Short-term effects of grassland renovation on CO₂ exchange of grasslands in a temperate humid region

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Abstract

To investigate the short-term effects of grassland renovation on carbon dioxide (CO₂) exchange of intensively managed grasslands in Japan, we conducted CO₂ flux measurements during the renovation process by using the eddy covariance and closed chamber methods in 2007, 2012, and 2013. The flux measurements were conducted at three grassland fields: two fields used for cutting, one receiving only chemical fertilizer (CF) and the other receiving composted cattle manure (CM) annually, and one field used for grazing (GM). Chamber measurements revealed flushes of CO₂ after plowing (14.45–23.94 μmol m⁻² s⁻¹) and subsequent disk harrowing (7.36 μmol m⁻² s⁻¹, in CF) followed by rapid drops of CO₂ flux, which were local and temporary phenomena. The mean CO₂ losses during renovation periods were calculated as 4.52–4.74, 6.00–6.81, and 4.57 g C m⁻² d⁻¹ in CF, CM, and GM, respectively; CF and CM calculation is based on eddy covariance measurements, including temporal flux variations and representing footprint areas. The amount of carbon input during renovation including non-harvested grass biomass (stubble and roots) and applied manure was estimated as 2.24–3.50 and 6.17–8.77 Mg C ha⁻¹ in CF and CM, respectively. Among them, carbon derived from plowed roots and manure is presumably resistant in soil, contributing to long-term soil organic carbon (SOC) accumulation. Our results also indicate that grassland renovation work does not affect short-term net CO₂ loss significantly, although it affects CO₂ emissions to a certain extent. We can therefore say that net CO₂ loss during renovation is mainly brought by the absence of vegetation in this site. Grasses contribute SOC accumulation through biomass allocation belowground; and it is thus recommended to shorten grassland renovation period, the duration without photosynthesis, to reduce CO₂ loss associated with grassland renovation.

Key words: Carbon dioxide, Closed static chamber, Eddy covariance, Grassland renovation, Soil organic carbon

1. Introduction

Grasslands play an important role in global carbon storage due to the large belowground carbon stock (Lauenroth and Milchunas, 1992; Gibson, 2009) and the long residence time of carbon due to the low frequency of soil disturbance (Jones and Donnelly, 2004; Gibson, 2009). The amount of organic carbon retained by grassland soil is greatly affected, both positively and negatively, by management practices (Conant *et al.*, 2001; Maia *et al.*, 2009). Soil organic carbon (SOC) in grassland is affected by nitrogen fertilization (Schuman *et al.*, 2002), grazing intensity (Reeder and Schuman, 2002; Chen *et al.*, 2015; Dlamini *et al.*, 2016), organic amendment application (Ryals *et al.*, 2014), irrigation (Kelliher *et al.*, 2012), harvesting (Franzluebbers and Stuedemann, 2009), and burning (Chaplot *et al.*, 2016). Grassland renovation can affect grassland SOC as well (Necpalova *et al.*, 2014).

The yields and forage quality of managed grasslands in temperate regions gradually decline due to the loss of the original sown sward composition associated with soil acidification, soil

compaction, weed invasion, and summer or winter killing (Hojito, 1998; Agricultural Production Bureau, Ministry of Agriculture, Forestry and Fisheries [MAFF], 2006; Velthof *et al.*, 2010). Thus, it is necessary to renovate temperate grasslands to maintain or increase productivity by plowing and reseeding occasionally; improving aeration, drainage, and chemical properties; removing weeds and pests; and introducing improved herbage varieties. Such renovation may have both positive and negative effects on carbon exchange in grasslands. Soil disturbance associated with grassland renovation should cause a temporary carbon dioxide (CO₂) flush from the soil or promote microbial decomposition of SOC, as is the case with tillage in cropland fields (Reicosky and Lindstrom, 1995; Reicosky *et al.*, 1997; Ellert and Janzen, 1999). At the same time, biomass production is increased, or at least maintained, by grassland renovation (Velthof *et al.*, 2010; de Koff *et al.*, 2011; Necpalova *et al.*, 2014), which may increase SOC through plant litter. Most studies to date have investigated the effects of tillage in grassland (Eriksen and Jensen, 2001; Grandy and Robertson, 2006; MacDonald *et al.*, 2010; Willems *et al.*, 2011) or land-use change (from or to grassland; Post and Kwon, 2000; Conant *et al.*, 2001; McLauchlan, 2006; Katsumo *et al.*, 2010) on SOC or CO₂ exchange. However, information on the effects of grassland renovation is limited. Evaluation of the renovation effects on carbon dynamics is important for estimating or predicting SOC changes in

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grasslands over a period of time on a regional or national scale.

In Japan, where the climate is temperate with ample precipitation, except at high elevations, sustaining a stable sward composition is sometimes difficult, especially in the region south of the Kanto district. Farmers in Japan are advised to renovate grasslands to restore declining productivity based on several regional criteria for soil physical and chemical properties and the degree of weed invasion (Agricultural Production Bureau, MAFF, 2006). Intervals between grassland renovation vary from several years to more than a decade, depending on farmers' judgements based on grassland conditions and their economic situation. In the future, these intervals may be shorter due to expected more frequent extreme weather events which cause grass declines than at present, making the evaluation of renovation effects even more important.

As a first step in evaluating the overall impact of grassland renovation, in this study we investigated CO₂ exchange during the grassland renovation process and quantified the short-term effects of the renovation on CO₂ exchange of intensively managed grassland in Japan. Grassland renovation events were studied in 2007, 2012, and 2013, based on CO₂ flux measurements with the eddy covariance and closed chamber methods. The measurements were conducted at three grassland fields: two fields are used for cutting, one receiving only chemical fertilizer and the other receiving composted cattle manure and chemical fertilizer, and the other field is used for grazing.

2. Materials and methods

2.1 Site description

The study was conducted at the Institute of Livestock and Grassland Science, National Agriculture and Food Research Organization (NARO), Forage and Livestock Research Station. The research station is located in Nasushiobara city in northern Tochigi Prefecture, Japan (36°55'N, 139°58'E, 320 m above sea level). The climate is temperate humid, with a mean annual temperature of 12.2°C and a mean annual precipitation of 1561 mm.

