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1 Soil carbon stocks and carbon sequestration rates in semi-natural grassland in Aso region,
2 Kumamoto, southern Japan

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4 Running title: Soil C sequestration in grassland in Aso, Japan

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Abstract

Global soil carbon (C) stocks account for approximately three times that found in the atmosphere. In the Aso mountain region of southern Japan, semi-natural grasslands have been maintained by annual harvests and/or burning for more than 1,000 years. Quantification of soil C stocks and C sequestration rates in Aso mountain ecosystem is needed to make well-informed, land-use decisions to maximize C sinks while minimizing C emissions. Soil cores were collected from six sites within 200 km² (767-937 m asl.) from the surface down to the k-Ah layer established 7,300 years ago by a volcanic eruption. The biological sources of the C stored in the Aso mountain ecosystem was investigated by combining C content at a number of sampling depths with age (using ¹⁴C dating) and δ¹³C isotopic fractionation. Quantification of plant phytoliths at several depths was used to make basic reconstructions of past vegetation and was linked with C-sequestration rates. The mean total C stock of all six sites was 232 Mg C ha⁻¹ (28-417 Mg C ha⁻¹), which equates to a soil C sequestration rate of 32 kg C ha⁻¹ yr⁻¹ over 7,300 years. Mean soil C sequestration rates over 34, 50 and 100 years were estimated by an equation regressing soil C sequestration rate against soil C accumulation interval, which was modeled to be 618, 483 and 332 kg C ha⁻¹ yr⁻¹, respectively. Such data allows for a deeper understanding in how much C could be sequestered in *Miscanthus* grasslands at different time scales. In Aso, tribe Andropogoneae (especially *Miscanthus* and *Schizochyrium* genera) and tribe Paniceae contributed between 64 and 100% of soil C based on δ¹³C abundance. We conclude that the semi-natural, C₄-dominated grassland system serves as an important C sink, and worthy of future conservation.

Introduction

Climate change and increasing atmospheric CO₂ are inextricably linked (IPCC, 2007). Estimates of global soil carbon (C) stocks are 2,500 Pg (organic C, 1,550 Pg; inorganic C, 950 Pg), which is 3.3 times higher than that of atmospheric C stocks (760 Pg) (Lal, 2004, 2008).

Grassland ecosystems can contribute to C mitigation through biomass feedstock production (substituting fossil fuels) and through C sequestration in the soil (Carpenter-Boggs *et al.*, 2003; Arshad *et al.*, 2004; Bronson *et al.*, 2004). Guo & Gifford (2002) reported that land-use change from cropland or forest to grassland can increase soil C stocks. Grassland ecosystems comprise approximately 41% of the terrestrial land area, except for areas of permanent ice cover (Adams *et al.*, 1990; White *et al.*, 2000). Moreover, soil organic matter (SOM) in temperate grasslands has been estimated to account for 331 Mg ha⁻¹, and grasslands contain 12% of global SOM (Conant *et al.* 2001; Schlesinger, 1977). Natural and semi-natural grasslands, which are comprised of several graminoid and forb species, including *Miscanthus sinensis*, account for 24% of grasslands (National Parks Association of Japan, 1996) and, in recent years, 4% (1,512 Mha) of Japan (Himiyama *et al.*, 1995). Many Japanese grasslands have been managed by annual harvesting for fuel and fodder and/or burning (Otaki, 1999; Stewart *et al.*, 2009). In the Aso region of southern Japan, the grassland vegetation is dominated mainly by *Miscanthus sinensis*, *Pleioblastus variegatus*, *Spodiopogon sibiricus*, *Arundinella hirta*, and *Imperata cylindrica* (Koyama & Ogawa, 1993; Yamamoto *et al.*, 2002). In this region, Andisols with high organic C content are widely distributed (Matsuyama & Saigusa, 1994; Toma *et al.*, 2011).

