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ORIGINAL ARTICLE

Manure application in managed grasslands can contribute to soil organic carbon sequestration: evidence from field experiments across Japan

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Abstract

We conducted field experiments in nine managed grassland sites spanning annual temperatures of 5.8–16.3 °C for 5 years to investigate the effects of farmyard manure (FYM) application and environmental conditions on soil organic carbon (SOC) stock changes. Experimental plots were established with three (zero, low, and high) levels of continuous FYM application at each site, and soil samples down to 30 cm depths were annually collected to determine SOC stocks. Annual changes in SOC stocks were analyzed by fitting a linear mixed-effect model, including the sites as random-effects. Based on the model, low and high level FYM application lead to 1.9–10.4 (4.8 on average) and 3.4–15.1 (8.4 on average) Mg C ha^{-1} year^{-1} of SOC increase across the sites, respectively. The random-effects for annual changes in SOC stocks were not correlated with soil type (andic vs. non-andic) but positively correlated with the duration, implying that the grassland maintenance without renovation contributed more to SOC sequestration than soil type. Simple simulations using the model showed that SOC stocks increased at all nine sites with recommended (low) level of FYM application. The simulation also revealed that SOC sequestration with the recommended level FYM application can be maintained even under a global change scenario with temperature rise of up to 2.8 °C above the current level. These results indicated that managed grasslands in Japan with the recommended level FYM application can contribute to climate change mitigation via SOC sequestration even under future climate change.

Keywords Soil organic carbon · Managed grasslands · Farmyard manure compost · Field experiments · Statistical model

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Introduction

Soil organic carbon (SOC) is the largest global carbon pool in terrestrial ecosystems (Bolin et al. 2001); therefore, even small SOC stock changes can have major impacts on atmospheric carbon dioxide (CO_2) concentration. Changes in SOC stocks can also affect food security, because SOC is the main component of soil organic matter (OM) that conserves water and nutrients and improves soil structure. The flow of carbon between the soil and the atmosphere is affected not only by climate variables and soil conditions, but also directly by land management.

In agricultural lands, SOC stocks can be increased with management practices, such as no-till farming, cover crops, or manure application, benefiting the land by both reducing CO_2 emission or increasing CO_2 absorption and achieving sustainable agriculture by improving soil quality (Lal 2004). Managed grasslands particularly play an important role in SOC sequestration among agricultural lands, because of their abundant belowground biomass and generally low decomposition rates (Anderson 1991; Gibson 2009). The amount of SOC sequestered in grasslands may be affected, both positively and negatively, by management (Abberton et al. 2011; Conant et al. 2017; Soussana et al. 2004). Livestock manure application is one of the major options to increase SOC sequestration because most livestock systems on grasslands generate large amounts of manure. Increase in carbon sequestration in grasslands by livestock manure application has been reported in various regions (Fortuna et al. 2003; Hirata et al. 2013; Jones et al. 2006).

In Japan, managed grasslands occupy 13.5% of the entire agricultural lands, which corresponds to 29.7% of the upland area (Ministry of Agriculture, Forestry and Fisheries [MAFF] 2020a). Despite this substantial percentage, much is unknown about SOC stocks in managed grasslands, partly because information on grassland soils have not been systematically collected in the past unlike soils under other agricultural management (Agricultural Production Bureau, MAFF 2008). Even the most comprehensive study evaluating nationwide grassland SOC stocks is only based on single timepoint estimations (Matsuura et al. 2012), and the changes in SOC stocks or factors affecting them still remain unknown. Therefore, it is important to evaluate them to introduce effective grassland management practices for increasing SOC sequestrations and acquiring accurate estimates of SOC stock changes in nationwide agricultural lands. Regarding grassland management, farmyard manure (FYM) compost application is one of the most important practices in Japan, because the livestock industry has been highly dependent on imported feed, causing various environmental problems due to inappropriate handling of livestock excreta. Accordingly, the Japanese government urges livestock farmers to effectively use excreta as compost and properly apply it on agricultural lands, also expecting to contribute to SOC sequestration (Agricultural Production Bureau, MAFF 2018).

The present study was conducted using field experiments in grassland sites across Japan that have been started in 2010 to investigate the changes in SOC stocks in managed grasslands and, through statistical model analyses, determine the effects of FYM compost application and environmental conditions on SOC stock changes. We hypothesized that soils with andic properties that result from the weathering of volcanic parent materials (FAO 2006) have positive effects on SOC stock change. The potential of SOC sequestration under current and future climate change conditions was also discussed based on statistical model analyses.

Materials and methods

Site description

Field experiments were conducted at nine sites across Japan, including the Tokachi (TKC), Niikappu (NKP), Ohu (OHU), Iwate (IWT), Nagano (NGN), Tottori (TTR), Kumamoto (KMM), and Miyazaki (MYZ) stations of the National Livestock Breeding Center (NLBC), and the head office of the NLBC at Fukushima Prefecture (FKS) (Table 1, Online Resource 1). The sites are located on a wide range of latitude from 31° 58′ N to 43° 04′ N, with mean annual air temperatures and precipitations ranging from 5.8 to 16.3 °C and from 841 to 2571 mm year^{-1}, respectively. The soil types in the sites vary among five soil groups; however, five of the sites have andic properties. The remaining four sites include Regosolic Andosols (Vitric Andosols; FAO 2006) which do not show andic properties.

Experimental design

The study was conducted from 2010 to 2015. Nine experimental plots (4 × 4 m or 5 × 5 m) were established with randomized block design in a grassland field at each site in 2010. The plots received three levels of FYM application treatment in triplicate. FYM application rates were 0, 30, and 60 Mg ha^{-1} year^{-1} on fresh weight basis, and these treatment groups are hereafter referred to as M00, M30, and M60, respectively. FYM compost was applied on soil surface annually after the final cut (during late autumn and winter). The treatment started after initial soil sampling (during October 2010 and February 2011) except in MYZ after the second soil sampling (November 2011).

