2eq ha^{-1} growing $season^{-1}$, respectively (Table 8). Including the approximation of BNPP in the calculation of the C budget for 2008 would have resulted in a C budget of 1.47 Mg C ha^{-1} growing $season^{-1}$. Given the high variability of belowground biomass measurements, and that biomass-C in May 2009 was not different than in May 2008, it is possible to assume the estimated BNPP value in 2008 would be similar for 2009. Under this assumption, including BNPP in the C budget calculation for 2009 would have resulted in a C budget of -0.23 Mg C ha^{-1} growing season^{-1}. The average of the C budget across years would be 0.62 Mg C ha^{-1} growing season^{-1}. Similarly, if the estimated BNPP value were included in the calculations of GWP in 2008 and 2009, GWP would have been -5.40 and 0.95 Mg CO_2eq ha^{-1} growing season^{-1}, respectively. The average of GWP across years would have been -2.22 Mg CO_2eq ha^{-1} growing season^{-1}.

Discussion

Environmental factors for carbon budget

The average C budget during this study was -0.31 Mg C ha^{-1} growing season^{-1}, which indicates C was released from the grassland ecosystem to the atmosphere. However, including the approximate value of BNPP, the average C budget of the *M. sinensis* grassland in Tomakomai would have been 0.62 Mg C ha^{-1} growing season^{-1}. Dominant components in the C budget appeared to be ANPP, Rh, and BNPP (Table 7). Total ANPP of *M. sinensis* and other plant species in our study site was low (1.94 to 2.80 Mg C ha^{-1} growing season^{-1}) compared to other studies. Yazaki *et al*. (2004) reported ANPP ranging from 11.4 to 12.1 Mg C ha^{-1} yr^{-1} for a *M. sinensis* grassland on an Andisol soil in Nagano, Japan (36°31'N, 138°21E, 1,315 m above sea level). They suggested the high ANPP of the *M. sinensis* grassland, relative to that of 10 western North American grasslands reported by Sims and Singh (1978), might have been due to high precipitation. Jones and Walsh (2001) reported dry weight yields of *M*. x *giganteus* increased with increasing precipitation and cumulative temperature during the growing season (1 May to 31 October). Dry weight yields of *M*. x *giganteus* maximized (19 Mg ha^{-1}) at approximately 400 mm of precipitation with a cumulative temperature of 2,740°C during the growing season based on data collected from several fields site throughout Europe (Clifton-Brown *et al*., 2001). Moreover, Sala *et al*. (1988) and Paruelo *et al*. (2010) reported a significant positive linear relationship between annual precipitation and ANPP in grassland ecosystems in the U.S. Midwest and south-central South America. Although Yazaki *et al*. (2004) considered high precipitation (1,288-1,396 mm) to contribute to high ANPP in the *M. sinensis* grassland in Nagano, ANPP at the Tomakomai study site was lower despite having comparable precipitation (1,075-1,263 mm). In addition, mean annual temperature at our study site ranged from 7.9 to 8.0°C, which is more conducive for growth than the mean annual temperatures of 6.1 to 6.5°C in the study by Yazaki *et al*. (2004). It appears the low ANPP at our study site was not the result of these climatic conditions.

Numata and Mitsudera (1969) reported height of *M. sinensis*, which can be used to estimate aboveground biomass (Yoshida, 1976), increased with increasing thickness of surface-soil A horizon in Miyagi, Japan. Thicker A horizons are often the result of soil development that can increase not only fertility levels but other soil properties that are important for plant development. Thickness of the A horizon in the study field of Yazaki *et al*. (2004) was 62 cm (Suzuki *et al*., 1999), which was deeper than that of our study site. The *M. sinensis* grassland at Tomakomai was established on a young volcanic ash soil that started to develop 269 years ago. In addition to the shallow depth of the A horizon at the study site, the entire rooting depth was limited by a water table 40 cm below the soil surface. Unfortunately, the study by Yazaki *et al*. (2004) provides no information about soil-fertility parameters. In an adjacent study to the Tomakomai grassland study site, application of P fertilizer elicited a significant yield response (data not published). Although *Miscanthus* is considered to have low-nutrient requirements (Heaton, *et al*., 2004; Lewandowski *et al*., 2003; Stewart *et al*. 2009), it is important to acknowledge insufficient availability of nutrients could negatively impact *M. sinensis* productivity. Insufficient fertility, possibly along with other soil or environmental constraints, may have limited ANPP at the *M. sinensis* grassland in Tomakomai.

Aboveground net primary production of *M. sinensis* and other plant species was lower in 2009 than in 2008 (Table 7). This was probably due to lower temperatures during the growing season in 2009 than in 2008 (Table 2). It is well known that net photosynthetic rates of C_3 and C_4 plant species increase with increasing temperature when temperatures are between 10 to 20°C (Berry & Björkman, 1980; Hikosaka, 2004). In addition, AET during the 2009 growing season, including the period of panicle formation, was 196°C lower than in 2008 (Table 2). Matumura (1997) reported biomass production of *M. sinensis* increased with increasing AET from spring until panicle formation.