Estimation of soil C change is essential to determine how much plant C contributes to soil C stock. Changes in soil mass C are, however, difficult to detect in short-term studies (Paustian *et al.*, 1997; Bellamy *et al.*, 2005; Koga *et al.*, 2006; Subke *et al.*, 2006) even with modern isotopic-fractionation techniques (Hansen *et al.*, 2004; Subke *et al.*, 2006; Clifton-Brown *et al.*, 2007; Toma *et al.*, 2011). One of the methods to estimate soil C accumulation rate is to estimate age with ^{14}C dating at a particular depth with the corresponding mass of soil C above the layer, divided by ^{14}C -date estimates (Schlesinger, 1990). This simple method underestimates soil C accumulation rates in deep soil horizons, because only recalcitrant soil C remains in deeper soil layers (Schlesinger, 1990). When soil C accumulation rates are measured in soil layers of different ages, a regression model between this parameter and duration of soil C accumulation can be fitted, which allows soil C accumulation rates for short time spans (several years to decades) to be projected. This projection can only be applied to ecosystems where similar vegetation structure is maintained in the long-term (>100 or >1,000 years), such as the Aso mountain grassland, which, unlike land used for agriculture, has retained a similar vegetation structure for >1,000 years (Miyabuchi & Sugiyama, 2006). *Miscanthus*, in particular *Miscanthus × giganteus*, has recently attracted attention as a renewable resource of biomass (Heaton *et al.*, 2004; Somerville *et al.*, 2010). Biomass production of *M. sinensis* in Aso was reported to be amongst the highest ($15 \text{ Mg ha}^{-1} \text{ yr}^{-1}$) of that studied in Japan (Stewart *et al.*, 2009; Toma *et al.*, 2010) and has been maintained and sustainably utilized for long periods of time. The Aso mountain grassland therefore makes an ideal study site to relate SOC dynamics and the contributing structure from different types of vegetation. Toma *et al.* (2010, 2012) demonstrated previously that soil C in the semi-natural

grasslands in Aso mainly comes from belowground roots and rhizomes, since annual harvest and/or burning removes most of aboveground biomass produced by the grasses from the ecosystem. To date, there are few studies detailing the contribution of C supply by belowground organs of both *Miscanthus* spp. and other plant species to soil C sequestration. Our main objectives were to 1) estimate the short- (several years) and long-term (>1,000 years) mean annual C sequestration rates, 2) quantify the average of soil C stock, and 3) to understand the interactions of plant species in establishing and maintaining this C sink.

Materials and Methods

Site description

The study was conducted in a semi-natural grassland on Andisols located on the northern rim and center of the Mt. Aso caldera in Kumamoto Prefecture, southern Japan, which was within an area of 225 km² (15 km × 15 km) (Fig. 1). In the region, *M. sinensis* vegetation is widely distributed (Fig. 2). The location of the sample sites, which were named site-A, site-B, site-C, site-D, site-E, and site-F, are shown in Fig. 1. Current land use in sites A, C, D, and F were meadows; site-B was grassland that had not been harvested for more than 40 years, and site E was a grazed pasture. The common management applied in each site was an annual burning event in each March. Mean annual precipitation and air temperature over a 30-year period (1971-2000) around the Aso area were 3,250 mm and 9.6°C, respectively. In the Aso region, the K-Ah horizon is a visually distinct volcanic ash layer deposited 7,300 years ago (Miyabuchi & Watanabe, 1997).

Soil sampling and analysis of C content, $\delta^{13}\text{C}$, ^{14}C dating, and plant phytolith

Soil samples were collected for calculating bulk density at 5-cm increments (79 cm³) in sites A-E and 10-cm increments (159 cm³) at site-F from the soil surface to the bottom of the K-Ah layer on 27 November 2009. Also, soil samples for measuring soil C content, soil $\delta^{13}\text{C}$, soil ^{14}C dating, and plant phytoliths were collected at the same depths.

Bulk density of each 5-cm or 10-cm increment was calculated by the weight of the samples dried in an oven at 105°C for 48 hr. Air-dried soil samples were sieved through a 2-mm mesh and crushed finely to powder. The total soil C content of powdered soil

samples was measured in an elemental analyzer (Vario EL III, Elemental, Hanau, Germany). In these volcanic soils, there is no inorganic C (Nelson & Sommers, 1996) and therefore, estimates of total organic C are equivalent to SOC derived from plants.