The grassland of all the sites were categorized as cultivated grassland (Allen et al. 2011), where no animals were grazed. The experimental fields of each site were used as intensively or extensively managed grassland before starting this study. The annual cutting frequency was 2–4 times, which differed

Table 1 Site characteristics

Site name	Experimental station	Latitude, longitude	Elevation (m a.s.l.[a])	T_{aN} (°C)	Pr_N (mm year^{-1})	Soil subgroup[b]	Vegetation	Cutting frequency	Previous grassland renovation
TKC	Tokachi station	43° 04′ N, 143° 10′ E	122	5.9	841	Haplic Pseudogley soils (Haplic Stagnosols)	*Phleum pratense* L., *Dactylis glomerata* L., *Trifolium pratense* L., *Trifolium repens* L.	2	1994
NKP	Niikappu station	42° 25′ N, 142° 28′ E	80	8.0	1032	Humic Regosolic Andosols (Umbric Vitric Andosols)	*Phleum pratense* L.	2	2005
OHU	Ohu station	40° 43′ N, 141° 08′ E	70	9.5	983	Cumulic non-Allophanic Andosols (Melanic Aluandic Andosols) [c]	*Phalaris arundinacea* L.	2	1996
IWT	Iwate station	39° 46′ N, 141° 08′ E	180	10.2	1266	Low-humic Allophanic Andosols (Silandic Andosols) [c]	*Dactylis glomerata* L., *Trifolium repens* L.	2–3	Autumn, 2007
FKS	Head office (Fukushima prefecture)	37° 09′ N, 140° 02′ E	820	11.5	1411	Haplic Allophanic Andosols (Umbric Silandic Andosols) [c]	*Phleum pratense* L.	3	October, 2008
NGN	Nagano station	36° 15′ N, 138° 30′ E	700	10.6	961	Humic Regosolic Andosols (Umbric Vitric Andosols)	*Dactylis glomerata* L., *Medicago sativa* L. (lc), *Echinochloa crus-galli* (L.) Beauv.	3	August 2002
TTR	Tottori station	35°30′ N, 133° 38′ E	40	14.7	1777	Cumulic non-Allophanic Andosols (Melanic Aluandic Andosols) [c]	*Dactylis glomerata* L., *Festuca arundinacea* Schreb., *Stellaria media* (L.) Villars	3	Around 1999
KMM	Kumamoto station	32° 51′ N, 130° 32′ E	0	16.3	1765	Lowland Reformed soils (Haplic Regosols (Transportic))	*Phalaris arundinacea* L., *Lolium multiflorum* Lam.	3–4	September 2007
MYZ	Miyazaki station	31° 58′ N, 130° 57′ E	230	16.3	2571	Low-humic Allophanic Andosols (Silandic Andosols) [c]	*Dactylis glomerata* L.	3–4	September–October 2010

T_{aN}, normal values (mean values during 1981–2010) of mean annual air temperature; Pr_N, normal values of annual precipitation; T_{aN} and Pr_N are data from nearby stations of the Automated Meteorological Data Acquisition System run by Japan Meteorological Agency

[a] Above sea level

[b] Comprehensive Soil Classification System of Japan, First Approximation (Obara et al. 2015). Soil names in parentheses indicate corresponding soils of the World Reference Base for Soil Resources 2006 (FAO, ISRIC and IUSS 2006)

[c] Soils with andic properties

depending on sites (Table 1). The timing of the previous grassland renovation (plowing and reseeding) also differed depending on sites, 0–16 years before the beginning of this study. Plant species or cutting schedule of each site were based on those generally used in each region.

FYM compost was produced in each site using the excreta from livestock raised in the site, with additional organic amendment (Online Resource 2). Recommended FYM application rate in managed grasslands differed depending on the prefecture, generally about 20–50 Mg ha^{-1} year^{-1} (MAFF 2020b), and the rates were not necessarily consistent with climate conditions or grass species. However, 30 Mg ha^{-1} year^{-1} is near-recommended application rate in most regions.

The amount of three major nutrients (N, P, and K; hereafter simply referred to as nutrient) supplied to each plot was designed to be comparable as much as possible. The application rates of chemical fertilizers in the M00 plots were determined based on the recommended levels for the regions where the sites are located. For M30 and M60 plots, nutrient which cannot be covered by the release from FYM was compensated by chemical fertilizers. Nutrient release from FYM was estimated as follows: the release rate of N was estimated using Uchida's model (Shiga et al. 1985) developed in Japan, and those of P_2O_5 and K_2O were estimated based on the handbook of animal waste management and utilization in Hokkaido (Research Team for Animal Waste Project, Hokkaido Agriculture and Animal Husbandry Experimental Stations 2004). Chemical fertilizer application was ceased, or the amount of chemical fertilizer application was reduced, in cases where estimated nutrient supply rates from FYM exceeded the regional recommended nutrient application rates (Online Resource 3).

Soil measurements

Soil samples from each plot were annually collected from a depth of 30 cm divided into two layers (0–5 cm and 5–30 cm) during the period between the final cut and subsequent FYM application to determine SOC stocks. Initial samples were collected in 2010 before the first FYM application treatment. The first layer in M30 and M60 plots includes organic layer starting the second year of the experiment. The organic layer tended to increase its thickness over the 5-year experiment due to the lack of plowing. In a long run, the organic layer will be plowed and mixed with mineral soil to a depth of approximately 30 cm when the grassland is renovated; therefore, samples were taken from the surface of the organic layer, and carbon content was evaluated.