Heterotrophic respiration was 2.27 and 3.11 Mg C ha^{-1} growing $season^{-1}$ in 2008 and 2009, respectively (Table 7). Due to snow cover and low temperatures (approximately 0°C) during the winter season at the Tomakomai study site (Fig. 3), CO_2 flux was likely very low. Moreover, nearly 0 mg C m^{-2} h^{-1} was reported in a field of reed canarygrass (*Phalaris arundinacea*) under similar growing conditions in Hokkaido as the *M. sinensis* grassland in Tomakomai during the winter season (Shimizu *et al*., 2009). Therefore, calculated Rh at the *M. sinensis* grassland likely approximates that of the entire year. In addition to the 20-cm deep root barriers installed in the soil around the bare plots, herbicide was applied 50-cm beyond the perimeter of the plots. Although the method for calculating Rh has been adopted (Subke *et al*., 2006), measured CO_2 flux may be influenced by decomposition of dead belowground plant materials and soil disturbance. It is still needed to improve the method for Rh calculation. However, calculated Rh in this study is adoptable since the effect of dead belowground plant materials and soil disturbance on Rh was removed as much as possible. Hence, the difference in Rh between two years might not be due to the intrusion of living plant roots into the bare plots. There is generally an optimal soil moisture range where soil CO_2 flux is maximized (Gulledge & Schimel, 1998; Harper *et al*., 2005, Yan *et al*., 2010). Harper *et al*. (2005) reported soil CO_2 flux increased with increasing soil temperature, but reached a maximum at approximately 30% of volumetric soil water content in a silty clay loam soil. Toma *et al*. (2010b) reported Q_{10}, which is a relative increase of CO_2 flux for every 10°C change in soil temperature, increased from 1.11 to 2.38 with increasing annual precipitation that increased from 1,015 to 1,576 mm. At the Tomakomai study site, precipitation during the growing season in 2009 was 20% higher (148 mm) than in 2008 (Table 2). In addition, exponential regression models of the B1 plot in 2009 was significantly higher than in 2008 (Fig. 3a), which may indicate higher moisture conditions in 2009, relative to 2008, were responsible for the increase in Rh during 2009.

It should be considered that CO_2 emissions from the study site might have included C that originated from the oxidation of organic matter accumulated when the site was a wetland. Increase in CO_2 emissions from drained wetlands is a well-known phenomenon (Nykänen *et al*., 1995; Wright & Reddy, 2001). However, CO_2 emission in our study was lower compared to other studies. Shimizu *et al*. (2009) reported Rh ranged from 3.6 to 3.9 Mg C ha^{-1} yr^{-1} in a field of reed canarygrass in Hokkaido, Japan (42°26'N, 142°29'E) on Andisols with the same parent material as the *M. sinensis* grassland in Tomakomai. Frank *et al*. (2004) reported Rh ranged from 4.52 to 5.09 Mg C ha^{-1} yr^{-1} in a switchgrass field on Mollisols in South Dakota, USA (46°46'N, 100°55'W). In those sites, mean annual temperatures were higher than at the Tomakomai site (Table 9). Although soil CO_2 flux is influenced by temperature (Boone *et al*., 1998), these studies were all in cooler northern latitudes where temperatures may not have such a strong influence because increasing rate of CO_2 flux may low at the low temperature compared with that at high temperature (Boone *et al*., 1998). More importantly, however, soil organic-C levels are considered to be the primary source of CO_2 produced in soil. At the Tomakomai site, soil C in the 0 to 30 cm depth increment was 55.7 Mg C ha^{-1} compared to 76.6 Mg C ha^{-1} in a reed canarygrass field reported by Shimizu *et al*. (2009). Soil CO_2 flux levels reported by Shimizu *et al*. (2009), which were higher than those measured in the *M. sinensis* grassland in Tomakomai, were likely due to the higher soil-C levels at their study site.

The amount of variability across sampling times made it difficult to estimate BNPP. Although it is a basic and important ecological variable, estimating BNPP has also been identified as a significant challenge in many other studies (Lauenroth, 2000; Midorikawa, 1978; Yazaki *et al*., 2004). Midorikawa (1978) and Yazaki *et al*. (2004) emphasized the difficulty of estimating BNPP in *M. sinensis* grasslands in Japan due to the large spatial variation in belowground biomass. The large amount of variability that can exist for this measurement was illustrated in a study on productivity levels of steppes and prairies in Uruguay, Argentina, and Brazil (Paruelo *et al*., 2010). BNPP ranged from 2.64 to 5.68 Mg C ha^{-1} growing season^{-1}. Cahill *et al*. (2009) reported that the ratio of BNPP to ANPP of C$_3$ (e.g. *Bromus inermis* Leyss., *Phleum pratense* L.) and C$_4$ (e.g. *Andropogen gerardii*, switchgrass, *Sorghastrum nutans*) species varied from 16 to 342% and 21 to 388%, respectively, in Wisconsin, USA (43°4'N, 89°49'W). Belowground net primary production of rhizomes of *M. sinensis* and roots of the entire plant community in the *M. sinensis* grassland in Tomakomai were approximated based on the growth pattern of rhizome and roots (Table 7). The strategy of *M. sinensis* to use stored reserves in the rhizome for re-growth, reported by Matumura (1998a), has been observed for other species, including alfalfa (*Medicago sativa* L.), which uses stored reserves in its roots, and *M*. x *giganteus* (Berg *et al*., 2009; Suzuki & Stuefer, 1999). Although our measurements provide only a crude estimate of BNPP, this value indicates the *M. sinensis* grassland ecosystem in Tomakomai might act as a sink of atmospheric C. Numata (1976) reported ANPP and BNPP of a *M. sinensis* grassland in Miyagi, Japan were 5.0 and 1.75 Mg ha^{-1} yr^{-1}, respectively. Based on other studies of the same grassland, Numata (1976) also reported BNPP of *M. sinensis* was 25 to 30% of ANPP. Considering ANPP of *M. sinensis* in our study was 0.93 to 1.14 Mg C ha^{-1} growing season^{-1}, if values reported by Numata (1976) are used, BNPP of *M. sinensis* could possibly range from 0.23 to 0.34 Mg C ha^{-1} yr^{-1}. This value range would be higher than our estimated values and would indicate that our estimate is very conservative. Given belowground biomass of *M. sinensis* and other plant species in Tomakomai was large compared with aboveground biomass may provide additional evidence suggesting this grassland represents a C sink.