Three grassland fields were provided for the study (Fig. 1): two adjacent grassland fields used only for cutting, one receiving chemical fertilizer alone (CF, 2.4 ha) and the other receiving composted cattle manure and supplementary chemical fertilizer (CM, 2.3 ha), and one grazing field that received composted cattle manure during the study period (GM, 0.5 ha). The soil of the fields is classified as a Haplic Brown Lowland soil (Kurashima *et al.*, 1993; as Haplic Fluvisols in the World Reference Base for Soil Resources; FAO, ISRIC, and IUSS, 2006). The CF and CM fields underwent grassland renovation in September 2001 and have been managed as grassland fields for cutting. After November 2004, CM received cattle manure composted with bark or sawdust on an annual basis at the rate of 15.1–32.0 Mg fresh weight ha⁻¹ yr⁻¹, and both fields have generally received about 200 kg N ha⁻¹ yr⁻¹, including the nutrient supply from the applied manure (Matsuura *et al.*, 2014). Grasses in CF and CM were cut four times per year, except in 2007, 2010, and 2012 (three times). The GM field has been managed as grazing land since 2006 (four head of cattle were grazed from April–May to October–November). The field received about 70 kg N ha⁻¹ yr⁻¹ and had not been renovated or manured between 2006 and the study period. Dominant plant cover prior to the study in CF and CM was *Dactylis glomerata* L. (orchardgrass) and *Lolium multiflorum* Lam. (Italian ryegrass) and that in GM was *D. glomerata*, *Agrostis gigantea* Roth (redtop), and *Festuca arundinacea* Schreb. (tall fescue).

2.2 Grassland renovation

Grassland renovation and measurements were conducted in 2007 and 2012 at the CF and CM fields and in 2013 at the GM field. The renovation work in CF and CM was conducted in parallel. The renovation procedures used in this study are standard ones practiced in Japan. The grassland fields were renovated during the end of August and the beginning of October, which is the most common period in this region to ensure sufficient growth of sown grasses to survive the winter.

Fig. 1. Layout of the experimental fields: two adjacent grassland fields used for cutting (a) and a grazing field (b). CF, the field receiving chemical fertilizer alone; CM, the field receiving composted cattle manure and supplementary chemical fertilizer.

The timing of each renovation operation is listed in Table 1. After cutting the third crop (in CF and CM) or summer grazing (in GM), a glyphosate-based herbicide was sprayed to kill the existing grasses and weeds, except in 2007, followed by lime application in GM. Next, composted cattle manure was applied to CM and GM, after which grassland fields were moldboard-plowed to a 200- to 300-mm depth and clods were crushed with disk harrows. In CF and CM, lime application and subsequent mixing of lime with soil by power harrows (in 2007) or disk harrows (in 2012) was then performed. Fertilizer application and seeding with broadcast spreaders followed by covering the soil using tooth harrows and compacting the soil using corrugated rollers were then conducted. In 2012, large stones were removed from CF by a stone picker before liming. The same amount of chemical fertilizer was applied to CF and CM during grassland renovation to ensure adequate initial grass growth. The number of tractors per field performing operations at the same time was at most three, two, and one in 2007, 2012, and 2013, respectively. We estimated the CO₂ emissions from fuel combustion by the renovation operations using tractors based on the inventory data of agricultural practices in Japan (Asai *et al.*, 2015; National Institute for Agro-Environmental Sciences, 2003a; 2003b).

The period of grassland renovation in the CF and CM fields was defined from the cutting of the third crop to the day before seedling emergence (24 and 40 d in 2007 and 2012, respectively), whereas that in GM was defined from the end of summer grazing to the day before seedling emergence (39 d). The amount of carbon input through manure application was 6.39 (in 2007) and 3.63 (in 2012) Mg C ha⁻¹ yr⁻¹ in CM and 2.71 Mg C ha⁻¹ yr⁻¹ in GM.

2.3 Eddy covariance measurements

Fluxes of CO₂, sensible heat, and latent heat were measured by the eddy covariance systems installed at the center of the CF and CM fields, and the fields' net ecosystem CO₂ exchange (NEE) rates were compared (Fig. 1a). Land around the systems was left unrenovated, and measurements were continued even during the renovation procedures.

The measurements were described by Matsuura *et al.* (2014), along with details of the setup and data processing. In short, the system consisted of a three-dimensional sonic anemometer-thermometer (CSAT-3, Campbell Scientific, Logan, UT, USA) and an open-path infrared CO₂/H₂O gas analyzer (LI-7500, Li-Cor, Lincoln, NE, USA) set up at a height of 2.3 m (CF) or 2.5 m (CM). The dominant wind directions during the grassland renovation period were SE–SSW and NW–NNE, and the minimal fetch of these directions were 68 and 76 m in CF and CM, respectively. The output signals of the sensors were sampled at 10-Hz frequency using a data logger (CR23X or CR1000, Campbell Scientific). The 10-Hz eddy covariance data were post-processed to determine CO₂ flux based on half-hourly runs. We used procedures to correct spike values (Vickers and Mahrt, 1997; Mano *et al.*, 2007), performed coordinate rotation (Wilczak *et al.*, 2001), corrected for frequency response losses (Massman, 2000; Massman, 2001) and for the influence of water vapor on sensible heat flux (Kaimal and Gaynor, 1991), and added a density term (Webb *et al.*, 1980). CO₂ storage was estimated from the difference of mean molar density between two continuous runs, and NEE was calculated as the sum of CO₂ flux and CO₂ storage.

Quality control of the fluxes was performed by means of quality tests based on meteorological and statistical theories (Mano *et al.*, 2007), basic tests for 10-Hz data (Vickers and Mahrt, 1997), nonstationarity and integral turbulence tests (Foken and Wichura, 1996), and a half-hourly spike test (Papale *et al.*, 2006). CO₂ flux under low-turbulence conditions was removed when the friction velocity was below 0.02 m s⁻¹, which was determined based on a statistical method described by Gu *et al.* (2005). Gaps of NEE were filled using flux calculation software (Ueyama *et al.*, 2012). Cumulative flux footprints were calculated with the footprint model of Kljun *et al.* (2004) in order to make sure that most of the footprint areas were within the minimal fetch of the fields.

2.4 Chamber measurements

CO₂ flux was also measured using the closed static chamber system in CF in 2012 and in GM in 2013. Part of the CF field

Table 1. Type of operations and the respective dates pertaining to grassland renovation in 2007, 2012, and 2013.

