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Title:

Aboveground plant biomass, carbon, and nitrogen dynamics before and after burning in a semi-natural grassland of *Miscanthus sinensis* in Kumamoto, Japan

Running title:

Burning impacts in a *M. sinensis* grassland

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Abstract

While burning has been used for several thousand years to maintain *Miscanthus sinensis* grasslands in Japan, there is little information about the nutrient dynamics in these ecosystems immediately after burning. We investigated the loss of aboveground biomass; carbon (C) and nitrogen (N) dynamics, surface soil C change before and after burning; and carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O) fluxes 2 hours after burning in a semi-natural *M. sinensis* grassland in Kumamoto, Japan. After burning, 98% of aboveground biomass and litter were consumed. Carbon remaining on the field was 102 kg C ha⁻¹. At least 43% of C was possibly lost due to decomposition. However, remaining C, which contained ash and charcoal, appeared to contribute to C accumulation in soil. Nearly 50kg N ha⁻¹ of total aboveground biomass and litter N was lost due to burning. There was no difference in the amount of 0-5cm surface-soil C before and after burning. Remaining litter on soil surface showed that burning appears to not have caused a reduction in soil C nor did it negatively impact the vegetative crown of *M. sinensis* in the soil. Compared to before the burning event, post-burning CO₂ and CH₄ fluxes from soil appeared to not be directly affected by burning. However, it appears the short time span of measurements of N₂O flux after burning sufficiently characterize the pattern of increasing N₂O fluxes immediately after burning. These findings indicate that direct impact of burning did not cause significant reductions in soil C reduction nor elevated CO₂ and CH₄ emissions from the soil relative to before the burning event.

Introduction

Due to the steadily rising global demand for energy and the strong likelihood of exhaustion of petroleum in the coming decades (Rühl, 2008), 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). Using bioenergy instead of fossil fuels also could potentially mitigate human-induced global climate change because carbon (C) in bioenergy mainly comes from atmospheric carbon dioxide (CO₂). Although maize (*Zea mays* L.) grain is currently a major source of biofuel, several plant functional types have been evaluated as candidate bioenergy crops (Heaton et al., 2008). Perennial grassland plant species, which are generally not competitive with food crop production such as switchgrass (*Panicum virgatum*) or *Miscanthus x giganteus*, appear to produce more energy effectively while mitigating greenhouse gas emissions (Davis et al., 2009). Grasslands are one of the most abundant land-cover types in the world; they comprise about 41% of global land area, except for areas of permanent ice cover (Adams et al., 1990; White et al., 2000). Grassland ecosystems have high C storage in soil (20 to 130 Mg C ha⁻¹) and are considered a potential sink or source of C (Carpenter-Boggs et al., 2003; Arshad et al., 2004; Bronson et al., 2004).

Over the past 20-30 years, much interest has been directed toward a highly productive sterile, perennial hybrid grass, *M. x giganteus*, for its use as a source of bioenergy (Jones and Walsh, 2001). *Miscanthus x giganteus* was first collected in Japan in 1935 and then cultivated throughout Europe (Jones and Walsh 2001). Although natural and semi-natural grasslands in Japan, which historically comprised 10% of the land area in Japan in the early 1900s (Imura and Shi 2004), but in recent years only

constitute 4% of the country (Himiyama *et al.*, 1995), are comprised of several graminoid and forb species, including *Miscanthus sinensis*, which, in addition to *Miscanthus sacchariflorus*, is one of the parent species of *M. x giganteus*. *M. sinensis* dominates most of these highly diverse grasslands. *Miscanthus sinensis* grasslands, which, in many locations, have been managed for hundreds of years in Japan (Matsumura and Iwata, 1976; Otaki, 1999), comprise about 24% of the grasslands in Japan (National Parks Association of Japan, 1996).

The *Miscanthus* genus, which utilizes the energy-efficient C₄ photosynthetic pathway, (Naidu *et al.*, 2003), is comprised of several species that are considered potential bioenergy crops because of their 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; Stewart *et al.*, 2009).