Comparison of C budget in M. sinensis grassland with other vegetations

Without taking into account BNPP, the average C budget was -0.31 Mg C ha^{-1} growing season^{-1}. Similar values were obtained by Yazaki *et al*. (2004). They reported the C budget of a *M. sinensis* grassland established in an Andisol soil in Nagano, Japan, varied from -1.0 to -0.5 Mg C ha^{-1} yr^{-1}. These values were also calculated without accounting for BNPP since it could not be measured due to large variability. When no mowing was done, the C budget varied from 3.57 to 4.03 Mg C ha^{-1} yr^{-1}. Values of C budget at the Tomakomai site, and that reported by Yazaki *et al*. (2004), were low compared with those reported for *M. x giganteus* under production management conditions (Clifton-Brown *et al*., 2007; Hansen *et al*., 2004, Table 9). Hansen *et al*. (2004) reported accumulated soil-C, which was detected by changes in δ^{13}C in soil under 9- and 16-year-old fields of *M.* x *giganteus* grown on Inceptisols was 0.78 and 1.12 Mg C ha^{-1}, respectively. In addition, Clifton-Brown *et al*. (2007) reported 0.59 Mg C ha^{-1} yr^{-1} of soil-C was derived from *M*. x *giganteus* production over a 15-year period on Inceptisols in Ireland (52°39'N, 07°50'W), which was based solely on BNPP given aboveground biomass had not been included in the calculation. Average estimated yields of *M*. x *giganteus* have been reported to be as high as 22.4 Mg ha^{-1} (Heaton *et al*., 2004), whereas aboveground biomass yield of *M. sinensis* ranges from 1.8 to 13 Mg ha^{-1} in grasslands in Japan (Stewart *et al*., 2009). Hence, C accumulation in *M. sinensis* grassland under natural conditions might be lower than that in cultivated *M*. x *giganteus* fields. For cultivated switchgrass, C accumulation rate ranged from 0 to 10 Mg C ha^{-1} yr^{-1} (Zan *et al*., 2001; Lee *et al*., 2007; Frank *et al*., 2004, Table 9). Except for the reported values of C budget in a switchgrass field where organic fertilizer was applied (Lee *et al*., 2007), C accumulation rate in switchgrass fields might be larger than for *M. sinensis* grasslands (Table 9).

Methane and nitrous oxide emissions

During the study period, negative CH_4 fluxes frequently occurred (Figs. 5a, b), which indicated atmospheric CH_4 was absorbed into the soil. Positive CH_4 fluxes occurred infrequently in August and September of 2008, which was likely due to relatively high soil moisture that may have caused hypoxic conditions. Low soil redox potential generally results in high CH_4 flux due to increased anaerobic respiration (Schimel *et al*., 1993; Conrad, 1995). This was further confirmed by the significant positive correlation between CH_4 flux and WFPS (0-10 cm) in the *M. sinensis* grassland in Tomakomai. Mori *et al*., (2008) also reported there was a significant positive correlation between CH_4 flux and soil moisture content in mixed orchardgrass (*Dactylis glomerata*) grasslands established on Andisols in Tochigi, Japan (36°55'N, 139°55'W). At the Tomakomai study site, CH_4 flux was approximately -50 µg C m^{-2} h^{-1}. Mosier *et al*. (1991) reported CH_4 flux ranged from -4.5 to -46 µg C m^{-2} h^{-1} in a shortgrass steppe in Colorado, USA (40°48'N, 104°45'W). Boeckx and Van Cleemput (2001) reported CH_4 fluxes in European grasslands varied from -5.6 to -35 µg C m^{-2} h^{-1}. Moreover, they suggested the average CH_4 oxidation capacity in grassland soils was -1.88 kg C ha^{-1} yr^{-1} (-2.5 kg CH_4 ha^{-1} yr^{-1}) which was similar to our results in 2008 (-1.07 kg C ha^{-1} growing season^{-1}) and 2009 (-

1.62 kg C ha^{-1} growing $season^{-1}$) (Table 6). Because CH_4 emission was nearly 0 µg C m^{-2} h^{-1} during the winter in a field of reed canarygrass on Andisols in Hokkaido, Japan with the same parent material as the *M. sinensis* grassland in Tomakomai (Hatano *et al*., 2008), CH_4 emission measured in Tomakomai during the growing season could be regarded as an annual CH_4 emission. As such, these data indicate the *M. sinensis* grassland in Tomakomai might act as a sink of atmospheric CH_4.

Nitrous oxide flux varied from -7.7 to 52 µg N m^{-2} h^{-1} (Fig. 5), which is similar in magnitude to what has been reported in other studies (Jørgensen *et al*., 1997; Mori *et al*., 2005; Mosier *et al*., 1991). Jørgensen *et al*. (1997) reported that N_2O flux from an unfertilized *M.* x *giganteus* field on Inceptisols ranged from -1 to 35 µg N m^{-2} h^{-1} in Hornum, Denmark. Mori *et al*. (2005) reported N_2O flux ranged from 1 to 122 µg N m^{-2} h^{-1} in an unfertilized, mixed orchardgrass grassland on Andisols in Tochigi, Japan. Mosier *et al*. (1991) reported N_2O flux in an unfertilized shortgrass steppe in Colorado ranged from 0 to 45 µg N m^{-2} h^{-1}. High N_2O flux was observed in April 2009 at the *M. sinensis* grassland in Tomakomai, which is during the period of heavy snowmelt in central Hokkaido. Tiedje (1994) and Flessa *et al*. (1995) reported high N_2O emissions from soil in the winter and early spring could be a general phenomenon in temperate and boreal climates where soils are subjected to periodic freezing and thawing. In August 2008 and 2009, N_2O flux slightly peaked to 13.8 and 14.5 ug N m^{-2} h^{-1}, respectively. Nitrous oxide is usually produced by nitrification and/or denitrification processes in soil (Bouwman *et al*., 2001; Tiedje, 1990). In August, anaerobic conditions might have occurred in soil pore space due to high precipitation. Anaerobic conditions in August also induced denitrification. Average annual N_2O emission at the Tomakomai study site was 0.22 kg