Operations	2007 (CF and CM)	2012 (CF and CM)	2013 (GM)
Cutting of the third crop	1 September	22 August	—
End of grazing	—	—	30 August
Lime application	—	—	10 September
Herbicide spray	—	27 August	10 September
Manure application	5 September [†]	29 August [†]	10 September
Moldboard plowing	12–13 September	4–5 September	17 September
Disk harrowing	13 September	11 September	19 September
Large stone removal	—	12 September [‡]	—
Lime application	13 September	18 September	—
Disk harrowing	—	22 September	—
Power harrowing	13 September	—	—
Chemical fertilizer application	14 September	25 September	1 October
Seed sowing	14–15 September	25 September	1 October
Soil covering (tooth harrowing)	15 September	25 September	1 October
Rolling	15 September	26 September	1 October
Seedling emergence	25 September	1 October	8 October [§]

[†] Applied to the CM field alone. [‡] Conducted in the CF field alone. [§] Seedling emergence occurred between 8 and 16 October, and the date of seedling emergence was assumed to be 8 October.

(about 5×20 m, Fig. 1a) where effects on the eddy covariance flux measurements were small and part of the GM field (about 5×20 m, Fig. 1b) that remained unrenovated (only sprayed with herbicide) were used for control chamber measurements. Four chambers were placed randomly in each renovated and unrenovated area, and individual chamber measurements were used as replicates.

Details of the system were presented previously by Matsuura *et al.* (2011). Briefly, the system consisted of an infrared CO₂ gas analyzer (GMP343 diffusion model, Vaisala, Helsinki, Finland) and a temperature/humidity sensor (HMP75, Vaisala) that penetrated through a chamber lid (white acrylic disk, diameter 465 mm); a data-logging device (MI70, Vaisala) for collecting data of CO₂ concentration, air temperature, and relative humidity inside a chamber; a temperature/pressure sensor (RS-12P, Espec Mic, Oguchi, Japan); and cylindrical stainless-steel chambers (inside diameter 390–415 mm, height 300 mm). Chambers were inserted to a target depth of 30 mm into the topsoil except after plowing when soil surface was rough; target depth after plowing was set at 50 mm to avoid air leakage or bypass below chambers as much as possible while ensuring enough chamber headspace. Chambers were temporarily removed during the series of operations for grassland renovation and reinstalled immediately after the operations. Each chamber was closed for 180 s, and CO₂ flux was calculated based on the CO₂ increase inside a chamber during the last 60–180 s, while taking atmospheric pressure and the dilution effect by water vapor inside the chamber into account.

Chamber measurements were made at about 0.5–1, 1–3 (only in 2012), 3–4, 5–6, and 24 h after plowing and subsequent disk harrowing and every 1–2 d (from 9:00 h to 11:00 h) after these operations except during rain. In 2013, measurements before plowing, about 0.5 h after tooth harrowing and 0.5 h after rolling were conducted in addition to the above measurements. Cumulative CO₂ emission was calculated using the trapezoidal rule. The flux values obtained from the last measurements before certain operations or from the first measurements after certain operations were temporally extrapolated up to the point of the operations for the calculations.

2.5 Supporting meteorological measurements

Air temperature was measured by a temperature/humidity sensor (HMP-45A, Vaisala) equipped with a stainless-steel ventilator (PVC-02-DC, Preed, Tokyo, Japan) at 2.5-m height. Precipitation was measured by a tipping bucket rain gauge (no model number, Nakaasa Instruments, Tokyo, Japan; or 52202, R. M. Young, Traverse City, MI, USA). These measurements were made at the center of the CM field near the eddy covariance system and the data were sampled every 10 s by a data logger (CR23X, Campbell Scientific). Air temperature was averaged for each 30-min period, and precipitation was summed over 30 min.

Soil temperature at 5.0 cm depth was measured using a temperature/pressure sensor (RS-12P, Espec Mic) with a temperature probe (RTH-1050, Espec Mic). Volumetric water content between the soil surface and a 6.0 cm depth was measured by a soil moisture sensor (ThetaProbe ML2x, Delta-T Devices, Cambridge, UK) connected to a hand-held data display (Soil Moisture Meter, Daiki Rika Kogyo, Kounosu, Japan). These measurements were made near each chamber simultaneously with the chamber measurements.

2.6 Soil sampling and analyses

Soil samples were collected using 100-mL stainless-steel cores (50 mm diameter, 51 mm length) several times before, during, and after the grassland renovation period from the renovated areas in CF in 2012 and in GM in 2013. Four or five cores were taken from the 0–5 and 10–20 cm layers in CF and 12 cores are taken from the 0–5 cm layer in GM each time. The three-phase volume ratio and bulk density of soils were determined based on solid and water volume measurements (digital actual volume meter DIK-1150, Daiki Rika Kogyo) and fresh and 105°C-oven-dried sample weight measurements.

In 2012, core samples (one 100-mL core for each layer) and composite samples were collected to determine bulk density and organic carbon concentration from the 0–5, 5–10, 10–20, and 20–30 cm layers at five locations each in the CF and CM fields. Samples were collected before and after grassland renovation (23–24 July and 20–21 November). The bulk density was determined as the mass of soil excluding coarse fragments >2 mm divided by the core sample volume. The organic carbon concentration of air-dried composite samples after passing through 2-mm mesh was determined by a dry combustion method (MT-700, Yanaco, Tokyo, Japan). The SOC stocks were calculated in an equivalent soil mass (Ellert and Battany, 1995) of 30-cm depth before renovation in each field.

2.7 Biometric measurements

Biometric measurements were conducted shortly before cutting the third crop in 2007 and 2012 in the CF and CM fields. Grass samples were clipped at about 5 cm above the ground at 9 to 12 locations in each field using a reciprocating grass mower with 1.3-m-wide cutter blades. The cutting areas and fresh weight of the samples were measured, and part of the fresh samples was oven-dried at 70°C and weighed. Total carbon of each dried sample was analyzed by a dry combustion method (MT-700, Yanaco).