Extensive research in Japan over the past several decades focused on the use of *M. sinensis*, which is native to Japan, as thatching material for roofs of traditional houses and buildings, organic fertilizer, and livestock feed (Stewart *et al.*, 2009). Most of the studies, however, about *M. sinensis* in Japan were focused on biomass production and vegetational characteristics in semi-natural *M. sinensis* grasslands. While this information is useful for better understanding the ecology and agronomy of *M. sinensis* and even other *Miscanthus* taxa such as *M. x giganteus*, there are other factors to consider if information related to this species and its role in the unique semi-natural grasslands in Japan can be utilized to further the field of bioenergy both as a source of plant improvement or as a stand-alone bioenergy crop. Also, while considerable effort has been expended in Europe and U.S. to understand the agronomics of *M. x giganteus*

for the past 20-30 years, most field-based studies have been 10 years or less in duration (Jones and Walsh, 2001). The longest duration of experiments have been, at most, 15 years (Clifton-Brown *et al.* 2007; Christian *et al.* 2008).

The unique semi-natural *M. sinensis* grasslands present opportunities to better understand not only the agronomic potential of the *Miscanthus* genus for long-term production for biomass or biofuel, but also its biogeochemistry. It is estimated that burning of *M. sinensis* grasslands, particularly in the Aso region of Kumamoto, has been estimated to have occurred for more than 10,000 years (Ogura *et al.*, 2002; Miyabuchi and Sugiyama, 2008). Burning as both a natural occurrence and as a management tool in several types of grassland throughout the world has been extensively documented (e.g., Anderson and Poth, 1996; Zepp *et al.*, 1996; Towne *et al.*, 2004; Castaldi and Fierro, 2005). Beneficial effects of burning have been demonstrated in grasslands. Zhang *et al.* (2008) reported the increase in light-saturated photosynthetic rate of *Agropyron cristatum* and *Cleistogenes squarrosa* in early July in burned grasslands in China compared to those that were not burned. Stem density of grass species also appears to increase due to burning relative to those not burned (Yamamoto *et al.*, 2002; Wang *et al.*, 2006). Knapp (1985) and Blair (1997) found that C₄ grasses, such as those found in North American tallgrass prairies, exhibited higher aboveground productivity when burned annually compared to grasses that were not burned.

In *M. sinensis* grasslands, burning is also important to maintain the vegetation composition of the fire-dependent ecosystem, which is species rich, and to also reduce the litter layer to allow for nutrient cycling (Iizumi, 1976; Yamamoto *et al.*, 2002). Yamamoto *et al.* (2002) also reported that aboveground biomass of *M. sinensis* in the

Aso region of Kumamoto was lower than in nearby grasslands where burning was stopped. They also found that where controlled burns had stopped, litter was calculated to be three to eight times greater than that found in controlled-burn sites.

To our knowledge, however, no work has been done to characterize C and nutrient dynamics or green house gas (GHG) emissions in *M. sinensis* grasslands subjected to annual burning events. Because the conditions of grassland ecosystems change drastically after burning, direct and indirect impacts of burning on nutrient cycling in *M. sinensis* grasslands could be considerably large. Direct impacts of burning, which occur during and just after burning, include C release to the atmosphere by burning of plant biomass and by heating of surface soil. Indirect impacts of burning, which occur after burning and are often long term in nature (i.e., several months or more), include nutrient loss from the soil surface by weathering and erosion (Fynn *et al.*, 2003). Moreover, increased microbial activity in soil also occurs due to rising soil temperatures and a higher number of wetting and drying cycles (Mills and Fey, 2004; Knicher, 2007). Thus, assessing the direct and indirect impacts of burning to C, N, and GHGs are important for evaluating C and nutrient cycling in *M. sinensis* grasslands.

In this study, we characterized the direct impacts of burning to the loss of plant biomass; dynamics of C and N; change in mass of C in surface soil; and change of CO₂, CH₄, and N₂O fluxes from the soil surface after burning in a *M. sinensis* grassland ecosystem, in which annual burning has occurred for more than 40 years, in Kumamoto, Japan. If high amounts of plant material (e.g. ash) remain on the soil surface after burning, this process could contribute to C accumulation in the soil due to the recalcitrant nature of this form of C, which is primarily in the form of charcoal (Lehmenn

et al., 2006; Marris, 2006). In addition, under certain conditions, we postulate that burning may not negatively impact C sequestration nor result in a change of GHG fluxes at significant levels from within the soil after burning. Outcomes of this study will provide important knowledge to the study of C and nutrient cycle and in the long-term management of *M. sinensis* grassland ecosystems with the intent that such information could be beneficial to subsequent development of not only *M. sinensis* as a bioenergy crop, but also other members of the genus.