N ha^{-1} growing $season^{-1}$. Because low N_2O emission during the winter, which ranged from 0.3 to 4.7 μg N m^{-2} h^{-1}, was reported in a field of reed canarygrass under similar growing conditions in Hokkaido as the *M. sinensis* grassland in Tomakomai (Jin *et al*., 2010), N_2O emission during the growing season at the Tomakomai study site likely represented CH_4 emission for the entire year. Jørgensen *et al*. (1997) reported average N_2O emission from an unfertilized *M*. x *giganteus* field on Inceptisols from April to August was 0.14 kg N ha^{-1}. Akiyama *et al*. (2006) estimated annual N_2O emission in unfertilized upland fields in Japan was 0.36 kg N ha^{-1} yr^{-1} for well-drained soils and 1.40 kg N ha^{-1} yr^{-1} for poorly-drained soils. Mori *et al*. (2005) reported that annual N_2O emissions from orchardgrass, white clover, and mixed orchardgrass-white clover grasslands were 0.39, 1.59, 0.67 kg N ha^{-1} yr^{-1}, respectively. Stehfest and Bouwman (2006) reported that estimated annual N_2O emissions in grasslands and steppe was 0.48 kg N ha^{-1} yr^{-1}, whereas annual N_2O emission in temperate forest was 0.22 kg N ha^{-1} yr^{-1}. It appears N_2O emission in the *M. sinensis* grassland was lower than in unfertilized upland fields or grasslands and similar to that of temperate forests.

Global warming potential in Miscanthus sinensis grassland

Average net GWP in this study was 1.22 Mg CO_2eq ha^{-1} growing $season^{-1}$, indicating that *M. sinensis* grassland at our study site accelerated global warming. However, including the approximated BNPP value results in average net GWP of -2.22 Mg CO_2eq ha^{-1} growing $season^{-1}$. Mosier *et al*. (2005) reported net GWP ranged from -0.80 to -0.48 Mg CO_2eq ha^{-1} yr^{-1} in a restored prairie, whereas it varied from -0.63 to 0.31 Mg CO_2eq ha^{-1} yr^{-1} in an upland fields under corn (*Zea mays*), soybean (*Glycine max*), and wheat

(*Triticum aestivum*) rotation in Colorado, USA (40°22’N, 103°7’W). Mosier *et al*. (2006) also reported net GWP in an agricultural field under corn-soybean rotation ranged from -1.3 from 2.5 Mg CO_2eq ha^{-1} yr^{-1} in Colorado, USA (40°39’N, 104°59’W). Mu *et al*. (2006) reported net GWP ranged from 0.75 to 1.8 Mg C ha^{-1} yr^{-1} in agricultural fields under different crop rotations in Hokkaido, Japan (43°14’N, 141°50’W). Relative to that reported above, net GWP in this study was relatively low when BNPP was considered in its calculation. This would indicate the *M. sinensis* grassland in Tomakomai may be helping to mitigate global warming. Similar to the C budget of the *M. sinensis* grassland in Tomakomai, NPP of *M. sinensis* and other species and Rh were the primary contributors to net GWP (Table 8).

Conclusions

Evaluation of the C budget and GHG emissions of the *M. sinensis* grassland in Tomakomai indicates the site may act as a C sink and help mitigate global warming. The most important factors for C budget and GWP in this grassland were ANPP, Rh, and BNPP, while CH_4 and N_2O emissions were not as important. Belowground biomass of *M. sinensis* and other plant species was large compared with aboveground biomass. The results of our study illustrate belowground biomass is not a trivial component for the characterization of C budgets and GHG emissions. The inherent variability in the measurement of belowground biomass makes it extremely difficult to accurately determine BNPP, which highlights the need to improve measurement techniques to better characterize the contribution of the belowground portion to the total C budget and GWP. Until variability in the measurement of the various components of the carbon budget and GWP parameters can be reduced or better measured, it will continue to be difficult to accurately estimate ecological benefits associated with these grasslands.

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Table 1. Physical and chemical characteristics of soil at a *Miscanthus sinensis* grassland in Tomakomai, Hokkaido, Japan

Horizon	Depth (cm)	Bulk density (g cm^{-3})	Soil texture				pH	CEC (cmol$_c$ kg^{-1})	TC (g C kg^{-1})	TN (g N kg^{-1})	C/N	P (Bray 2) (mg P kg^{-1})	Exchangeable K (mg K kg^{-1})
			Sand	Silt	Clay								
				(%)									
Ah1	0-5	0.20	76.8	23.2	0	SL	5.5	18.7	94.76	7.39	12.8	5.10	165
Ah2	5-13	0.68	86.0	14.0	0	S	5.65	9.48	63.25	4.59	13.8	42.5	189
C	13-16	0.89	97.4	2.60	0	S	5.76	2.07	9.55	0.53	18.0	24.7	174
2Ab	16-18	0.90	93.6	5.36	1.07	S	5.61	6.49	27.43	1.80	15.2	33.4	34.5
2Bb	18-22	0.91	99.0	0.52	0.52	S	5.72	3.80	6.75	0.34	20.1	50.6	27.0
2C1	22-29	0.94	99.5	0.51	0	S	5.82	0.57	2.65	0.11	24.8	36.1	44.9
2C2	+29	1.05	99.5	0.51	0	S	5.69	0.56	2.20	0.11	20.8	31.8	29.0

Table 2. Climate data of a *Miscanthus sinensis* grassland in Tomakomai, Hokkaido, Japan. The growing seasons in 2008 and 2009 were 6 May to 16 November and 9 April to 8 November, respectively.

Year	Mean temperature (°C)	Precipitation (mm)	AET[§] (°C)	AET from 1 May to panicle formation[†] (°C)
Annual				
30-year average[‡]	7.6	1190		
2008	7.9	1075		
2009	8.0	1263		
Growing season				
30-year average[‡]	14.7	870	2522	
2008	14.6	819	2612	1471
2009	13.8	967	2589	1275

§ Accumulative effective temperature (AET) is accumulative daily mean temperature over 10°C.

† Panicle formation began on 18 August 2008 and 5 August 2009

‡ 30-year average was from 1980 to 2009.

Table 3. Seasonal change of mean biomass carbon (C) of living and dead aboveground biomasses of *Miscanthus sinensis* and other plants in a grassland in Tomakomai, Hokkaido, Japan. Values between parentheses represent standard deviations (*n* = 8).