Stable isotope ratios for $^{13}\text{C}/^{12}\text{C}$ were determined in soil samples taken from sites A to D. Samples were generally recovered from five to nine depths in the soil profile. $^{13}\text{C}/^{12}\text{C}$ was measured in powdered samples with an Accelerator Mass Spectrometer (AMS, Beta Analytic Inc., Miami, Florida, USA) and expressed in units relative to the international standard Pee Dee Belemnite (PDB) in standard δ notation (‰) as follows:

$$\delta^{13}\text{C} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000,$$

where R_{sample} is the isotope ratio $^{13}\text{C}/^{12}\text{C}$ of the sample and R_{standard} is the $^{13}\text{C}/^{12}\text{C}$ of the PDB standard (Balesdent *et al.*, 1987). Proportion of C (X_{C4}) of C_4 plant species-derived C in the soil was calculated as follows:

$$X_{\text{C4}} = (\delta^{13}\text{C}_{\text{soil}} - \delta^{13}\text{C}_{\text{C3}}) / (\delta^{13}\text{C}_{\text{C4}} - \delta^{13}\text{C}_{\text{C3}}) \times 100,$$

where $\delta^{13}\text{C}_{\text{soil}}$ is $\delta^{13}\text{C}$ of collected soil, $\delta^{13}\text{C}_{\text{C3}}$ is $\delta^{13}\text{C}$ of C derived from C_3 plant species, and $\delta^{13}\text{C}_{\text{C4}}$ is $\delta^{13}\text{C}$ of C derived from C_4 plant species. Values of $\delta^{13}\text{C}_{\text{C3}}$ and $\delta^{13}\text{C}_{\text{C4}}$ were cited from reported values of mean $\delta^{13}\text{C}$ of C_3 plant species (-28 ‰, Yoneyama, 2008) or rhizome of *M. sinensis* (-14.7 ‰, Toma *et al.*, 2012). Because *M. sinensis* was the dominant plant species relative to soil C accumulation in this study, its value of $\delta^{13}\text{C}$ for was used to represent that of C_4 plant species (shown in Results).

Soil samples from a depth of 30- to 35-cm were also used for C dating using AMS. More samples were used in site B where the sampling depth was shallower (10-15-cm) and deeper (50-55-cm) due to local soil conditions. The conventional ^{14}C ages were calculated using the Libby half-life of 5,568 years and soil $\delta^{13}\text{C}$. Corrections to calendar

years were performed by a program compiled by Talma & Vogel (1993) based on calibration data sets from INTCAL 98 (Stuiver *et al.*, 1998). Soil C sequestration rate was calculated by total soil mass C, which was the sum of soil mass C above the ¹⁴C-date-measured soil layers or the K-Ah layer, and was then divided by corresponding ¹⁴C dates or 7,300 years (K-Ah layer).

We modified Two Compartments of the Exponential Decay model (TCED model) developed by Murayama *et al.* (1990) by fitting a curve between mean soil C sequestration rate and soil C accumulation interval. The TCED model consisted of labile (C_1) and non-labile (C_2) fractions as shown in the following equation,

$$Y_t = C_1 \exp(-k_1 t) + C_2 \exp(-k_2 t) \quad \text{equation 1}$$

where Y_t is the amount of residues remaining at time t . C_1 and C_2 are the initial proportions decomposed according to the rate constants k_1 and k_2 , respectively. Mean annual soil C sequestration rate for T year period (R_T) was shown by the sum from t equals 1 to T of Y_t over T as shown in the following equation,

$$R_T = (\sum_{t=1}^T Y_t) / T \quad \text{equation 2}$$

KaleidaGraph (var. Win 4.1, HULINKS, Tokyo, Japan) was used for fitting the equation to the relationship between mean annual soil C sequestration rate and soil C accumulation interval.

Identification and quantification of plant phytoliths in the soil followed methods described by Fujiwara (1976) and Miyabuchi *et al.* (2012). Briefly, soil samples were oven dried at 105°C for 24 hr. Glass beads (diameter of 40 µm) were added to the sample at a rate of 0.02 g per 1 g of soil to provide a visual marker. Organic matter in the samples was removed by loss on ignition at 550°C for 6 hours before dispersion in an ultrasonic

bath (300 W, 42 kHz) for 10 minutes. Particles coarser than 20 µm were extracted by a precipitation method (Miyabuchi *et al.*, 2011). Identification and counting of plant phytoliths were performed under a polarizing microscope at 400× magnification and continued until more than 400 glass beads were counted. Plant phytolith content was calculated by the following equation:

$$\text{Phytolith content} = (Gg \times Pc) / Gc,$$

where Gg is the total number of glass beads in the sample equivalent to 1 g, Pc is the number of grains of one phytolith morphotype counted in the scan, and Gc is the number of glass beads counted in the scan. Identification of phytolith morphotypes was based on Fujiwara (1976), Fujiwara & Sasaki (1978), Kondo & Sase (1986), Sugiyama & Fujiwara (1986), Sugiyama *et al.* (1988), and Sugiyama (1999, 2001). With this technique, tribes Andropogoneae and Paniceae were identified. In addition, the *Miscanthus* was the only genus that could be identified in tribe Andropogoneae. The classification of Suzuki (1996) was used for subfamily Bambusoideae. Aboveground biomass production of identified plant species in collected soil layers (5-cm or 10-cm thickness) was calculated with the following equation:

$$\begin{aligned} \text{Aboveground biomass production (kg m}^{-2} \text{ cm depth}^{-1}) &= \text{number of phytolith per 1 g} \\ &\text{soil} \times F \text{ (gram of dry plant biomass per 1 phytolith)} \times \text{bulk density (kg m}^{-2} \text{ cm depth}^{-1}) \times \\ &5 \text{ or } 10 \text{ (cm depth)} \end{aligned}$$

where F is the conversion factor for estimating aboveground biomass production from number of plant phytoliths. Conversion factors ($\times 10^{-5}$) of each identified plant species were 2.94 (*Oryza sativa*), 8.4 (*Panicum* type), 1.24 (*Miscanthus* type), 0.33 (*Schizachyrium* type), 1.16 (*Pleioblastus* sect. *Nipponocalamus*), 0.48 (*Pleioblastus* sect.

Nezasa), 0.75 (*Sasa* sect. *Sasa*), and 0.3 (*Sasa* sect. *Crassinodi*) (Sugiyama, 2000; Sugiyama *et al.*, 2002; Inoue *et al.*, 2001; Watanabe *et al.*, 1996).

Estimation of regional soil carbon storage and mean soil C sequestration rate in semi-natural grassland

Soil C stock in semi-natural grassland was calculated by regression between soil C sequestration rate and duration of soil C accumulation. Soil C stock was estimated for 7,300 years, because similar vegetation in semi-natural grassland in the Aso region was reported to be retained for approximately 10, 000 years (Miyabuchi & Sugiyama, 2006; Miyabuchi *et al.*, 2010). Total soil C stock for 7,300 years and soil C sequestration rate in semi-natural grassland in the Aso region was estimated in the area of current semi-natural grassland (11,000 ha; Ohtaki, 1999). Total soil C sequestration rate in natural and semi-natural grassland in Japan (1,512 Mha; Himiyama *et al.*, 1995) was also calculated.

Statistical analysis

Statistical analyses were performed using “R” (version 2.10.1, R Development Core Team, 2005). Regression analysis between C content and total number of plant phytoliths, or soil mass C and calculated biomass production were performed by the least squares method.

Results

Soil C contents at six sites ranged from 5 to 252 g C kg⁻¹, and generally decreased with depth (Fig. 3). In contrast with soil C, bulk densities increased with depth from 0.12 to 0.43 g cm⁻³. Soil C stocks estimated from soil C and bulk densities above K-Ah ranged from 28.1 Mg C ha⁻¹ (site-E) to 417 Mg C ha⁻¹ (Site-B). Soil C sequestration rates throughout the soil profile estimated from ¹⁴C dating showed that these significantly decreased with increasing soil C accumulation interval (Fig. 4). Toma *et al.* (2012) reported that mean soil sequestration rate during recent 47 years in site-B was calculated to 503 kg C ha⁻¹ yr⁻¹. Including this, mean soil C sequestration rate was fitted into equation 1. C_1 and C_2 in equation 1 were 1910 and 193.4, respectively. The decomposition rate constant for labile (k_1) and non-labile (k_2) fractions were 0.123 and 0.001, respectively. Coefficient of determination of the fitting curve was 0.984 (Fig. 4). This model showed the soil C sequestration rate for 1-, 34-, 50-, and 100-yr durations were 1,885, 617, 483, and 332 kg C ha⁻¹ yr⁻¹, respectively. Soil-C sequestration rate for 7,300 years in sites A-F were 38, 57, 32, 10, 4, and 50 kg C ha⁻¹ yr⁻¹, respectively. Soil C sequestration rate for 7,300 years were less than 3 % compared with sequestered C for a recent 1-year period. Soil $\delta^{13}\text{C}$ ranged from -14.0 to -21 ‰ (Average: -16.8 ‰, CV: 7.76 %, Fig. 3). Soil C derived from C₄ plant species varied from 53 to 100 % (Average: 84 %, Fig. 3).