Materials and Methods

Site description

The study was conducted in a semi-natural grassland of *M. sinensis* located in the northern rim of the Mt. Aso caldera in Kumamoto, Japan (33°01.58'N, 131°03.89'E, 794 m above sea level) on Cumulic Non-allophanic Andosols (Cultivated Soil Classification Committee, 1995; Matsuyama and Saigusa, 1994) or Melanudans (USDA, 1999). A distinct feature of the soil profile, which was useful in calculating the average C accumulation rate, was the 2AB horizon, commonly known in Japan as K-Ah, which is chemically and visually distinct and was deposited 7,300 years ago due to a nearby volcanic eruption (Miyabuchi and Watanabe, 1997).

The terrain of the study site was relatively flat and less susceptible to erosion compared to surrounding fields and provided more uniform conditions for measuring nutrient cycling. Selected physical and chemical properties of the soil are listed in Table 1. Soil C and N amounts on the top 30 cm were 20.2 kg C m⁻² and 1.04 kg N m⁻², respectively. Soil textures, which varied by depth in the soil profile, were clay loam, loam, or sandy loam (Table 1). Bulk densities of each horizon within the profile ranged from 0.30-0.33 g cm⁻³ (Table 1). Bray(2) P and exchangeable K, which were higher in the top horizon, gradually decreased in deeper horizons (Table 2). The site has a 30-year mean annual precipitation of 3250 mm and air temperature of 9.6°C.

Plant species with high relative dominance were *Miscanthus sinensis* (47.1%), *Arundinella hirta* (9.86%), *Pleiblastus argenteostriatus glaber* (5.45%), *Amphicarpaea bracteata* subsp. *edgeworthii* var. *japonica* (6.02%), *Artemisia indica* var. *maximowiczii* (3.56%), *Lespedeza bicolor* (2.99%), *Pteridium aquilinum* subsp. *japonicum* (2.77%),

and *Lespedeza cuneata* (2.02%). The only management applied for 40 years and possibly longer was an annual burning event each March. Aboveground biomass at this site was not harvested over the 40-year management period. The study was initiated on 21 March 2009 with a 1-hr burn event beginning at 10:00 AM. The fire was ignited by a handheld burner from the leeward side of the study site. Burning and smoke emission had ended by 12:00 PM on 21 March.

Aboveground biomass and litter measurements before and after burning

Aboveground biomass and litter of *M. sinensis* and other plants in the study site were collected from 16 1-m² square areas with similar amounts of biomass; eight of the areas were used for measurements before burning on 26 February and the remaining eight for measurements immediately after burning on 21 March. Litter samples after burning also contained ash because they could not be separated. Samples were oven-dried at 70°C for 48 hours, then weighed, grounded, and analyzed for C and N by an elemental analyzer (Vario EL III, Elemental, Hanau, Germany).

Amount of C in surface-level soil

Undisturbed soil samples (0-5 cm) were collected with stainless steel cores (100 cm³) from each of the eight replications before and after burning. The cores were divided into the 0-1 cm, 1-2 cm, 2-3 cm, and 3-5 cm depth increments. Bulk density and C content of soil in each layer were calculated (Logsdon and Cambardella, 2000). Soil C concentration was analyzed with an elemental analyzer.

Measurement of CO₂, CH₄, and N₂O fluxes

We measured CO₂, CH₄, and N₂O fluxes with a closed-chamber method described by Toma and Hatano (2007) with four replications before and after burning. Stainless steel bases were installed 30 min. before the first measurement. The bases were removed prior to burning and re-installed immediately thereafter. Before burning, gas fluxes were measured at 1:00 PM on 25, 11:00 AM and 4:00 PM on 26 February; 10:00 AM and 2:00 PM on 27 February; 2:00 AM on 28 February; and 2:00 PM on 28 February. Fluxes of GHG measured before burning were considered a daytime baseline. Gas fluxes were measured 2, 4, 7, 11, 23, 31, 45 hours after burning from 12:00 PM on 21 March to 7:00 AM on 23 March. A 250 ml gas sample was collected into a tedlar bag (500mL) for CO₂ determination at 0 and 6 minutes from the time the chambers were closed, which was based on the method of Nakano *et al.* (2004). Carbon dioxide concentrations were measured with a CO₂ analyzer (ZFP-9, Fuji Electric Systems, Tokyo, Japan). Vacuum-10 mL vials sealed with butyl rubber stoppers (SVF-10, Nichiden-Rika, Kobe, Japan) were used to collect 20-ml CH₄ and N₂O gas samples at 0, 15, and 30 min after chamber closure. Methane and N₂O concentrations were determined by a gas chromatograph equipped with flame ionization detector (FID) (GC-8A, Shimadzu, Kyoto, Japan) for CH₄ and electron capture detector (ECD) (GC-14B, Shimadzu, Kyoto, Japan) for N₂O.