		Aboveground biomass C (Mg C ha^{-1})		
		M. sinensis		Other plants
Year	Date	Living	Dead	
2008	18 May	-	1.00 (0.61)	0.45 (0.44)
	1 Sep	0.54 (0.19)	0.45 (0.26)	2.12 (0.31)
	4 Nov	-	1.28 (0.83)	1.31 (0.30)
2009	11 May	-	0.83 (0.30)	0.52 (0.32)
	1 Sep	0.55 (0.41)	0.29 (0.26)	1.54 (0.14)
	28 Oct	-	0.94 (0.62)	0.85 (0.41)

Table 4. Seasonal change of mean biomass carbon of belowground biomass of rhizomes of *Miscanthus sinensis* and roots of *M. sinensis* and other plant species in a grassland in Tomakomai, Hokkaido Japan. Values between parentheses are standard deviations ($n = 8$).

Year	Day-Month	Belowground Rhizome of *M. sinensis*	Root of *M. sinensis* and other plant species	Total
2008	18-May	1.57 (0.62)	7.89 (1.66)	9.46 (1.66)
	1-Sep	1.18 (0.40)	8.68 (1.71)	9.86 (1.76)
	4-Nov	1.34 (0.58)	8.23(1.50)	9.57 (1.50)
2009	11-May	1.32 (0.75)	7.49 (4.03)	8.81 (4.04)

Table 5. Heterotrophic respiration, Decomposition of dead belowground plant material, and CO_2 flux induced by soil disturbance during growing season in a *Miscanthus sinensis* grassland in Tomakomai, Hokkaido, Japan. Values between parentheses represent one-sided 95% confidence interval.

Year	Heterotrophic respiration	Decomposition of dead belowground plant material	CO_2 flux induced by soil disturbance
		(Mg C ha^{-1} growing $season^{-1}$)	
2008	2.27 (0.31)	0.55 (0.86)	-0.56 (2.03)
2009	3.11 (1.25)	0.29 (0.36)	-0.90 (0.97)

Table 6. Average methane (CH_4) and nitrous oxide (N_2O) emissions during the growing season in a *Miscanthus sinensis* grassland in Tomakomai, Hokkaido, Japan. Values between parentheses represent one-sided 95% confidence interval.

Year	CH_4 emission (kg C ha^{-1} growing $season^{-1}$)	N_2O emission (kg N ha^{-1} growing $season^{-1}$)
2008	-1.07 (0.59)	0.07 (0.06)
2009	-1.62 (0.25)	0.36 (0.18)
Average	-1.34	0.22

Table 7. Aboveground net primary production (ANPP) of *Miscanthus sinensis* and other plant species, belowground net primary production (BNPP), heterotrophic respiration (Rh), methane (CH_4) emission, and carbon budget during growing season in a *M. sinensis* grassland in Tomakomai, Hokkaido, Japan. Negative values indicate C was emitted from the ecosystem. Values between parentheses represent one-sided 95% confidence interval.

	ANPP (Mg C ha^{-1} growing season^{-1})		BNPP (Mg C ha^{-1} growing season^{-1})		Rh	CH_4 emission	Carbon budget
Year	*M. sinensis*	Other plants species	Rhizomes of *M. sinensis*	Roots of M. sinensis and other plants	(Mg C ha^{-1} growing season^{-1})	(kg C ha^{-1} growing season^{-1})	(Mg C ha^{-1} growing season^{-1})
2008	1.14 (1.17)	1.66 (0.64)	0.15 (0.06)§	0.79 (0.24)§	2.27 (0.31)	-1.07 (0.59)	0.53 (2.69)
2009	0.93 (0.74)	1.01 (0.44)	-	-	3.11 (1.25)	-1.62 (0.25)	-1.17 (1.41)
Average	1.04	1.34	-	-	2.69	-1.34	-0.31

§ Values were not included into the C budget

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Table 8. Above- and belowground net primary production (ANPP and BNPP) of *Miscanthus sinensis* and other plant species; heterotrophic respiration (Rh); methane (CH_4) and nitrous oxide (N_2O) emissions; and global warming potential (GWP) in a *M. sinensis* grassland in Tomakomai, Hokkaido, Japan. Negative values indicate the mitigation of global warming. Values between parentheses represent one-sided 95% confidence interval.

Year	ANPP (Mg CO_2eq ha^{-1} growing $season^{-1}$)		BNPP (Mg CO_2eq ha^{-1} growing $season^{-1}$)		Rh (Mg CO_2eq ha^{-1} growing $season^{-1}$)	CH_4 emission (Mg CO_2eq ha^{-1} growing $season^{-1}$)	N_2O emission (Mg CO_2eq ha^{-1} growing $season^{-1}$)	Net GWP (Mg CO_2eq ha^{-1} growing $season^{-1}$)
	M. sinensis	Other plants species	Rhizomes of *M. sinensis*	Roots of *M. sinensis* and other plants species				
2008	-4.18 (4.29)	-6.09 (2.35)	-0.55 (0.22)§	-2.90 (0.88)§	8.32 (1.12)	-0.03 (0.02)	0.03 (0.03)	-1.95 (5.02)
2009	-3.41 (2.71)	-3.71 (1.61)	-	-	11.4 (4.60)	-0.05 (0.01)	0.17 (0.08)	4.40 (5.58)
Average	-3.80	-4.90	-	-	9.86	-0.04	0.10	1.22

§ Values were not included in the calculation net GWP.

Table 9. Tabulated values of carbon (C) accumulation under *Miscanthus sinensis*, *Miscanthus x giganteus*, and switchgrass (*Panicum virgatum*) fields.