2.8 Statistical analysis

All the statistical analyses were performed with the R software, version 3.3.0 (R Core Team, 2016). Student's two-sample *t*-test (or Welch's two-sample *t*-test when variances were unequal) was performed to analyze the effect of treatments or years on chamber measurements, soil organic carbon content, and biomass. Correlation analysis and multiple linear regression analyses were conducted to assess the effects of environmental parameters on the chamber measurements; CO₂ flux data after the post-tillage flush subsided were used for these analyses. The differences in soil physical properties among each sampling were analyzed with Tukey-Kramer's honestly significant difference test.

3. Results

3.1 CO₂ exchange in grassland fields for cutting (in 2007 and 2012)

The data-acquisition rates of NEE measured by the eddy covariance systems during grassland renovation periods in CF and CM after quality control were 62.8% and 61.6% in 2007 and 61.0% and 62.1% in 2012, respectively. The footprint analyses showed that, in both years, more than 90% and 80% of the observed fluxes for the dominant wind direction during these periods originated from within the minimal fetch of the CF and CM fields, respectively.

During grassland renovation periods, variations in daily mean air temperature were relatively small and the air temperature averaged 22.2 and 22.7°C in 2007 and 2012, respectively (Figs. 2a, 3a). Several precipitation events occurred during these periods, and the precipitation totaled 297.0 and 276.7 mm in 2007 and 2012, respectively (Figs. 2b, 3b). Renovation coincided with the period of autumnal rain, and rainfall affected by an autumn rain front sometimes continued for several days. In 2012, chamber measurements from 15 September to the end of the renovation could not be conducted due to rain. In the CF field in 2012, the soil temperature of the renovated area was 0.55°C higher, on average, than that of the unrenovated area (Fig. 3a), and the volumetric soil water content of the renovated area was 0.20 m³ m⁻³ lower, on average, than that of the unrenovated area during plowing and rolling (Fig. 3b).

According to chamber measurements (in 2012), a large CO₂ flush of 23.94 μmol m⁻² s⁻¹ was observed 16 min (average of four chamber measurements; the same applies hereafter) after plowing (Fig. 3c). The CO₂ flux dropped after about 5 h and then remained at less than 4.4 μmol m⁻² s⁻¹. A small flush (7.36 μmol

m⁻² s⁻¹) of CO₂ was also observed by chamber measurements 31 min after subsequent disk harrowing, dropping to the pre-harrowing level in less than 3 h.

Daily mean NEE showed similar trends to daily mean air temperature, with occasional declines or dips following precipitation events (*e.g.*, 6 and 12 September 2007, and 21 and 24 September 2012; Figs. 2c, 2d, 3c, 3d). Although clear flushes of CO₂ were not observed by eddy covariance measurements, daily mean NEE showed temporary increase in CO₂ emission after plowing in 2012 (Fig. 3c, d). In 2007, while daily mean NEE showed no increase or decrease after plowing, increase in CO₂ emission after disk harrowing and power harrowing was observed in CF (Fig. 2c). The average CO₂ emission during plowing and rolling tended to be smaller than that during manuring and plowing both in 2007 and 2012 (Table 2). The difference in average CO₂ emission between the fields during manuring and plowing was slightly larger than that during plowing and rolling.

The mean CO₂ losses during grassland renovation periods in CF and CM were 4.52 and 6.81 g C m⁻² d⁻¹ (108.4 and 163.3 g

Fig. 2. Environmental parameters and CO₂ fluxes in 2007: (a) air temperature, (b) precipitation, (c) NEE in the CF field, and (d) NEE in the CM field. Dashed vertical lines indicate the beginning of each operation for grassland renovation (MP, moldboard plow; DH, disk harrow; PH, power harrow; SC, soil covering; CR, corrugated roller).

C m⁻² in total over 24 d) in 2007 and 4.74 and 6.00 g C m⁻² d⁻¹ (189.5 and 240.0 g C m⁻² in total over 40 d) in 2012, respectively (Table 2). The mean CO₂ loss during plowing and rolling based on chamber measurements in the CF field in 2012 (3.84 g C m⁻² d⁻¹) was smaller than that based on eddy covariance measurements (5.02 g C m⁻² d⁻¹). The total CO₂ emission rates during plowing and rolling calculated from chamber measurements were 88.4 g C m⁻² in the renovated area in CF and 95.6 g C m⁻² in the unrenovated area in CF, with no statistically significant difference between them ($p = 0.53$).

3.2 CO₂ exchange in a grazing land field (in 2013)

The daily mean air temperature during the grassland renovation period in 2013 was 20.3°C (Fig. 4a). The total amount of precipitation during this period was 275.1 mm, which was similar to that in 2007 and 2012 (Fig. 4b), but the amount of precipitation after plowing was quite small. During plowing and rolling, the soil

temperature in the renovated area was 1.22°C higher, on average, than that in the unrenovated area (Fig. 4a), and the volumetric soil water content in the renovated area was 0.17 m³ m⁻³ lower, on average, than that in the unrenovated area (Fig. 4b). These trends are similar to those observed in 2007 and 2012.

A large CO₂ flush (14.45 μmol m⁻² s⁻¹) was observed 26 min after plowing, followed by a decrease of the CO₂ flux after about 6 h (Fig. 4c). The CO₂ flux remained at 4.13–4.67 μmol m⁻² s⁻¹ afterward. No flush of CO₂ was observed after disk harrowing. The CO₂ flux remained at a constant level afterward, with the flux in the renovated area tending to be higher than that in the unrenovated area. The mean CO₂ emission rates during the end of grazing and plowing were at the same level as that during plowing and rolling, although we should note that the CO₂ emission before plowing was estimated based on just a few measurements (Table 2, Fig. 4c).

The mean CO₂ loss during the grassland renovation period was 4.57 g C m⁻² d⁻¹ (178.3 g C m⁻² in total over 39 d; Table 2). The

Fig. 3. Environmental parameters and CO₂ fluxes in 2012: (a) air temperature and soil temperature, (b) precipitation and volumetric soil water content, (c) NEE and chamber-measured CO₂ flux in the CF field, and (d) NEE in the CM field. Dashed vertical lines indicate the beginning of each operation for grassland renovation (MP, moldboard plow; DH, disk harrow; SC, soil covering; CR, corrugated roller). The subscript Ch indicates the beginning of an operation in the area where chamber measurements were conducted. The subscript UR indicates values of the unrenovated area. Plots for chamber measurements are not connected with a line if certain renovation operations were conducted between them. Error bars indicate standard deviations ($n=4$).