Fluxes of CO₂, CH₄, and N₂O were calculated by 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 (1.977×10⁶ mg m⁻³ for CO₂, 0.717 ×10⁶ mg m⁻³ for CH₄, and 1.978 × 10⁶ mg m⁻³ for N₂O); 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 ($\text{m}^3 \text{ m}^{-3} \text{ h}^{-1}$); T is the air temperature ($^{\circ}\text{C}$), and α is a conversion factor for CO_2 to C ($=12/44$), CH_4 to C ($=12/16$), or N_2O to N ($=28/44$).

Ancillary measurements

Soil temperature was measured with a thermometer (CT-413WR, CUSTOM, Tokyo, Japan) at 5 cm of soil depth and volumetric soil water content averaged over the top 6 cm of the soil was measured with a moisture sensor (DIK-311D, Daiki Rika Kogyo Co., Ltd., Saitama, Japan) during gas sample collection. Continuous hourly soil temperature measurements were also collected at 5 cm of soil depth from February 26 until the end of the study (Ondotori Jr.TR-52S, T&D Corporation, Nagano, Japan). Total soil porosity and volumetric water content measurements were used to calculate water-filled pore space. Air temperature and precipitation information was collected from a nearby weather station.

Statistical analysis

Aboveground biomass, litter, soil C comparisons, and CO_2 , CH_4 and N_2O fluxes before and after burning were performed using Student's t -test for normally distributed data and Mann-Whitney's U -test for data not normally distributed as determined by a Chi-square test (Ichihara, 1990).

Results

We calculated the average C accumulation rate within the soil profile at the study site over the past 7,300 years that was possible given the 2AB horizon. Average C and N accumulation over the past 7,300 years were $58.0 \text{ kg C ha}^{-1} \text{ yr}^{-1}$ and $2.60 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, respectively.

After burning, aboveground biomass and litter and ash were significantly lower than those prior to burning ($P < 0.01$) (Table 2). Loss of aboveground biomass and litter due to burning was calculated 98.5% of that before burning. In addition, prior to burning, C and N in aboveground biomass and litter were significantly higher than aboveground biomass and litter and ash after burning (Table 2). Released C and N in aboveground biomass and litter were 98.6% and 96.7% of C and N of aboveground biomass and litter prior to burning, respectively. Only 1.4% and 3.3% of C and N of aboveground biomass and litter prior to burning remained after the burn event (Table 2). Mean C accumulation constituted nearly 57% of remaining C after burning (Table 2). Mass of C before and after burning did not significantly differ in surface soil from 0 to 1cm, 1 to 2cm, 2 to 3cm, and 3 to 5cm (Table 2).

When CO_2 , CH_4 , and N_2O fluxes were measured in February, average of soil temperature at 5cm depth and WFPS were 8.6°C and 14.1%, respectively (Fig. 2). Average fluxes of CO_2 , CH_4 and N_2O before burning were $72.4 \text{ mg C m}^{-2} \text{ h}^{-1}$, $-39.0 \text{ } \mu\text{g C m}^{-2} \text{ h}^{-1}$, and $-0.70 \text{ } \mu\text{g N m}^{-2} \text{ h}^{-1}$ (Fig. 2).

As burning commenced from 8:00 AM to 12:00 AM in 21 March, air and soil temperature rose up from 8.4°C to 15°C and 6.08°C to 11.7°C , respectively. Precipitation began from 0:00 AM on 22 March and continued for 18 hours. Total

accumulated rainfall was 65.5 mm. Over the entire measurement period, CO₂, CH₄, and N₂O fluxes after burning ranged from 35.8 to 111 mg C m⁻² hr⁻¹, -56.2 to -7.47 μg C m⁻² hr⁻¹, and -2.08 to 31.4 μg N m⁻² hr⁻¹, respectively (Fig. 2). However, CO₂, CH₄, and N₂O fluxes beginning at the end of the burn event until precipitation began ranged from 35.8 to 95.3 mg C m⁻² hr⁻¹, -56.2 to -7.47 μg C m⁻² hr⁻¹, and 2.59 to 31.4 μg N m⁻² hr⁻¹, respectively (Fig. 2). Carbon dioxide and CH₄ fluxes before and after burning were within similar ranges (Fig. 2). However, average of N₂O fluxes 1 day after burning significantly increased from -0.70 to 48.2 μg N m⁻² hr⁻¹ ($P < 0.05$) (Fig. 2).