Vegetation	Grassland type	Location	Coordinates	Air temperature (°C)	Precipitation (mm)	Elevation (m)	Soil type	Management	Method	C accumulation (Mg C ha^{-1} yr^{-1})	Authors
Miscanthus sinensis	Natural	Hokkaido, Japan	42°40'N, 141°45'E	7.6	1190	2	Andisols	Natural	E	-1.16 — 0.53	This study
	Semi-natural	Nagano, Japan	36°31'N, 138°21'E	6.5	1126	1315	Andisols	Mowing	E	-1.0 — -0.5	Yazaki et al. (2004)
Miscanthus x giganteus	Intensive	North Jutland, Denmark	56°50'N, 9°26'E	7.4	706	30	Inceptisols	Mowing	δ^{13}C	0.78 — 1.12	Hansen et al. (2004)
	Intensive	Co. Tipperary, Ureland	52°39'N, 7°50'W	9.9	1004	90	Inceptisols	Mowing, fertilization (chemical)	δ^{13}C	0.59	Clifton-Brown et al. (2007)
Switchgrass	Intensive	Quebec, Canada	42°25'N, 75°56'W	6.5	1062	370	N/A	Mowing, fertilization (organic)	SCC	3.5	Zan et al. (2001)
(*Panicum virgatum*)	Intensive	South Dakota, USA	44°10'N, 96°41'W	6.3	602	470	Molisols	Mowing, fertilization (chemical)	SCC	2.4	Lee et al. (2007)
	Intensive							Mowing, fertilization (organic)	SCC	4	Lee et al. (2007)
	Intensive							Mowing	SCC	0	Lee et al. (2007)
	Intensive	South Dakota, USA	46°46'N, 100°55'W	4	404	588	Molisols	Mowing, fertilization (chemical)	E	5.61 — 9.99	Frank et al. (2004)

Natural = grassland was naturally established

Semi-natural = grassland was naturally established, but mowing, fertilization, grazing or burning frequently occurred

Intensive = grassland was established artificially

E = ecological method

δ^{13}C = isotopic δ^{13}C analysis

SCC = soil C change

Figure legends

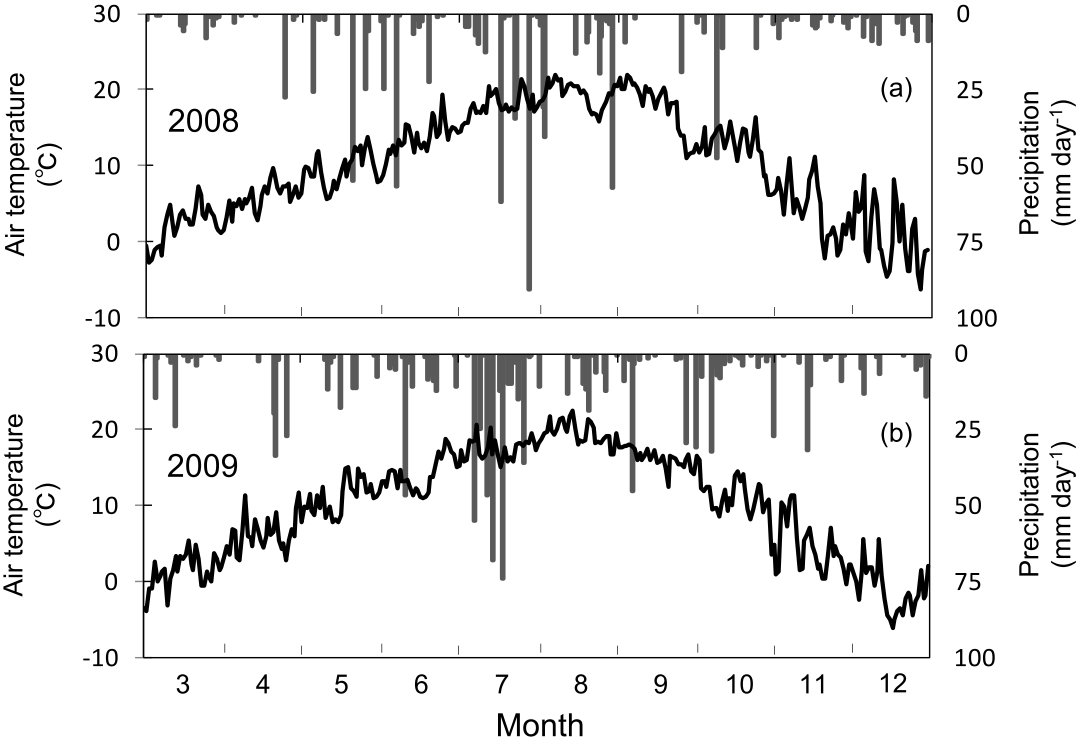
Fig. 1 Seasonal change of air temperature (black line) and precipitation (gray bar) in 2008 (a) and 2009 (b) in a *Miscanthus sinensis* grassland in Tomakomai, Hokkaido, Japan.

Fig. 2 Seasonal change of soil temperature at 5 cm (a, b), water-filled pore space (WFPS) (c, d), ammonium (NH_4^+) concentration (e, f), and nitrate (NO_3^-) concentration (g, h) in the vegetation plots in 2008 and 2009 in a *Miscanthus sinensis* grassland in Tomakomai, Hokkaido, Japan. Filled circles and black lines represent the values of 0-10-cm depth of soil. Open circles and dotted lines represent the values of 10-20-cm depth increment of soil. Error bars represent standard deviation (SD) values.

Fig. 3 Relationships between soil temperature at 5-cm depth and CO_2 flux in B1 (a), B2 (b), and B3 (c) plots in 2008 and 2009 (b) in a *Miscanthus sinensis* grassland in Tomakomai, Hokkaido, Japan. Filled and open circles represent values in 2008 and 2009, respectively. Solid and dotted lines represent exponential regression models between CO_2 flux and soil temperature in 2008 and 2009, respectively. The model is $F = a*\exp(b*T)$, where F is the CO_2 flux (mg C m^{-2} hr^{-1}) and T is soil temperature at 5 cm depth (℃). Asterisk represents significant difference in regression models among years (**, $P < 0.01$).

Fig. 4 Seasonal change of heterotrophic respiration in the vegetation plots in 2008 (a) and 2009 (b) in a *Miscanthus sinensis* grassland in Tomakomai, Hokkaido, Japan.