Triplicate core samples were taken from the middle of each layer to determine bulk density using 100-ml stainless-steel cores. The core samples were oven-dried at 105 °C and weighed. Then, the remaining coarse fragments (gravel and coarse OM) in the core samples that were filtered on a 1-mm sieve were oven-dried at 105 °C and weighed. Composite samples to determine carbon concentration were taken from each whole layer and passed through a 1-mm sieve after air-drying. Part of the sieved soil was ground to < 0.5-mm particles, and the total carbon concentration was determined using a dry combustion method (JM1000CN; J-Science Lab, Kyoto, Japan or MT-700; Yanaco, Tokyo, Japan).

In FKS site, soils from surrounding fields were dumped over the experimental plots due to strong winds in early spring in 2013, making it difficult to analyze changes in bulk densities or organic carbon stocks; therefore, the data acquired in 2010–2012 were used for this site's analyses.

SOC stock per unit area should be corrected for the mass and volume of coarse fragments. However, it is difficult to determine the volume of coarse fragments. Therefore, SOC stock per unit area was calculated without measuring the volume of coarse fragments by defining soil bulk density as the oven-dried mass of soil, excluding coarse fragments divided by the core volume as follows:

$$CS = \sum_{i=1}^{k} (CC_i \times BD_i \times d_i) \times 10 \quad (1)$$

$$BD_i = [W_{Si} - (W_{Gi} + W_{Oi})]/V_{Si} \times 10^{-3} \quad (2)$$

where CS is the SOC stock per unit area (Mg C ha^{-1}), CC_i is the SOC concentration (g C kg^{-1}), BD_i is soil bulk density (Mg m^{-3}), d_i is thickness of the soil layer (m), W_{Si} is the mass of the core soil sample (kg), W_{Gi} is the mass of gravel > 1 mm (kg), W_{Oi} is the mass of coarse OM > 1 mm (kg), and V_{Si} is the volume of the core (m^3). The subscript i indicates layer number, and $k = 2$ in this study.

When Eqs. (1) and (2) were combined, the equation to find CS becomes the same equation as the one that Expert Panel on Soil (2006) or Rodeghiero et al. (2009) proposed to correct the mass and volume of coarse fragments. We quantified SOC stocks in equivalent soil mass in each site based on mineral soil mass to eliminate the effects of changes in soil OM on the equivalent soil mass (Toriyama et al. 2011), which was adjusted from the method proposed by Ellert and Bettany (1995). The initial soil mass is chosen as the reference soil mass in each site.

Biometric and farmyard manure measurements

Biometric measurements were conducted immediately before each grass cutting to evaluate the amount of annual dry matter yield (Y). Grass samples were clipped approximately 5–10 cm above the ground from a 50 × 50-cm area in each plot, and their fresh weights were measured. Part of the samples were oven-dried at 70 °C for > 48 h and weighed.

FYM samples were collected from the compost stockpile immediately before or after application to determine the amount of carbon input through FYM application (CM). After measuring fresh weight, the samples were air-dried and ground to measure the total carbon concentration using the dry combustion method (JM1000CN or MT-700).

Meteorological data

Mean air temperature (T_a) and accumulated precipitation (Pr) during the period between each annual soil sampling were calculated using the data from the Automated Meteorological Data Acquisition System near each site (Japan Meteorological Agency 2020).

Statistical analysis

All statistical analyses were performed using the R software, version 3.5.2 (R Core Team 2018). Tukey's honestly significant difference test was conducted to confirm whether the initial values of soil bulk density and SOC concentration/stocks of each soil layer in each treatment were not significantly different. Annual changes in bulk density and SOC concentration of each soil layer (ΔBD_i and ΔCC_i) and SOC stocks of 0–30 cm depth (ΔCS) were analyzed by fitting linear mixed-effect model using the lmer function in the lmerTest package (Kuznetsova et al. 2017); the sites were included as random-effects, expecting that the effects of soil type differences will be observed. In particular, regarding ΔCS, we hypothesized that soils with andic properties have large random term values. The explanatory variables (fixed effects) included soil parameters of the previous year corresponding to each objective variable (BD_{ip}, CC_{ip}, and CS_p), and CM, Y, T_a, and Pr during the previous year. Model selection was conducted using the dredge function in the MuMIn package based on Akaike's Information Criterion (AIC), with lower AIC values indicating the preferred model, and the models with the lowest AIC values were selected. However, models with small (< 2) AIC differences relative to the smallest AIC value in the set of models (ΔAIC) also provided substantial support (Burnham and Anderson 2002). Therefore, the validity of the lowest-AIC-value models was examined whether they have a reasonable set of variables by comparing them with models having an ΔAIC < 2 upon model selection. The selected models were re-fitted, and the fixed effects and their corresponding *p*-values, as well as the random-effects, were calculated.

Results

Carbon input through farmyard manure application

The applied FYM had carbon concentrations of 166.7–428.3 g C kg^{-1} on dry weight basis and 85.6–261.3 g C kg^{-1} on fresh weight basis, which differed depending on the site (Online Resource 2). The amount of carbon input through FYM application were 2.6–7.8 (6.0 on average) and 5.1–15.7 (12.0 on average) Mg C ha^{-1} $year^{-1}$ in M30 and M60, respectively. Relatively large variations among the applied years were observed in the amount of carbon input compared with the carbon concentration due to the varying water content of FYM among applied years.

Bulk density

The initial bulk density of each site ranged from 0.40 to 1.06 and from 0.37 to 0.98 Mg m^{-3} in the first and second layer, respectively (Online Resource 4). We confirmed no significant difference in the initial bulk density among the treatment groups with three exceptions: between M00 and M60 of the first layer in NKP ($p = 0.042$) and OHU ($p = 0.029$), and that of the second layer in NGN ($p = 0.039$). Decreasing tendency in bulk density of the first layer was observed in M30 and M60 in most sites. The fitted linear mixed-effect model revealed that BD_{1p}, CM, and Y have significant negative effects ($p < 0.01$), and T_a has a positive effect (not significant [n.s.]) on ΔBD_1 (Online Resources 7 and 8). BD_{2p} and T_a showed significant negative ($p < 0.01$) and positive (n.s.) effects on ΔBD_2, respectively. No significant relationship was found for random-effects between ΔBD_1 or ΔBD_2 and the different soil types.