Phytoliths were quantified in soil for family Poaceae for the C₄ *Miscanthus* (Fig. 5a, b) and other genera of Andropogoneae (Fig. 5b), Paniceae (Fig. 5c), and genus *Zoysia*, and for the C₃ genus *Oryza*, genus *Pleioblastus*, and genus *Sasa*. Phytolith concentrations from trees, such as genus *Fagus* and genus *Quercus* ranged from 0 to 4.8% (Fig. 6).

Phytoliths from Poaceae accounted for up to 75% of total plant phytoliths. Contribution of plant species to biomass derived from three dominant types of C₄ plants varied among sampling sites (Fig. 7). Paniceae was dominant 10-25 cm in site-A (69-81%), 0-55 cm in site-B (52-70%), 20-25 cm in site-C (56%), 30-35 cm in site-E (56%), and 0-5 cm in site-F (55%). In the other sites and soil depths, *Miscanthus* was dominant (53-100%), except for 30-35 cm in site-A. Phytoliths in site-A possibly included a morphotype that appeared to be that of *Miscanthus*, but not specific to the known *Miscanthus* morphotype. Soil C content and mass C increased with the total number of phytoliths in soil (Fig. 8) and calculated biomass production by Poaceae (Fig. 9).

The mean soil C stock in semi-natural grassland was calculated to be 204 Mg C ha⁻¹ by multiplying mean soil C sequestration rate for 7,300 years and soil C accumulation interval of 7,300 years in Fig. 4. Thus, total soil C stock in semi-natural grassland in Aso region was 2.2 Tg C. Mean soil C sequestration rate for 1, 10, and 100 years in semi-natural grassland in Aso region was 21, 14, and 3.7 Gg C yr⁻¹. When mean soil C sequestration rate in natural grassland, which was established naturally and had not received any management, was assumed to be same with semi-natural grassland in Japan, mean soil C sequestration rate for 1 and 50 years in natural and semi-natural grassland in Japan was estimated to 2.8 and 0.7 Tg C yr⁻¹, respectively.

Discussion

Short and long term mean annual C sequestration rates and soil C stock

Our estimation of mean soil C sequestration could be considered a representative value of this phenomenon in the Aso region because equation 2 for estimating soil C sequestration rate was established using all the data collected in sampling sites and the recent (47 years) mean soil C sequestration rate from Toma *et al.* (2012). Since mean soil C sequestration data at 47 years interval from Toma *et al.* (2012) was estimated in a comparison study of soil $\delta^{13}\text{C}$ between *M. sinensis* grassland and *Cryptomeria japonica* forest plantation, the data of soil C sequestration in our study was not influenced by the nuclear testing, which confounds analysis of ^{14}C data of soil-C sequestration. Estimated mean soil C sequestration using this regression model could significantly vary depending on the number of data points collected for mean soil C sequestration occurring over short timeframes (years to decades). Estimated mean soil C sequestration calculated by equation 1 and 2 was $1,885 \text{ kg C ha}^{-1} \text{ yr}^{-1}$ over a 1-year soil C accumulation interval. However, including data, which were based on soil C accumulation in deeper soil layers (100-cm depth) in a cultivated *M. × giganteus* field (Hansen *et al.* 2004), into our data set (Table 1) resulted in 1-yr soil C sequestration estimates that varied from 881-5,026 $\text{kg C ha}^{-1} \text{ yr}^{-1}$. This variation represented our estimation of mean soil C sequestration within recent years at short time scales. Coefficient of variation (CV) for estimates of mean soil C sequestration rates based on the regression model using data from our study and one including the data from Hansen *et al.* (2004) were more than 40% for 10-year projections. However, CV values decreased with increasing years of interval, and became less than 5% for the estimate of soil-C sequestration rate over a 34-yr interval. This indicates that

the calculated mean soil C sequestration over a 34-yr interval was a more accurate value. Thus, mean soil C sequestration calculated was $618 \text{ kg C ha}^{-1} \text{ yr}^{-1}$ at 34 year soil C accumulation interval and decreased with increasing soil C accumulation interval, e.g. from 1,232 (10 years) to 28 (7,300 years) $\text{kg C ha}^{-1} \text{ yr}^{-1}$. Estimated mean soil C sequestration rates for recent years intervals were, of course, possible values in semi-natural grassland in Aso region. However, for future studies, additional data of mean soil C sequestration data at recent years intervals, particularly of *M. × giganteus* under cultivated conditions, are required for improving the certainty of the regression model in our study.