Fig. 5 Seasonal change of methane (CH_4) (a, b) and nitrous oxide (N_2O) (c, d) fluxes in vegetation plots in 2008 and 2009 in a *Miscanthus sinensis* grassland in Tomakomai, Hokkaido, Japan. Error bars represent standard deviation (SD) values.



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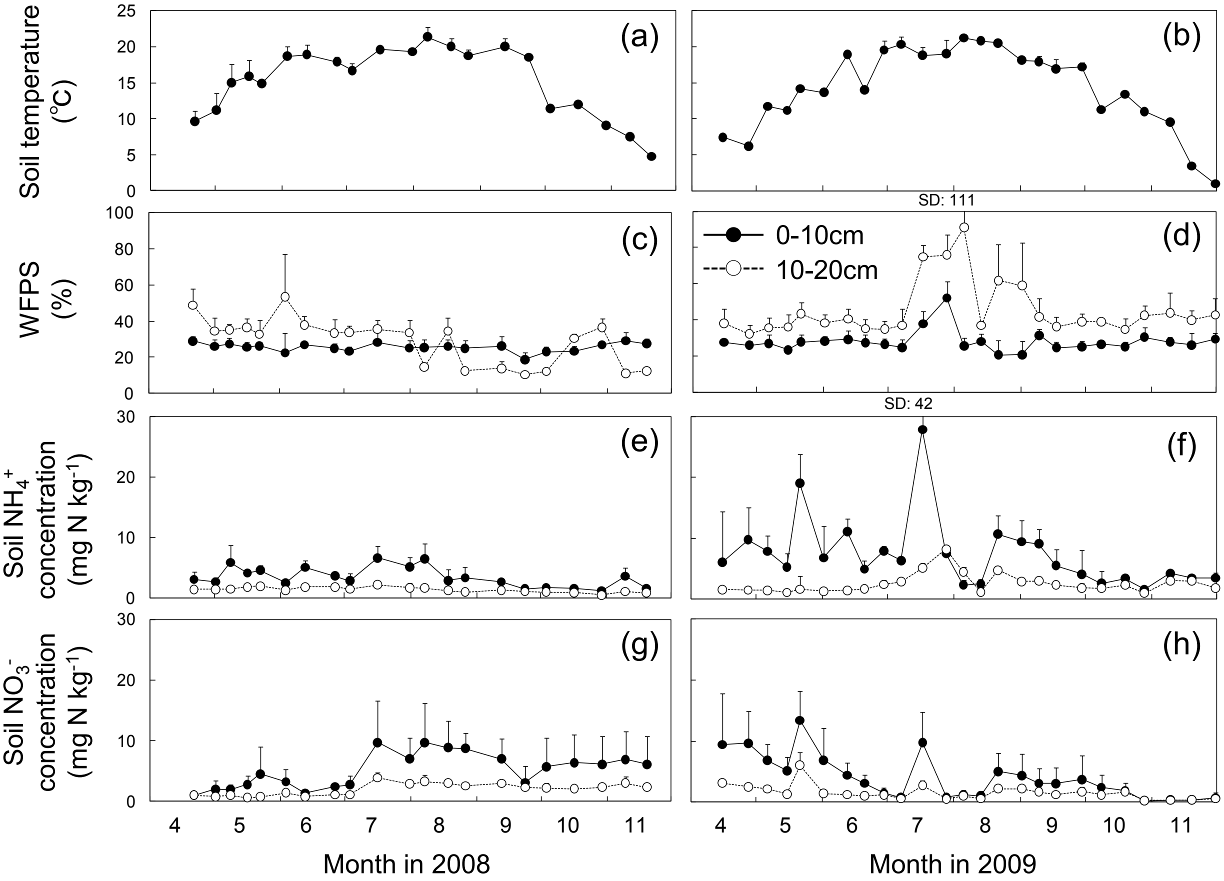

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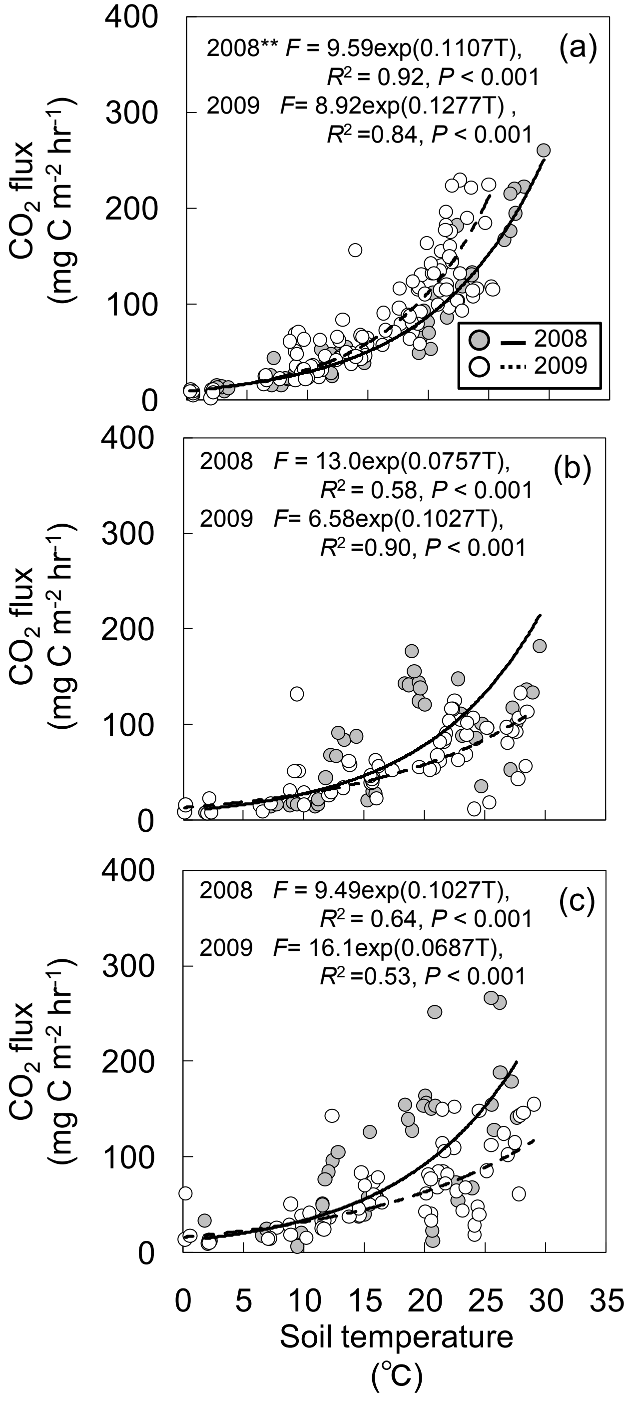