Carbon concentration

The initial carbon concentration ranged from 19.2 to 122.6 and from 6.8 to 110.9 g C kg^{-1} in the first and second layers, respectively (Online Resource 5). No significant difference in carbon concentration among the treatment groups in the initial year was observed except between M00 and M60 of the first layer in TKC ($p = 0.022$). The carbon concentration of the first layer showed an increased tendency that increased as FYM application rate or site latitude increased. On another note, carbon concentration trend of the second layer was unclear. The fitted model for ΔCC_1 included significantly negative interaction effects ($p < 0.01$) of CM and T_a, in addition to significantly negative ($p < 0.01$) and positive ($p < 0.01$) effects of CC_{1p} and CM, respectively, and only T_a showed a positive effect on ΔCC_1 (n.s.) (Online Resources 7 and 8). The model for ΔCC_2 was affected negatively by CC_{2p} ($p < 0.01$) and T_a (n.s.), whereas it was positively affected by CM ($p = 0.015$). No significant difference was observed in the random terms of the fitted models between soil types. The random-effects for both ΔCC_1 and ΔCC_2 tended to increase with the number of years after previous grassland renovation, but no significant correlation was observed.

Carbon stocks

The initial values of SOC stocks per unit area to a depth of 30 cm ranged from 13.4 to 187.5 Mg C ha^{-1} (Fig. 1, Online Resource 6). We confirmed no significant difference in SOC stocks among the treatments in the initial year, except between M00 and M60 in TKC in the first layer ($p = 0.024$) or 0–30 cm depth ($p = 0.049$). SOC stocks in the second layer showed no clear trend, and an increasing tendency in SOC stocks was observed in the first layer in several sites, particularly in M30 and M60, resulting in the increased tendency in SOC stocks of 0–30 cm depth (Fig. 1). Generally, high-latitude sites tended to have higher SOC stocks in 30 cm depths than low-latitude sites. However, there are a few exceptions such as IWT or TTR sites. The model for ΔCS revealed significantly negative and positive effects of CS_p ($p < 0.01$) and CM ($p < 0.01$), respectively (Table 2). T_a had an insignificantly negative effect on ΔCS. No significant difference was observed in the random-effects for ΔCS between soil types, and the hypothesis that soils with andic properties have large random terms was not supported. However, the random-effects showed increasing tendency with the number of years after previous grassland renovation, and they had marginally significant correlation ($p = 0.051$, Fig. 2).

Evaluation of soil organic carbon stock changes with simulations using the fitted model

SOC stock changes of 0–30 cm depth in each site were estimated using the fitted model to evaluate the current state of SOC stocks and the changes in SOC sequestration under future warming conditions. As ΔCS was negatively affected by CS_p, the estimates of SOC stock changes calculated from the initial values took a saturation curve, and they converged in about 10 years for all nine sites. This is partly because the models obtained in this study were estimated from relatively short-period (5-year) data, and it is not appropriate to use them for long-term estimation. Thus, the estimated 1-year changes in SOC stocks from the initial values were analyzed using simple simulations with specific parameters for each site as follows: *CM* as the mean values during the study period, current state of T_a as the normal values (T_{aN}; annual means during 1981–2010), and CS_p as the initial values averaged over all treatments.

First, the estimates under current T_a conditions were calculated (Table 3). The estimation of the amount of carbon input through FYM compost application necessary for $\Delta CS > 0$ Mg C ha^{-1} year^{-1} had negative values in five sites, indicating that SOC can be increased without applying FYM. The other four sites needed 0.8–3.4 Mg C ha^{-1} year^{-1} of carbon input, which corresponded to 5.0–14.3 Mg ha^{-1} year^{-1} of FYM application, to maintain SOC stocks. SOC increase from the initial stocks in M00 averaged over all sites was estimated as − 2.0 to 5.7 (1.3 on average) Mg C ha^{-1} year^{-1}. Furthermore, 30 Mg ha^{-1} year^{-1} of FYM application led to an increase in SOC stocks in every site, and the increasing rate from the initial value was estimated as 1.9–10.4 (4.8 on average) Mg C ha^{-1} year^{-1} on average. The estimated SOC increase in M60 was at an average of 3.4–15.1 (8.4 on average) Mg C ha^{-1} year^{-1}.

Then, ΔCS from the initial SOC stocks under increased T_a conditions from T_{aN} to T_{aN} + 6.0 °C was simulated for each treatment (Fig. 3). It should be noted that the simulations did not consider changes in grass yield, precipitation, or soil environment under increased T_a conditions, only taking into account the explanatory variables included in the fitted model for ΔCS. The estimations of the nationwide average air temperature increase from the current levels in Japan under four Representative Concentration Pathway (RCP) scenarios (Sasaki et al. 2015) are also indicated in the figures. ΔCS linearly decreased with T_a increase. The number of sites with $\Delta CS >$ 0 Mg C ha^{-1} year^{-1} decreased along with the increase in T_a, whereas ΔCS remained positive until T_a rose to T_{aN} + 3.3 °C in M00, and the mean T_a at which ΔCS turns to negative was T_{aN} + 0.73 °C on average of nine sites. SOC enhancement in M30, where T_a at which ΔCS turns to negative was T_{aN} + 2.8 °C on average, can be maintained even under warming conditions up to T_{aN} + 6.0 °C. FYM application at the rate of 60 Mg ha^{-1} year^{-1} led to further SOC increase, and ΔCS remained positive even under 8.7 °C T_a increase, with T_a at which ΔCS turns to negative being T_{aN} + 4.8 °C on average. The differences of simulated ΔCS values among the sites in M30 or M60 under warming conditions were not the same as those in M00, because ΔCS was affected by *CM* of each site. Some of the sites have low T_a values at which ΔCS turns to negative despite high-carbon FYM application, as found in M30 in NKP or FKS site (Online Resources 2). The sites with low ΔCS values in M00 and low-carbon FYM application in M30 and M60, as shown in NGN or TTR site, also have low T_a values at which ΔCS turns to negative in all FYM application regime. In contrast, some sites have relatively high T_a values at which ΔCS turns to negative in all FYM application regime, as found in IWT or KMM site which have low initial SOC stocks.