Table 2. Total and average CO₂ emissions during grassland renovation periods.

Year and measurement method	Management period	Duration (d)	Cumulative CO ₂ emission of each field (g C m ⁻²)			Average CO ₂ emission of each field (g C m ⁻² d ⁻¹)		
			CF	CM	GM	CF	CM	GM
2007 (EC [‡])	Cutting–plowing	11	62.2	86.0		5.18	7.82	
	Cutting–manuring	4	19.8	23.9		4.94	5.98	
	Manuring–plowing	8/7 [†]	42.4	62.1		5.30	8.87	
	Plowing–rolling	3/4 [†]	12.1	27.7		4.03	6.92	
	Rolling–seedling emergence	9	34.2	49.6		3.80	5.51	
	Total	24	108.4	163.3		4.52	6.81	
2012 (EC [‡])	Cutting–plowing	13	62.9	91.2		4.84	6.51	
	Cutting–manuring	7	32.3	39.5		4.61	5.65	
	Manuring–plowing	6/7 [†]	30.7	51.6		5.11	7.37	
	Plowing–rolling	23/22 [†]	115.4	139.8		5.02	6.35	
	Rolling–seedling emergence	4	11.2	9.1		2.80	2.26	
	Total	40	189.5	240.0		4.74	6.00	
2012 (Ch)	Plowing–rolling	22	88.4 [§] (95.6)			3.84 (4.35)		
2013 (Ch)	End of grazing–plowing	18			83.6 [¶]			4.64
	Plowing–rolling	15			72.2 (56.0)			4.81 (3.73)
	Rolling–seedling emergence	6			22.5			3.76
	Total	39			178.3			4.57

EC, eddy covariance; Ch, chamber. Values in parentheses represent emissions of unrenovated areas. [†] Durations for CF/CM. [‡] Cumulative CO₂ emissions are estimated if 100% of the footprint was renovated. [§] Cumulative CO₂ emissions during the beginning of the plowing day and when plowing started were estimated using measurements of the unrenovated area. [¶] Estimated using a mean value of the measurements conducted before plowing.

Fig. 4. Environmental parameters and CO₂ fluxes in 2013: (a) air temperature and soil temperature, (b) precipitation and volumetric soil water content, and (c) chamber-measured CO₂ flux in the GM field. Dashed vertical lines indicate the beginning of each operation for grassland renovation (MP, moldboard plow; DH, disk harrow; SC, soil covering; CR, corrugated roller). The subscript UR indicates values of the unrenovated area. Plots for chamber measurements are not connected with a line if certain renovation operations were conducted between them. Error bars indicate standard deviations ($n=4$).

mean CO₂ loss during plowing and rolling, the period permitting comparison between the treatments, tended to be larger in the renovated area (4.81 g C m⁻² d⁻¹) than in the unrenovated area (3.73 g C m⁻² d⁻¹). However, there was no significant difference in the cumulative CO₂ emission during this period between the two areas (renovated 72.2 g C m⁻², unrenovated 56.0 g C m⁻²; $p = 0.076$).

3.3 Physical properties and organic carbon content of soils

After plowing in 2012 and 2013, the air ratio for 0–5 cm depth increased significantly and declines occurred in water and solid volume ratios and bulk density (Figs. 5a, c). The air ratio increased and the water volume ratio decreased during grassland

renovation, with the solid ratio remaining at the same level, in both years. After rolling, most of these soil physical properties returned to the levels before plowing in 2012, whereas all the properties remained at the same level in 2013. Soil physical properties at 10–20 cm depth showed similar trends as those of 0–5 cm depth after plowing (Fig. 5b). However, the amount of change was relatively small compared to those at the shallower depth, and soil physical properties returned to the original levels after disk harrowing.

The SOC content of the 0–5 cm layer in CM significantly decreased and that of deeper (10–30 cm) layers tended to increase after grassland renovation in 2012 (Table 3). The SOC concentration in CF showed a similar tendency, but there was no significant difference after renovation. The SOC stocks to a 30-cm depth before grassland renovation were 131.97 and 122.56 Mg C ha⁻¹ in CF and CM, respectively. The SOC stocks after renovation showed decreasing tendency compared to those before renovation in both fields (129.61 and 119.35 Mg C ha⁻¹ in CF and CM, respectively), although no significant difference was found between them.

3.4 Biomass production before grassland renovation

Biomass of the third crop in CF and CM were 1.08 and 1.14 Mg C ha⁻¹ in 2007 and 1.68 and 1.22 Mg C ha⁻¹ in 2012, respectively. Harvest biomass in 2012 were significantly different between the fields ($p < 0.01$). The harvest biomass in 2012 tended to be larger than that in 2007, with a significant difference in CF ($p < 0.01$).

3.5 Relationships between CO₂ flux and environmental factors

The CO₂ fluxes measured by closed chambers in the renovated areas were correlated with several environmental factors of the soil surface layer (Table 4). CO₂ flux was significantly correlated with the volumetric soil water content (negative correlation) alone in CF in 2012 and with the air ratio (positive), soil temperature (positive), and volumetric soil water content (negative) in GM. When the data obtained in CF and GM were analyzed together, a significant correlation was found between CO₂ flux and all the environmental factors investigated except soil temperature (air ratio, positive; solid volume ratio, negative; bulk density, negative; volumetric soil water content, negative). However, multicollinearity was detected among several soil parameters by an analysis based on variance inflation factors, and multiple regression analyses show no statistically significant model to predict CO₂ flux based on environmental factors. The correlation coefficient between soil temperature and CO₂ flux for the two fields was small compared to that for each individual field. This is partly because the responses of chamber-measured CO₂ flux (F_c) to soil temperature (T_s) differed between the fields (CF in 2012, $F_c = 0.917 \exp(0.049 T_s)$, $p = 0.373$ and 0.231 for the first and second coefficients, respectively; GM, $F_c = 1.423 \exp(0.051 T_s)$, $p = 0.009$ and 0.002 for the first and second coefficients, respectively).