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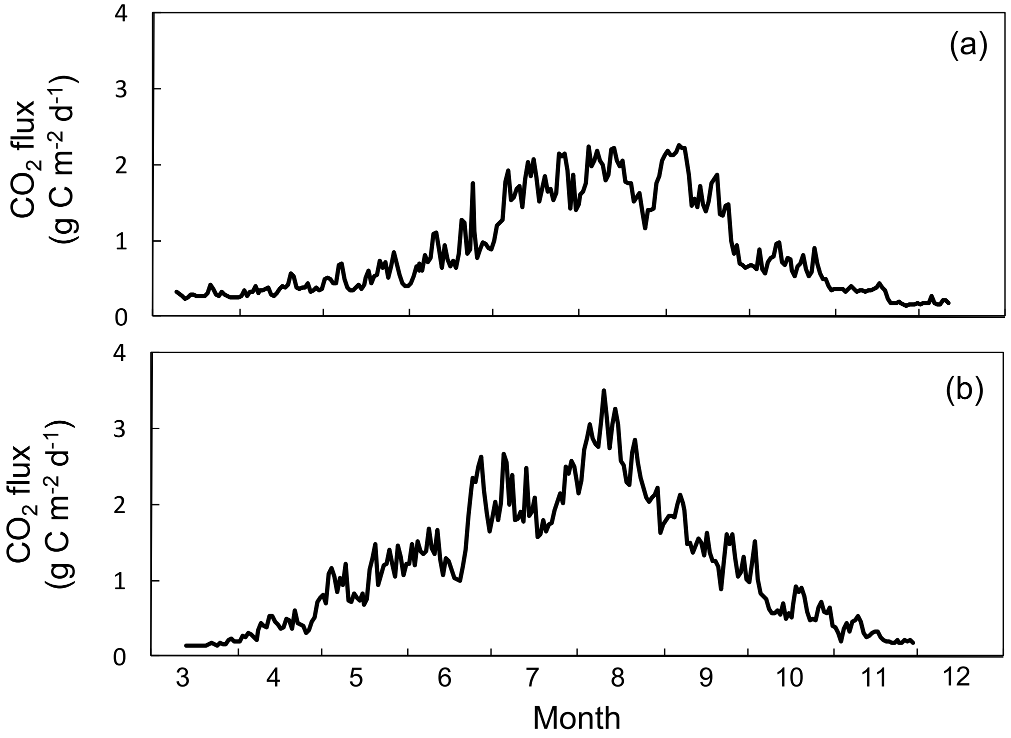

Fig. 4 Seasonal change of heterotrophic respiration in the vegetation plot in 2008 (a) and 2009 (b) in a *Miscanthus sinensis* grassland in Tomakomai, Hokkaido, Japan.

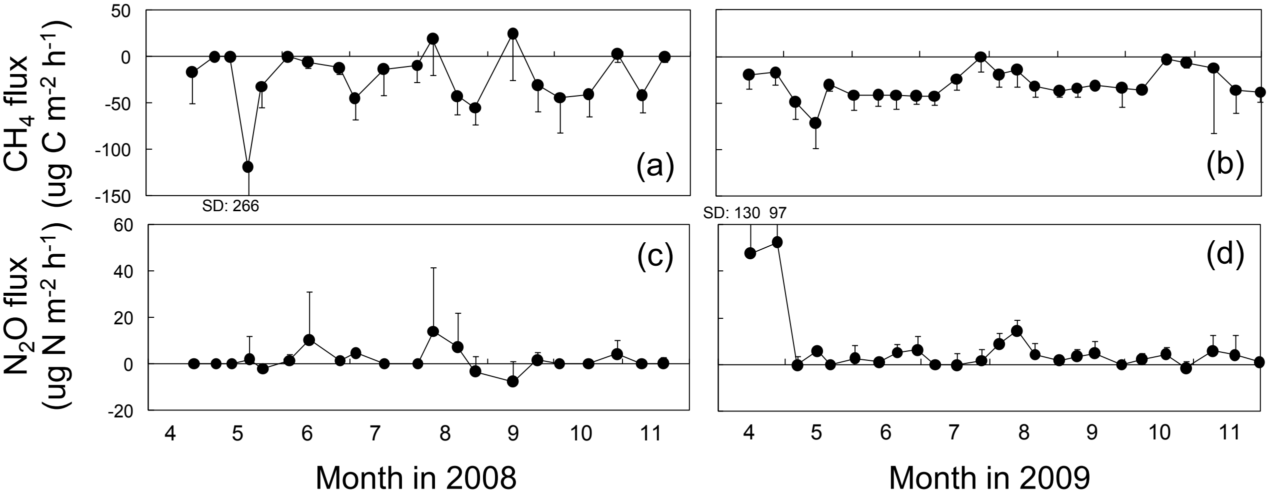

Fig. 5 Seasonal change of methane (CH_4) (a, b) and nitrous oxide (N_2O) (c, d) fluxes in a vegetation plot in 2008 and 2009 in a *Miscanthus sinensis* grassland in Tomakomai, Hokkaido, Japan. Error bars represent standard deviation (SD) values.