Table 2 Results of linear mixed-effect model analyses for annual soil organic carbon (SOC) stock changes

Fixed-effect				Random-effect	
Parameter	Estimate	SE	*p*-value	Parameter	Estimate
(Intercept)	82.728	21.601	< 0.001	TKC	19.044
CS_p	− 0.543	0.044	< 0.001	NKP	8.014
CM	0.595	0.111	< 0.001	OHU	25.625
T_a	− 1.733	1.617	0.289	IWT	− 31.927
				FKS	− 4.116
				NGN	− 13.614
				TTR	39.894
				KMM	− 42.194
				MYZ	− 0.726

Linear mixed-effect model for annual SOC stock changes (ΔCS, Mg C ha^{-1} year^{-1}) was expressed as $\Delta CS \sim CS_p + CM + T_a + (1 \mid \text{site})$; refer to Table 1 for the abbreviations of the site names (parameters for random-effects)

CS_p, SOC stock of the previous year (Mg C ha^{-1}); *CM*, the amount of carbon input through farmyard manure application (Mg C ha^{-1}); T_a, mean air temperature between each annual soil sampling (°C); (1 | site), random term; *SE*, standard error

Discussion

Response of soil parameters to environmental and management factors

We analyzed the annual changes in soil parameters ΔBD_i, ΔCC_i, and ΔCS by fitting linear mixed-effect models. The negative effects of the corresponding soil parameters of the previous year in the fitted models led to convergence of the modeled parameters, indicating the possibility of reaching new steady states. Convergence time for the SOC stock changes estimated from the model was approximately 10 years, which was relatively a short period, compared with literature values (West and Post 2002; West and Six 2007). The effects of soil parameters of the previous year may have been probably overestimated in the models. This is not only because the estimation was based on limited timepoint data, but also because annual changes in soil parameters were