Discussion

Loss of aboveground biomass and biomass carbon and nitrogen by burning

Possibly due to the large spatial variation of vegetative crown size of *M. sinensis* clumps (Numata, 1975), standard deviations of mean values in Table 2 were higher than might be expected. Although, most of the aboveground biomass and litter were completely burned, some of the unburned litter remained on the ground after burning (Table 2). Iwanami (1972) reported that 92 to 96% of aboveground biomass of *M. sinensis* and other plant species was consumed by burning in a similar *M. sinensis* grassland in Kawatabi, Miyagi, Japan (38°44'N, 140°45'E).

In a laboratory experiment on effects of burning on biomass, remaining mass of straw of spring wheat (*Triticum aestivum*), oat (*Avena sativa*), and flax (*Linum usitatissimum*) were only 13%, 8%, and 4%, respectively (Heard *et al.* 2006). Iwanami (1973) reported that when 10 Mg dry wt. ha⁻¹, which is similar in scale to the 10.8 Mg dry wt. ha⁻¹ of *M. sinensis* measured in our study, was burned in a *M. sinensis* grassland in Kawatabi only the aboveground biomass was completely consumed by burning. The belowground biomass only partially burned to a depth of 2 mm. Burning occurred only in the outer margins of the vegetative crown. This would indicate that the crown of *M. sinensis* is likely not disturbed by burning, which might be advantageous for maintaining the vegetational composition of the *M. sinensis* in semi-natural *M. sinensis* grasslands (Yamamoto *et al.* 2002). Kitchen *et al.* (2009) reported that over a 13-year period, root biomass was greater in annually burned prairies than in those that were not burned in Kansas, USA, which confirms similar work in *M. sinensis* grasslands in Japan (Iwanami, 1973).

Given that little, if any, belowground biomass C appears to be lost during burning of *M. sinensis* grasslands, the calculation of C budgets of these ecosystems is possibly simplified. However, detailed studies to measure changes in belowground biomass during burning have not been conducted to completely validate this approach.

Similar to the 98.6% loss of C in aboveground biomass and litter in our study (Table 2), Heard *et al.* (2006) also reported that loss of C in burned straw of winter wheat, oat, and flax were estimated to be 90.6%, 96.3%, and 96.9%, respectively. Only 102 kg C ha⁻¹ of aboveground biomass and litter was left on the *M. sinensis* grassland surface (Table 2). Average C accumulation during 7,300-year-period, which followed the volcanic eruption, at the rate of 58.0 kg C ha⁻¹ y⁻¹ was lower than accumulated soil C under 9- (778 kg C ha⁻¹) and 16- (1125 kg C ha⁻¹) year-old fields of *M. x giganteus* grown on Typic Haplumbrept (Hansen *et al.* 2004), but higher than that under 2 to 5 year-old cultivated switchgrass (0 kg ha⁻¹ yr⁻¹) where C inputs equaled outputs (Sartori *et al.*, 2006). Also, Yazaki *et al.* (2004) reported that in mowed, but unharvested *M. sinensis* grasslands on Andisols located near Nagano Japan, C accumulation rate (3570-4030 kg C ha⁻¹ yr⁻¹) was 62-69 times higher than our calculation. In this study, average C accumulation was 56.9% of remaining C after burning (Tables 3). Therefore, at least 43.1% of C, which remained on the field after burning, was possibly lost to the atmosphere.

Carbon in the form of charcoal, which remained on the soil surface after burning, is known to decompose at slow rates compared with fresh organic C (Seiler and Crutzen, 1980; Skjemstad *et al.*, 2002; Lehmann *et al.*, 2006). Compared with aboveground biomass in *M. sinensis* grasslands, large amounts of underground biomass are

generally stored belowground (Stewart *et al.*, 2009). Carbon in belowground biomass may contribute to C accumulation in soil. However, there is a possibility that charcoal in the residual ash, which was left on the field after burning, may ultimately contribute to C accumulation in soil because of its recalcitrant nature (Lehmann *et al.*, 2006; Marris, 2006).