4. Discussion

4.1 CO₂ exchange during grassland renovation and regulating factors

Changes in daily mean NEE were probably affected both by the renovation operation and environmental conditions such as air or soil temperature and precipitation, as suggested by the significant correlations between chamber-measured CO₂ fluxes and

Fig. 5. Changes in the three-phase volume ratio of soils and bulk density before, during, and after grassland renovation periods: (a) 0–5 cm layer of the CF field in 2012, (b) 10–20 cm layer of the CF field in 2012, and (c) 0–5 cm layer of the GM field in 2013. Statistically significant differences were found between values with different letters ($p < 0.05$). BD, bulk density; BP, before plowing; AP, after plowing; AD, after disk harrowing; AR, after rolling. Subscripts indicate the timing of soil sampling in days after each operation (e.g., AP_{3d}, soil samples collected 3 d after plowing).

environmental factors (Figs. 2, 3, Table 4), and it was difficult to determine the extent of their effects on CO₂ emissions based on the eddy covariance measurements conducted in renovated areas alone. However, net and mean CO₂ losses during grassland renovation over footprint areas were evaluated based on continuous NEE data as discussed below (see section 4.2 and 4.3).

Chamber measurements revealed flushes of CO₂ soon (16–31 min) after plowing (in 2012 and 2013) or disk harrowing (in 2012), followed by rapid drops of CO₂ flux after these operations (Figs. 3c, 4c). These trends are similar to those reported in previous studies (Reicosky and Lindstrom, 1995; Reicosky *et al.*, 1997; Ellert and Janzen, 1999; Gesch *et al.*, 2007; Willems *et al.*, 2011). The primary flush can be explained by the release of a high concentration of CO₂ from large voids in the soil (Reicosky and Lindstrom, 1995; Ellert and Janzen, 1999) and the release of dissolved CO₂ from soil water caused by evaporation of water and exposure of

air–water interfaces to atmospheric pressures and concentrations (Reicosky and Lindstrom, 1995) as a result of soil inversion. The secondary flush is due to the release of CO₂ trapped in pores caused by the destruction of large soil aggregates (Reicosky *et al.*, 1997). The release of dissolved CO₂ may also contribute to the secondary flush. The observed increase in air ratio and decrease in water volume ratio or volumetric soil water content (Fig. 5) and significant correlations between these parameters and chamber-measured CO₂ fluxes (Table 4) support this explanation.

Rapid drops of CO₂ flux observed by chamber measurements after plowing or disk harrowing suggest that a large CO₂ concentration was released immediately after these operations. Several studies reported larger CO₂ flux with more immediate measurements after tillage than observed in the present study (Table 5; Reicosky *et al.*, 1997; Willems *et al.*, 2011). Because the flush of CO₂ from the soil occurs along with tillage, it is quite difficult

Table 3. Changes in soil organic carbon concentration before and after grassland renovation in 2012.

Field	Timing of soil sampling	SOC concentration at four depths (g kg ⁻¹)				SOC stock to 30-cm depth [†] (Mg C ha ⁻¹)
		0–5 cm	5–10 cm	10–20 cm	20–30 cm	
CF	Before renovation	57.15	48.38	49.92	44.54	131.97
	After renovation	47.85	49.17	52.56	44.59	129.61
CM	Before renovation	58.62	37.11	40.34	28.21	122.56
	After renovation	35.51*	36.23	46.40	31.94	119.35

Asterisk indicates statistically significant difference from before renovation (**p* < 0.05).

[†] The SOC stock after renovation was calculated in an equivalent soil mass (Ellert and Battany, 1995) of 30-cm depth before renovation in each field.

Table 4. Pearson's correlation coefficient between soil parameters and chamber-measured CO₂ fluxes in the renovated areas.

Field (year)	<i>n</i>	<i>n'</i>	<i>A</i>	<i>S</i>	<i>BD</i>	<i>T_s</i>	<i>VWC</i>
CF (2012)	4	11	0.839	−0.893	−0.903	0.446	−0.69*
GM (2013)	6	12	0.851*	−0.494	−0.511	0.808**	−0.867**
CF and GM (2012–2013)	10	23	0.869**	−0.862**	−0.874**	0.164	−0.697**

Asterisks indicate statistically significant correlations (**p* < 0.05, ***p* < 0.01). *n*, number of observations for *A*, *S* and *BD*; *n'* number of observations for *T_s* and *VWC*, which include data not corresponding to the measurements of soil physical properties; *A*, air ratio; *S*, solid volume ratio; *BD*, bulk density; *T_s*, soil temperature; *VWC*, volumetric soil water content. Measurement depth: *A*, *S*, and *BD*, 0–5 cm; *T_s*, 5.0 cm; *VWC*, 6.0 cm.

Table 5. Comparison of CO₂ emission associated with grassland renovation between this and previous studies.

Reference	Location	Management	DAR (yr)	Treatment	Peak CO ₂ emission		Average CO ₂ emission	
					Value (μmol m ⁻² s ⁻¹)	TAT	Value (g C m ⁻² d ⁻¹)	DAV (d)
Reicosky <i>et al.</i> (1997)	USA	Cut, fertilized	>30	MP, DH	272.73	< 2.5 min	116	1
	USA	Cut, fertilized	>30	CP, DH	181.82	< 2.5 min	116	1
Willems <i>et al.</i> (2011)	Ireland	Grazed/cut, manured	5	Plow	43.62	8 min	3.05	7
Grandy and Robertson (2006)	USA	Cut (cut biomass left in place)	46	MP, DH	16.04	< 2 d	5.45	33
MacDonald <i>et al.</i> (2010)	Canada	Cut	7	S, MP, TH	5.45	< 1 d	1.64	75
	Canada	Cut, manured	7	S, MP, TH	4.55	< 1 d	0.92	75
Rutledge <i>et al.</i> (2014)	New Zealand	Grazed, fertilized	5–8	S, MP, PH, R	—	< 1 d	3.86–7.84	39–43
This study (CF)	Japan	Cut, fertilized	5 or 6	MP, DH, PH, TH, R	23.94	16 min	4.52–4.74	24–40
This study (CM)	Japan	Cut, fertilized, manured (annually)	5 or 6	S, MP, DH, DH, TH, R	—	—	6.00–6.81	24–40
This study (GM)	Japan	Grazed, fertilized, manured (once)	>7	S, MP, DH, TH, R	14.45	26 min	4.57	39