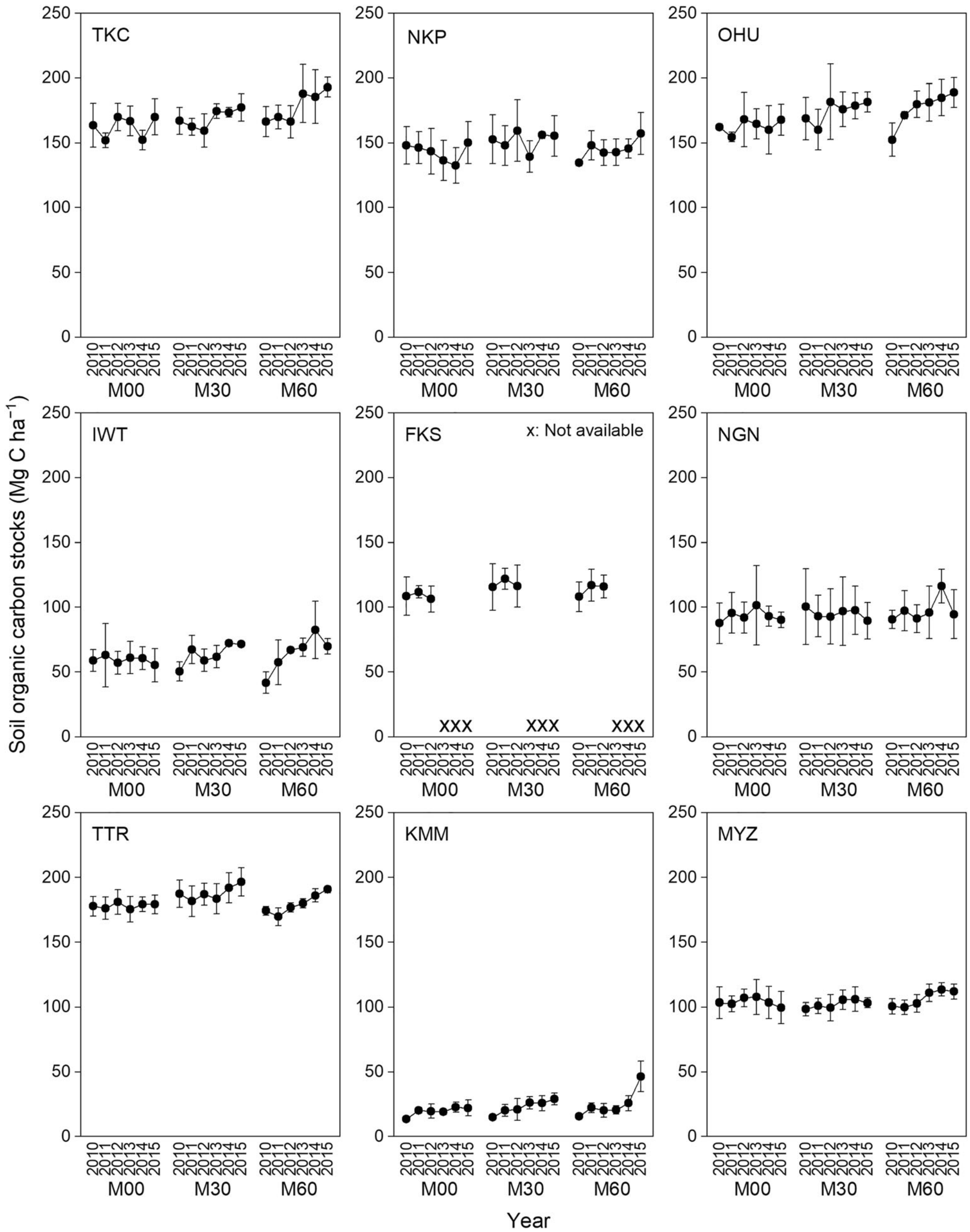

Fig. 1 Annual variations in soil organic carbon stocks in equivalent soil mass based on mineral soil mass. Error bars indicate standard deviations (n = 3). M00: without farmyard manure (FYM) application; M30: 30Mg ha^{-1} year^{-1} of FYM application; M60: 60Mg ha^{-1} year^{-1} of FYM application. Refer to Table 1 for the abbreviations of the site names. In FKS site, soils from surrounding fields were dumped over the experimental plots due to strong winds in early spring in 2013, making it difficult to analyze changes in bulk densities or organic carbon stocks; therefore, the data acquired in 2010–2012 were used for this site's analyses

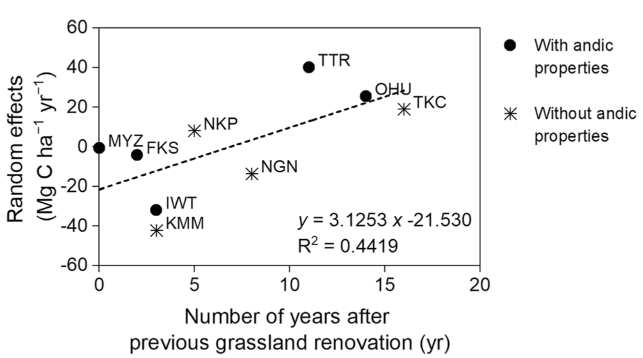

Fig. 2 Relationship between the number of years after previous grassland renovation and the random-effects obtained for the model for annual soil organic carbon stock changes. Refer to Table 1 for the abbreviations of the site names

estimated using the corresponding parameters of the previous year, and both of which have large data variabilities, often leading to SOC stock data fluctuation (Fig. 1). Despite these limitations, the effects of environmental and biometric parameters on the modeled soil parameters were reasonable. Therefore, the models helped to understand or evaluate at least short-term changes in soil parameters.

There were significant negative effects of *CM* on ΔBD_1, implying that OM input affected surface soil bulk density (Online Resource 8). Furthermore, a decrease in bulk density, along with the increase in soil OM concentration, was observed by Adams (1973). This is interpreted as a dilution effect caused by the mixing of coarsely structured OM with denser mineral fractions of the soil (Haynes and Naidu 1998).

Table 3 The amount of farmyard manure (FYM) compost and its carbon content necessary for soil organic carbon (SOC) changes > 0 Mg ha^{-1} year^{-1}, and SOC increase in each treatment, which was estimated from the fitted model (Mg C ha^{-1} year^{-1})

Site	C_{input} ($\Delta CS > 0$)	FYM_{input} ($\Delta CS > 0$)	Estimated SOC increase[a]		
			M00	M30	M60
TKC	− 2.8	− 13.9	1.7	5.2	8.8
NKP	3.4	14.3	− 2.0	2.2	6.4
OHU	− 7.2	− 31.2	4.3	8.4	12.6
IWT	− 9.6	− 36.9	5.7	10.4	15.1
FKS	2.5	9.9	− 1.5	3.0	7.4
NGN	− 0.6	− 7.1	0.4	1.9	3.4
TTR	0.8	5.0	− 0.5	2.5	5.4
KMM	− 7.2	− 44.2	4.3	7.1	10.0
MYZ	1.6	8.0	− 1.0	2.7	6.3
Mean	− 2.1	− 10.7	1.3	4.8	8.4

C_{input} ($\Delta CS > 0$): the amount of carbon input through FYM application necessary for SOC changes > 0 Mg C ha^{-1} year^{-1} ; FYM_{input} ($\Delta CS > 0$): the amount of FYM application necessary for SOC changes > 0 Mg C ha^{-1} year^{-1} . M00: without FYM application; M30: 30 Mg ha^{-1} year^{-1} of FYM application; M60: 60 Mg ha^{-1} year^{-1} of FYM application. Refer to Table 1 for the abbreviations of the site names

[a] SOC stock changes estimated from the initial values using the fitted model with the following parameters for each site: *CM* as the mean values during the study period, current state of T_a as the normal values (annual means during 1981–2010), and CS_p as the initial values averaged over all treatments

The decrease in bulk density associated with OM input was reported by Weil and Kroontje (1979) and Khaleel et al. (1981). *Y* also showed negative effects on ΔBD_1; however, since ΔCC or ΔCS was not affected by *Y*, the effects of *Y* was unrelated to OM input, but it may have been caused by increased root activity and aggregation promoted by residue decomposition products in the soil surface layer (Shaver 2010). The negative effect of T_a on ΔBD_1 and ΔBD_2 possibly reflected the increase in OM decomposition due to high temperature. The effects of *CM* and T_a on ΔCC_1, ΔCC_2, and ΔCS were reasonable (Table 2, Online Resource 8). The positive and negative effects of *CM* and T_a indicated the increased SOC due to FYM application and increase in OM decomposition due to high temperature, respectively. The negative interaction effects of *CM* and T_a on ΔCC_1 suggested that warming-induced decomposition may exceed the positive effect of *CM* on ΔCC_1, implying that temperature variation was more important than the FYM application for SOC sequestration. The fixed-effect structure of the models for the second layer, the absent effects of *CM* and *Y* on ΔBD_2, and the lack of interaction effects of *CM* and T_a on ΔCC_2 are explained by the fact that the effects of coarsely structured OM and the supply of grass residue or labile OM were limited to the surface soil under the no-till management of the grassland plots during the study period.