Heard *et al.* (2006) also reported that loss of N in burned straw of winter wheat, oat, and flax were estimated to 98.2%, 99.0%, and 99.8%, respectively. These values are quite similar with what was found in our study (96.7%). Additionally, since mineral N generally increases in soil after burning (Murphy *et al.*, 2006; Knicher, 2007), it is possible that additional N may have been lost through leaching after burning. On the other hand, N accumulation rate was $2.60 \text{ kg N ha}^{-1} \text{ y}^{-1}$. Therefore, addition of N due to biological fixation of N_2 by other species, such as *Lespedeza bicolor* and *Lespedeza cuneata*, which are common in *M. sinensis* grasslands, and wet and dry N deposition possibly contributed to N accumulation in the soil over time. This requires further investigation, but it should be considered that in grassland ecosystems, nearly 20% of the biomass N may come from biological N_2 fixation (Pakrou and Dillon, 2000; Kimura *et al.*, 2007). Also, it has been reported that N_2 -fixing clostridia and nondiazotrophic bacteria symbiotically provide N to *M. sinensis* plants (Ye *et al.*, 2005; Stewart *et al.*, 2009). Moreover, nearly $10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ of N deposition were reported in agricultural fields in Japan (Kimura *et al.*, 2007; Hayashi *et al.*, 2007).

Carbon in surface soil

Mass of C in surface soil (i.e., 0-1 cm, 1-2 cm, 2-3 cm, and 3-5 cm depth) did not

significantly change after burning (Fig. 1). Since some litter remained unburned, it is unlikely that the burning temperature and/or the short duration of burning, were sufficient to have an impact on reducing soil C. Increases in soil temperature in surface soil at burning are generally minimal in this grassland ecosystem even if temperatures at the air-surface interface are greater than 100°C (Iwanami, 1972). Iwanami (1972) reported that soil at 1-cm depth increased in the range of 8-14°C when compared with the temperature before burning when 3-5 Mg dry wt. ha⁻¹ was burned in a *M. sinensis* grassland in Kawatabi. In this study, soil temperature at the 5-cm depth increased from 7.54 to 10.6°C during the 1-hour burn period (Fig. 2). This increase followed the same pattern as ambient air temperature and likely indicates temperature changes were due to increased sunlight radiation, but not to burning. While CO₂ fluxes from the soil were not measured during burning, if it is assumed there is a lag time in the amount of organic matter decomposition and concomitant increases in CO₂ fluxes due to increased temperature, the data collected two hours after burning indicate very small fluxes occurred. At 12:00 pm, CO₂ flux was 95.3 mg C m⁻² hr⁻¹. This corresponds to about 0.01% of soil C in the top 5 cm of soil and indicates that burning did not directly disturb soil C in the surface soil. As mentioned, burning does not appear to be detrimental to C accumulation in surface soils in this grassland system.

Change of CO₂, CH₄, and N₂O fluxes after burning

The CO₂ flux, which included microbial and root respiration, during the winter was lower than the values observed in a *M. sinensis* grassland growing on Andisols near Nagano, Japan in November (200-300 mg C m⁻² hr⁻¹). The CH₄ flux was nearly within

the same range (about $-30-0 \mu\text{g C m}^{-2} \text{ hr}^{-1}$) as that in an unfertilized and unburned orchardgrass pasture during winter on a volcanic ash soil in Nasu, Japan (Mori *et al.* 2005). Nitrous oxide was a little lower than the value ($0-25 \mu\text{g N m}^{-2} \text{ hr}^{-1}$) of Jorgensen *et al.* (1997) in a *M. x giganteus* field on Typic Haplumbrept in Denmark.

When measurement of CO_2 , CH_4 , and N_2O fluxes began at 12:00 PM, burning and smoke emission had ended. Due to a rain event that began at 12:00 am on 22 March, only flux data collected before that time was analyzed given that rain can drastically change soil conditions due to changes in temperature and moisture. Considering that several factors influence GHG flux from soil (Conrad 1995; Bouwman *et al.*, 2001), caution is needed when comparing measured GHG fluxes of other grassland ecosystems where burning occurs. After burning, there were significant differences of CO_2 and CH_4 fluxes between before and after burning. However, N_2O fluxes one day after burning appear to have been affected by burning. Only N_2O fluxes significantly increased compared with that before burning ($P < 0.05$).