DAR, duration after previous renovation; TAT, time after primary tillage to the first observation; DAV, duration for averaging; S, spray; CP, chisel plow; MP, moldboard plow; DH, disk harrow; TH, tooth harrow; R, roller.

to measure the true peak values following tillage by using the chamber system. Chamber measurements, therefore, are likely to underestimate the total amount of CO₂ released following tillage. In contrast to chamber measurements, no clear flushes of CO₂ were observed in eddy covariance measurements. During plowing or disk harrowing operations when moving CO₂ sources are dominant and CO₂ flush intensity changes within a short period, CO₂ transport by horizontal advection is presumably not negligible and steady-state conditions cannot be assumed. Such conditions are not in agreement with the principal assumptions for the eddy covariance method. Thus, data quality of the eddy covariance measurements during these operations should be low and it is difficult to observe the flushes of CO₂ following tillage using this method. Due to the limitation of these measurement methods, we probably could not observe the true values of CO₂ flush during renovation. However, in the present study, measurements with these methods show a whole picture of the short-term CO₂ exchange during grassland renovation periods, including local and temporary phenomena such as the flushes of CO₂ following plowing or disk harrowing and continuous CO₂ flux representing footprint areas. CO₂ fluxes close to the true peak values following tillage can be observed if chamber measurements are made soon after these operations or if eddy covariance measurements are made with a low measuring height to ensure tillage operation within a footprint finishes before post-tillage flush subsides. Each measurement method has advantages and disadvantages and it is advisable to use both methods to evaluate CO₂ exchange during grassland renovation, as conducted in this study.

Despite a flush or temporary increase in CO₂ emission after plowing or disk harrowing, net CO₂ loss during plowing and rolling calculated from chamber measurements showed no significant difference between the renovated and unrenovated area both in CF and GM. Tillage, in general, induces CO₂ emission from soils due to increased aeration (Quincke *et al.*, 2007) and increased microbial degradation of organic matter resulting from aggregate disruption (Eriksen and Jensen, 2001; Grandy and Robertson, 2006). Increase in microbial activity is also induced by soil temperature increase after tillage (Grandy and Robertson, 2006). Removal of vegetation cover by tillage causes increase in soil heat fluxes with the absence of transpiration and plant shade, causing increase in average soil temperature (Monteith and Unsworth, 2013). In contrast, several studies reported that CO₂ emission from renovated grassland is smaller than that from unrenovated grassland (Yamulki and Jarvis, 2002; MacDonald *et al.*, 2010; Willems *et al.*, 2011). Reduction of CO₂ emission in renovated grassland is attributed to the contribution of both heterotrophic and autotrophic respiration. MacDonald *et al.* (2010) reported that CO₂ production by soil microflora was reduced after a poorly drained grassland was plowed. Quincke *et al.* (2007) noted that moldboard plowing, which buries labile organic matter at a depth where microbial activity is decreased due to lower temperature, does not increase cumulative CO₂ loss from the soil. Willems *et al.* (2011) reported that autotrophic respiration contributes to higher CO₂ emission from unplowed grassland than from plowed grassland. According to a meta-analysis by Subke *et al.* (2006), an average of 33% (10–42%) of soil respiration is autotrophic in temperate grasslands.

In the present study, average CO₂ emission during grassland renovation was likely determined by the balance between the increasing and decreasing effects of grassland renovation on CO₂ emission: the former factors include increased aeration and soil temperature after plowing (Table 4, Figs. 3a, 4a) and the latter include burial of plant biomass and annually applied manure into the subsoil. Our results suggest that these effects on CO₂ emission were balanced. Larger CO₂ emission rates during cutting and plowing in 2007 than in 2012 were explained by the fact that grasslands were not sprayed with herbicide in 2007 and autotrophic respiration from grass stubble and roots contributed to this difference. Reduced autotrophic respiration by plowing the living biomass into the subsoil likely have led to a decrease in CO₂ emission in 2007. In the GM field, which had been managed extensively, the decreasing effect of renovation on CO₂ emission may be small because of the burial of a limited amount of organic matter including cattle dung and composted cattle manure applied during renovation.

Significant correlations were found between chamber-measured CO₂ flux and several environmental factors, even though the measurements were made over different fields and years (Table 4). High correlations between CO₂ flux and air ratio (positive correlation), solid volume ratio (negative), and bulk density (negative) of the renovated area indicate that CO₂ flux is affected largely by changes in aeration during the grassland renovation process. The different response of CO₂ flux to soil temperature between the fields (see section 3.5) implies that the amount of organic matter (plowed plant biomass, manure, and surface litter), the effects of which could not be analyzed directly in this study, also affect CO₂ flux during grassland renovation. One reason for our inability to develop a significant multiple regression model of environmental factors is probably the limited number of observations. Many factors such as the meteorological and soil environment, quantity and quality of plowed organic matter, and SOC content have multiple effects on CO₂ flux during grassland renovation. Although the evaluation of those effects is challenging, gathering data under various conditions is necessary to better understand the factors regulating CO₂ exchange during renovation.

4.2 Short-term carbon loss and carbon input associated with grassland renovation

The average rates of respiratory CO₂ loss during the grassland renovation periods were 4.52–4.74, 6.00–6.81, and 4.57 g C m⁻² d⁻¹ in CF, CM, and GM, respectively (Table 2). Larger CO₂ emissions in CM than CF were mainly due to decomposition of the applied manure. Smaller CO₂ emission in GM than CM may be due to less organic matter input in this field. These values are within the range reported in the literature (Table 5; Grandy and Robertson, 2006; MacDonald *et al.*, 2010; Rutledge *et al.*, 2014). Net CO₂ emissions during the renovation periods, which represent short-term carbon loss associated with grassland renovation, were 108.4–189.5, 163.3–240.0, and 178.3 g C m⁻² in the CF, CM, and GM fields, respectively (Table 2). Estimated CO₂ emissions from fuel combustion by renovation operations were 4.9–5.2, 5.6, and 5.2 g C m⁻² in the CF, CM, and GM fields, respectively. The ratios of CO₂ emissions from fuel combustion to those from the soil during renovation periods were within 5%

(CF, 2.6–4.8%; CM, 2.3–3.4%; and GM, 2.9%).