The effects of site-specific factors on soil organic carbon changes

Andic properties are largely controlled by the presence of reactive metals (particularly aluminum and iron) mainly originated from volcanic materials, and these metal phases can bind with OM to form organo-mineral complexes (Boudot et al. 1989; Higashi 1983; Wagai et al. 2020), contributing to OM stabilization in the soils (Torn et al. 1997). We thus hypothesized that soils with andic properties have large random terms, which was not supported because no significant difference was observed in the random-effects for ΔCS between soils with andic and non-andic properties. Two possible reasons for this are as follows. First, in this study, FYM applied to the soil surface was not plowed and mixed with soil during the experiments, not having enough chance to interact with the reactive metal phases (Hopkins et al. 2009) even in soils with andic properties. Second, chemical weathering to form reactive metal phases is relatively slow even for volcanic materials (Dahlgren et al. 1997; Shoji et al. 1993). However, further characterization of organo-mineral interactions may reveal the mineralogical control of SOC sequestration.

Interestingly, the random-effects for ΔCS were positively correlated with the number of years after previous grassland renovation (Fig. 2). Long-term SOC sequestration in grasslands up to many decades has been reported (Klumpp and Fornara 2018). The results of the model analyses may result from the development of soil aggregates that induce physical protection of organic carbon over the course of the no-till management after reseeding (Razafimbelo et al. 2008; Six et al. 2002). The fixed effects of FYM application on annual SOC changes calculated with the average carbon input in M30 was 3.6 Mg C ha^{-1} year^{-1} of SOC stock increase (6.0 × 0.595; Online Resource 2, Table 2), which corresponded to 1.1 times the size of the random-effects per year after grassland renovation (3.6 divided by the slope of the regression line, 3.1; Fig.

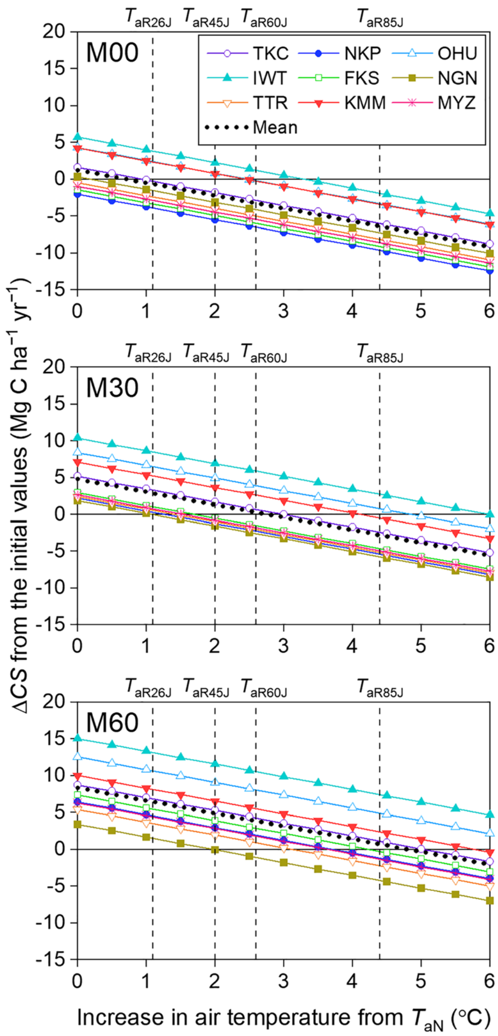

Fig. 3 Responses of modeled soil organic carbon (SOC) changes from the initial SOC stocks under increased air temperature conditions from the normal values in M00 (without farmyard manure [FYM] application), M30 (30 Mg ha^{-1} year^{-1} of FYM application), and M60 (60 Mg ha^{-1} year^{-1} of FYM application) treatments. Response for each site and averaged response over all sites are indicated. ΔCS, annual changes in SOC stocks; T_a, mean air temperature between each annual soil sampling; T_{aN}, normal values of air temperature (annual means during 1981–2010). T_{aR26J}, T_{aR45J}, T_{aR60J}, and T_{aR80J} indicate the estimations of nationwide averaged air temperature increase from the current level in Japan under four Representative Concentration Pathways scenario, RCP 2.6 (1.1 °C increase), RCP 4.5 (2.0 °C), RCP 6.0 (2.6 °C), and RCP 8.5 (4.4 °C), respectively (Sasaki et al. 2015)

2). The results indicated that the effect of maintaining the grasslands for 1 year is almost the same as 30-Mg ha^{-1} year^{-1} FYM application in terms of SOC sequestration. Maintaining grasslands in their high productivity and favorable vegetation state can reduce the frequency of grassland renovation; increase the rate of SOC sequestration; and at the same time, reduce labor and cost for renovation.