Considering that aboveground biomass in semi-natural grassland in Aso has been harvested or burned every year for more than 1,000 years or possibly for more than 10,000 years (Otaki 1999; Stewart *et al.* 2009), continuous use of semi-natural grassland by human and C sequestration in soil could be compatible. Soil C has been, thus, increased 63 Mg C ha^{-1} over a 100-year period, notwithstanding that soil C has been released due to heterotrophic respiration, wind and water erosion. Cultivation activity in agricultural fields, in which aboveground and/or belowground products were harvested and removed from fields, often causes soil C consumption (Coleman *et al.*, 1997; Yazaki *et al.*, 2004; Shimizu *et al.*, 2009). However, the semi-natural grasslands in Aso are potentially atmospheric C sink despite the continual harvesting aboveground biomass.

Calculated soil C stock in our study (204 Mg C ha^{-1}) was larger compared with than in all land use types (40 to 131 Mg C ha^{-1}) in Spain, in which Rodríguez-Murillo (2001) analyzed soil C stock data collected from >1,000 soil profiles. Soil C stock in semi-natural grassland in Aso was nearly two times higher than in meadows in Spain

(Rodríguez-Murillo, 2001). Soil C stock in Andisols in Spain (244 Mg C ha⁻¹) was within the range of that calculated in our study, and had the highest soil C stock among soil groups in Spain, except for Histosols. This suggests that vegetation of *M. sinensis* on Andisols induced higher soil C stock in the Aso region. Calculated value of k_1 and k_2 in equation 1 represented decomposition rate constants for labile and non-labile organic matter, respectively (Murayama *et al.* 1990). Organic matter with lower values of k_1 and k_2 decomposes slowly. In the study by Murayama *et al.* (1990), k_1 and k_2 values were 2.75 and 0.014 for rice straw and 0.205-0.249 and 0.033-0.0083 for wheat straw. Both sets of constants were higher relative to our study. Organic matter with high C:N ratios, such as *M. sinensis* (267; Toma *et al.*, 2012), generally have more recalcitrant decomposition rates relative to those with lower C:N ratios (Toma *et al.*, 2007), such as rice (35) and wheat (128) straw (Murayama *et al.* 1990). It is likely that soil C at the study sites in Aso derived primarily from *M. sinensis* plant material, which is a more stable type of organic matter relative to rice and wheat straw. Moreover, lower values of k_1 and k_2 could indicate the existence of charcoal C, which is also a very stable form of C found in burned grasslands. As such, C supply from charcoal and partially decomposed organic matter from vegetation and charcoal potentially explains the high soil C values at our study sites.

Soil C stock and soil C sequestration rate in semi-natural grassland in Aso region were estimated to be 2.2 Tg C over the 7,300-year period and, on average, 21 Gg C yr⁻¹, respectively. Annual C emission from households in Japan in 2008 was 1,375 kg C yr⁻¹ (CGER, 2010). Consequently, C emission from approximately 15,000 households was absorbed in the semi-natural grassland in Aso. Moreover, soil C sequestration rate in natural and semi-natural grassland in Japan (2.8 Tg C yr⁻¹) was equivalent to 5 % of total

C emission in 2008 from cars in Japan (CGER, 2010). Thus, soil C sequestration in semi-natural grassland serves as an important C stock and sink. Estimation of soil C sequestration rates in semi-natural grassland in Aso region was based on the assumption that all of the grassland in the Aso region was adequately managed for soil C stock, such as harvest and burning, and should be considered as optimal (or maximal) values of soil C sequestration. In addition, it should be noted that soil C sequestration rate is usually influenced by topographic characteristics (soil erosion, etc.). The soil C stock in our study site ranged from 28-417 Mg C ha⁻¹ over 7,300 years. Because we did not have enough information to analyze the spatial variation of soil C stock, future work needs to focus on characterizing this variation and also the mechanisms of soil C accumulation in the Aso region. Given the heterogeneity in topography in the Aso grasslands, such estimates will be useful in improving the estimation of average soil C sequestration rates in regions with undulating terrain.