In 2012, when the amount of SOC was measured before grassland renovation, net CO₂ losses calculated from eddy covariance measurements during grassland renovation were 1.44% (189.5 g C m⁻² / 131.97 Mg C ha⁻¹) of the carbon stock in the top 30 cm of soils in CF and 1.96% (240.0 g C m⁻² / 122.56 Mg C ha⁻¹) in CM (Tables 2, 3). These values are similar to those calculated for New Zealand dairy pasture (0.86–1.91%, extrapolated from measurements for 0–10 cm depth by Rutledge *et al.* (2014)). Although previous studies have reported a SOC decrease or increase following conversion of grassland to or from other land-use types (Post and Kwon, 2000; Conant *et al.*, 2001), little is known about carbon loss from the soil associated with grassland renovation and SOC recovery following renovation. Necpalova *et al.* (2014) noted a significant SOC decrease following grassland renovation and no evidence of SOC recovery during the 3 yr after renovation. However, when biomass or SOC-rich soil surface layer is buried in the subsoil layer during renovation, the decomposition rate of the biomass or SOC will be decreased due to lower temperature, which should improve SOC accumulation in grasslands.

The SOC stocks after renovation showed decreasing tendency in both fields in this study (Table 3). However, biomass buried during grassland renovation can contribute to long-term SOC accumulation. We estimated the amount of plowed biomass and evaluated its effects on carbon balance. The reported ratios of non-harvested biomass (stubble and roots) to harvested biomass of grasses varied from 0.91 to 24.5 according to plant species, environmental conditions, and seasons (Sakai *et al.*, 1969; Janicka, 2004; Kacorzuk *et al.*, 2013). We adopted the ratios for *D. glomerata* cut in late summer (stubble / harvested parts 0.63, roots / harvested parts 1.45; Sakai *et al.*, 1969) and estimated the plowed biomass of stubble and roots as 0.81 (0.68–1.06 in CF and 0.72–0.77 in CM) and 1.85 (1.56–2.44 in CF and 1.65–1.77 in CM) Mg C ha⁻¹ on average, respectively. Accordingly, the amount of carbon input during renovation including stubble, roots, and applied manure (other than surface litter) was estimated as 2.24–3.50 and 6.17–8.77 Mg C ha⁻¹ in CF and CM, respectively. Many studies report decomposition rate or residence time of biomass in soil. Rasse *et al.* (2005) summarized several studies and estimated that mean residence time of root-derived carbon in soil is 2.4 times that of shoot-derived carbon. Puget and Drinkwater (2001) reported that 49% of the carbon derived from grass roots was present in the soil after five month, whereas only 13% of the shoot-derived carbon remained. According to Gerzabek *et al.* (1997), half-lives of organic carbon originating from green manure and animal manure was 3.3 and 7.0 yr, respectively. Shiga *et al.* (1985) investigated decomposition process of various organic amendments by glass-fiber filter bag method and found that plant shoot, plant root, and livestock manure largely consists of fraction with a half-life of 0.15, 1.5, and 15 yr, respectively. It can be presumed from these findings that stubble-derived carbon is labile and less responsible for long-term SOC accumulation. On the other hand, carbon derived from grass roots and livestock manure is presumably more resistant in soil. Decomposition rate of biomass buried in deeper layer will be decreased due to lower temperature, as suggested from the changes in the difference in CO₂ emission rate between

the fields after plowing (Table 2). On the assumption that only the buried biomass of grass roots and manure applied during renovation contribute to long-term SOC accumulation, at most 1.56–2.44 and 5.40–8.04 Mg C ha⁻¹ of carbon can be accumulated in CF and CM soils, respectively.

4.3 Effects of practices pertaining to grassland renovation on CO₂ emissions

As discussed above, grassland renovation work does not affect short-term net CO₂ loss significantly, although it affects CO₂ emissions to a certain extent by changing soil environment (Table 4). We can, therefore, say that net CO₂ loss during renovation periods is mainly brought by the absence of vegetation in this site. The ratios of net CO₂ emission during renovation in 2007 to that in 2012 were 0.57 (108.4 g C m⁻² / 189.5 g C m⁻²) and 0.68 (163.3 g C m⁻² / 240.0 g C m⁻²) in the CF and CM fields, respectively, and these ratios are close to the ratio of the renovation period, 0.6 (24 d / 40 d, Table 2). These results indicate that respiratory CO₂ loss did not decrease over time during renovation periods and that shortening the durations of renovation will decrease net CO₂ loss during renovation almost in proportion to period shortening. Grasses, in general, allocate more biomass (*i.e.*, more carbon) to belowground part than to aboveground part as discussed above (Sakai *et al.*, 1969; Janicka, 2004; Kacorzuk *et al.*, 2013), contributing to SOC accumulation (Rasse *et al.*, 2005; Gibson, 2009) even though aboveground biomass is harvested. Thus, it is recommended to complete grassland renovation process as quickly as possible and shorten the duration without photosynthesis in order to reduce net CO₂ loss associated with grassland renovation.

Chamber measurements revealed a flush of CO₂ after soil disturbance such as plowing and disk harrowing. The largest flush was observed after plowing, which is the first and largest soil disturbance in the grassland renovation process. Several studies noted that the amount of CO₂ emission after tillage varies depending on the tillage tools (La Scala *et al.*, 2001; Reicosky *et al.*, 2005; Gesch *et al.*, 2007; Quincke *et al.*, 2007) or tillage depth (Reicosky and Archer, 2007); larger and deeper disturbance induces larger CO₂ emission. In Japan, simple grassland renovation methods using a rotary harrow with shallow tillage depth instead of a moldboard plow and a disk harrow or using a no-till drill seeder without other soil-disturbing operations are sometimes used in order to lower costs, decrease labor, and reduce the risk of soil loss (Agricultural Production Bureau, MAFF, 2006). At the same time, these simple methods can reduce CO₂ emission during the grassland renovation process.

Our findings are based on short-term measurements around the grassland renovation period. To evaluate the overall impact of renovation on the carbon dynamics in managed grasslands including growth of sown grasses or decomposition of buried biomass, it will be necessary to conduct long-term monitoring of CO₂ exchange with paired (renovated and unrenovated) observations or to compare the measurements before and after renovation. In addition, it is important to compare the carbon loss associated with grassland renovation with the degree of the recovery or increase in yields after renovation, especially when evaluating the effects of the renovation method.

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