Potential increase in soil organic carbon stocks by farmyard manure application

The estimates calculated with the fitted model for ΔCS showed that SOC increased in M00 at a rate of 1.3 Mg C ha^{-1} year^{-1} when averaged over all sites (Table 3). However, SOC decreased in four sites without FYM application, implying that several managed grasslands in Japan need OM input to maintain SOC stocks. The FYM application rate of 30 Mg ha^{-1} year^{-1}, the recommended level in most regions in Japan, can potentially increase SOC in all nine sites (4.8 Mg C ha^{-1} year^{-1} on average). Moreover, 60-Mg ha^{-1} year^{-1} FYM application may lead to further SOC increase, but the 30-Mg ha^{-1} year^{-1} option is more feasible in most regions for mineral balance of forage grasses (Agricultural Production Bureau, MAFF 2007). Several short-term and long-term experimental studies have demonstrated that OM input increased SOC in managed grasslands (1.3 Mg C ha^{-1} year^{-1} during 2 years in Germany (Steinbeiss et al. 2008); 2.6–8.1 Mg C ha^{-1} year^{-1} during 6 years in the UK (Jones et al. 2006); − 0.06 to 1.6 Mg C ha^{-1} year^{-1} during 12 years in the USA (Franzluebbers and Stuedemann 2009); 3.7–4.4 Mg C ha^{-1} year^{-1} during 20 years in Canada (Angers et al. 2010); 0.31–0.86 Mg C ha^{-1} year^{-1} during 43 years in Ireland (Fornara et al. 2016); and 0.066–0.15 Mg C ha^{-1} year^{-1} during 126 years in the UK (Hopkins et al. 2009)). Yagasaki and Shirato (2014) used a model simulation based on nationwide soil inventory data to show that the SOC of the top 30 cm in managed grasslands in Japan increased by 0.9 Mg C ha^{-1} year^{-1}, with 4.1–5.1 Mg C ha^{-1} year^{-1} of organic carbon input through manure (including slurry and excreta) application during 1990–2000, although studies on SOC changes in Japanese grasslands are quite limited. Short-term studies (3–4 years) that used a micrometeorological approach indicated that ecosystem carbon in managed grasslands in Japan was lost by − 0.9 to − 0.1 Mg C ha^{-1} year^{-1} without FYM application, but it increased with 30-Mg ha^{-1} year^{-1} FYM application by 0.8–2.1 Mg C ha^{-1} year^{-1} (Limin et al. 2015; Matsuura et al. 2014). The estimates in the current study is comparable to the literature values of the increased SOC rate in Japanese grasslands, which are relatively high compared with those in other regions.

Recent organic carbon input through manure application in managed grasslands in Japan was estimated at 4.0 Mg C ha^{-1} year^{-1} (2000–2008) (Yagasaki and Shirato 2014), which is 2.0 Mg C ha^{-1} year^{-1} less than the carbon input with M30 FYM application (6.0 Mg C ha^{-1} year^{-1} on average, Online Resource 2). Based on the fitted model, FYM application at 2.0 Mg C ha^{-1} year^{-1} less than 30 Mg ha^{-1} year^{-1} calculated for each site (i.e., FYM application equivalent to 4.0 Mg C ha^{-1} year^{-1} on average) can increase SOC stocks by 3.6 Mg C ha^{-1} year^{-1} on average. Thus, managed grasslands in Japan entirely have the potential to sequester roughly an additional

1.2 Mg C ha^{-1} $year^{-1}$ (4.8 − 3.6 Mg C ha^{-1} $year^{-1}$) of SOC if FYM is applied up to the recommended level of 30 Mg ha^{-1} $year^{-1}$, whereas the potential depends on FYM quality, as well as climate, vegetation, or soil conditions. Actually, the amount of FYM production is unevenly distributed depending on regions; several places have a surplus of FYM, whereas others allow further application (Agricultural Production Bureau, MAFF 2018; Mishima et al. 2008). Therefore, it would be impossible to apply FYM to all managed grasslands up to the recommended level; however, SOC sequestration can be increased by distributing and applying surplus FYM to grasslands permitting additional application.

Soil organic carbon sequestration potential under future climate change conditions

The simulations under warming conditions showed that the averaged T_a at which ΔCS turns to negative in M00 was T_{aN} + 0.73 °C, which is below the nationwide average of T_a increase under RCP 2.6 scenarios (1.1 °C increase) (Fig. 3). Furthermore, 30 Mg ha^{-1} $year^{-1}$ of FYM application increased SOC stocks at all nine sites and can maintain SOC enhancement up to 2.8 °C increase on average, which was slightly above the averaged nationwide T_a increase under RCP 6.0 scenarios (2.6 °C increase). These simulation results revealed that the managed grasslands in Japan, with the recommended level of FYM application, have the potential to increase SOC sequestration under future climate changes. The simulation also showed the differences among the sites for SOC sequestration potential under warming scenarios. Some of the sites which have low initial SOC stocks have relatively large potential to increase SOC stocks even without FYM application as IWT or KMM site. In contrast, the sites having low ΔCS values in M00, particularly sites with low-carbon FYM application such as NGN or TTR, would need high-carbon FYM application to increase SOC stocks under warming conditions. The results showed that SOC stocks can be increased or decreased depending on future grassland management strategies. Several sites have relatively low T_a values at which ΔCS turns to negative even with high-carbon FYM application, as found in NKP or FKS site, implying that SOC sequestration of these grasslands may be vulnerable to future climate changes even after applying the recommended level of FYM.

Conclusions

The simulations based on a linear mixed-effect model signified that the managed grasslands in Japan applied with FYM at the recommended level (30 Mg ha^{-1} $year^{-1}$) can increase SOC stocks even under future warming conditions. The model also revealed that long-term maintenance of grasslands without renovation has an effect of increasing SOC sequestration rate and, at the same time, improving soil quality and saving labor and cost. Even though SOC in some grasslands may be vulnerable to future climate changes, proper grassland management including proper FYM application can potentially mitigate climate change effects. The findings in this study will contribute to better understanding of carbon dynamics in managed grasslands, as well as the effects of grassland managements.

One of the limitations of this study is that the analyses were conducted with statistical models based on limited timepoint data. Another limitation is that the models used in this study took into account the decomposition of the applied FYM only in the next year of the application. A further study using process-based carbon models, such as RothC or CENTURY, should be conducted with longer term soil data to understand more detailed SOC sequestration processes and obtain more reliable SOC change estimations. Quantitative evaluation of soil types such as the content of clay/silt or reactive metals, including the effects of grassland renovation, would also be necessary to understand the processes and mechanisms behind carbon stabilization and SOC sequestration.

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Declarations

Conflict of interest The authors declare no competing interests.

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