Besides that reported in our study, there appears to be little, if any, information about changes in GHG fluxes from soil before and after burning in a grassland ecosystem. Levine *et al.* (1996) reported no differences in N_2O fluxes before and after burning in savannas in South Africa. However, our results may indicate frequent measurement of N_2O fluxes at least one day after burning might be needed to more accurately quantify N_2O emission after burning, particularly if the increase of N_2O fluxes after burning is higher than that of mean annual N_2O fluxes. The range of N_2O fluxes after burning (2.59 to $31.4 \mu\text{g N m}^{-2} \text{ hr}^{-1}$) was similar to values measured over the period of a year in an unfertilized and unburned orchardgrass pasture on a volcanic ash soil in Nasu, Japan

(1-10 $\mu\text{g N m}^{-2} \text{ hr}^{-1}$) (Mori *et al.*, 2005) and that measured from April to July in a *M. x giganteus* field on Typic Haplumbrept in Denmark (0-50 $\mu\text{g N m}^{-2} \text{ hr}^{-1}$) (Jordensen *et al.*, 1997). Although future investigations of longer duration will be required to determine a more accurate estimate of the contribution of gas emissions due to burning, burning may not directly cause substantial increases in CO_2 , CH_4 and N_2O fluxes as they relate to total annual gas emissions.

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Table 1. Physical and chemical characteristics in each soil horizon in in a semi-natural *Miscanthus sinensis* grassland in Aso, Kumamoto, Japan.

Horizon	Depth (cm)	Bulk density (g cm ⁻³)	Sand (%)	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)
Ah1	0-24	0.33	40.6	39.9	19.5	CL	5.31	47.7	221	11.8	18.7	24.4	132
Ah2	24-49	0.33	62.1	30.2	7.67	L	5.33	51.0	196	7.85	25.0	4.07	26.8
Ah3	49-64	0.32	68.8	24.4	6.77	SL	5.48	48.1	154	5.64	27.3	3.22	27.5
2AB	64-75	0.32	68.1	23.7	8.19	SL	5.48	38.7	52.5	2.02	26.0	1.65	16.7
3Ab	75-100	0.30	60.9	25.8	13.3	L	5.73	43.8	114	3.96	28.7	1.76	17.0

CL, clay loam; L, loam, SL, silty loam; CEC, cation exchange capacity, TC, total carbon; TN, total nitrogen; C/N, carbon/nitrogen ratio; P, phosphorus; K, potassium

Table 2. The amount of dry biomass, carbon, and nitrogen of plant and litter before and after the burning in a semi-natural *Miscanthus sinensis* grassland in Aso, Kumamoto, Japan. Asterisks represent significant ($P<0.01$) difference between sum of aboveground biomass and litter before and after burning (Mann-Whitney U -test).

Measured parameters	Biomass dry weight		Carbon		Nitrogen	
	Average	SD	Average	SD	Average	SD
Before burning						
<i>M. sinensis</i> (kg ha ⁻¹)	10,823	7,013	5,249	3,459	16.8	11.3
Other plant (kg ha ⁻¹)	156	139	78.5	68.6	0.95	1.12
Litter (kg ha ⁻¹)	5,218	1,785	2,238	790	34.5	9.53
Subtotal (kg ha ⁻¹)	16,197**	7,238	7,566**	3,549	52.2**	14.8
After burning						
<i>M. sinensis</i> and other species (kg ha ⁻¹)	102	122	48	61	0.3	0.38
Litter and ash § (kg ha ⁻¹)	146	34	54.8	14.1	1.38	0.35
Subtotal (kg ha ⁻¹)	247	127	102	62.6	1.68	0.52
Loss by burning (kg ha ⁻¹)	15,950	7,240	7,463	3,550	50.5	14.8

§ After burning, litter and ash could not be separated

Figure legends

Fig. 1. Mass of soil C in surface soil (0-1cm, 1-2cm, 2-3cm, and 3-5cm) before and after burning. Solid squares are mass of C before burning. Open squares are mass of C after burning. Error bars are standard deviations of the means. NS indicates non-significant at the $P < 0.05$ probability level.

Fig. 2. Change of the air temperature (a), precipitation (a), soil temperature (b), water-filled pore space (WFPS) (c), CO₂ flux (d), CH₄ flux (e), and N₂O flux (f). Error bars are standard deviations of the means. Vertical line indicates when the controlled burn was initiated. Vertical dotted line shows when precipitation began.