Interactions of plant species and management regimes in establishing and maintaining this C sink

From the analysis of soil $\delta^{13}\text{C}$, average of soil C that originated from C₄ plants was 84%. Except for soil layer at 30-35-cm depth in Site-A (53 %), more than 72 % of total C came from C₄ plants, which suggests that the contribution of C by C₄ plants (Paniceae, Andropogoneae, *Miscanthus*, and *Zoysia*) was larger compared to that supplied by C₃ plants (*Oryza*, *Pleioblastus*, and *Sasa*), since the composition of dominant species has not changed greatly for more than 1,000 years due to annual burning practices (Ogura *et al.*, 2002; Stewart *et al.*, 2009). Dominant species among identified C₄ plants by phytolith

analysis were mostly from the tribe Andropogoneae (notably the genus *Miscanthus*) and tribe Paniceae (Fig. 7). In the Aso region, *M. sinensis* is widely distributed. The average height of *M. sinensis*, which is a perennial, rhizomatous species, averages 2 m in height (Stewart *et al.*, 2009). On the other hand, *Paspalum thunbergii* is a perennial species with height ranging from 40-90 cm (Ohwi, 1982). *P. thunbergii*, which belongs to tribe Paniceae, is native to the Aso region (Suzuki & Abe, 1959). Though biomass production of *P. thunbergii* has not been studied, annual biomass production of *M. sinensis* was potentially larger than that of *P. thunbergii* based on height differences. In addition, given that *P. thunbergii* is non-rhizomatous indicates C supply by its belowground organs might be low compared with *M. sinensis* (Ohwi, 1982). In the contemporary vegetational makeup, *M. sinensis* is the dominant species in the Aso grassland ecosystem and has the highest biomass relative to sympatric species. Therefore, contribution of genus *Miscanthus* plant to soil C was larger than Paniceae plants because belowground biomass was mainly affected by soil C accumulation through harvest and burning practices.

As mentioned, C supply by belowground organs might contribute to soil C accumulation. Annual burning in site-B consumed more than 98 % of aboveground biomass and litter (Toma *et al.*, 2010). Toma *et al.* (2010) reported only 102 kg C ha⁻¹ yr⁻¹ of aboveground biomass C was remained after burning. At a minimum, larger amounts of C potentially came from belowground biomass C than from aboveground biomass C during sequestration over the past 100 yr, especially considering that the C sequestration rate over 100 years (332 kg C ha⁻¹ yr⁻¹) in our study was still more than twice the C supply from aboveground biomass after burning. Moreover, the positive correlation between the biomass production of Poaceae and soil mass C showed the possibility of

large contributions of belowground biomass to soil C accumulation (Fig. 9). Most of the phytoliths, which are composed of silica and are synthesized in leaves, especially in epidermal cells of species in Poaceae (Kondo & Sase, 1986), originated from aboveground biomass. Kondo (1996) reported the amount of silica in roots and rhizomes in *Poaceae* was only 1% compared with that in aboveground organs. Silica content in root of *Sasa nipponica*, which was Poaceae, was only 7.1-26% of that in leaf (Fu *et al.*, 2001). Positive correlations between above- and belowground biomass of plant have been observed in temperate bogs (Murphy & Moore, 2010), *Spartina alterniflora* (Gross *et al.*, 1991), *Spartina maritima* (Castillo *et al.*, 2008), and loblolly pine (*Pinus taeda*) (Coyle *et al.*, 2008). Therefore, Fig. 9 shows how soil mass C increases with increasing total amount of biomass production (sum of above- and belowground biomass production). In our study site, however, most of aboveground biomass, except for fallen leaves, was removed to the out of grassland ecosystem due to burning or harvest. Thus, increasing of soil mass C depended on increasing belowground biomass production.

As discussed above, belowground biomass C of Poaceae appears to contribute to soil C accumulation at our study sites. Similar with our study, positive relationships between soil C content and total number of plant phytoliths over the past 13,500 years in the Aso region was reported (Miyabuchi & Sugiyama, 2008). Carbon supply by plant material was an important controlling factor for soil C accumulation in spite of factors contributing to C loss, such as soil and wind erosion, soil C leaching, and soil organic C decomposition. Among the identified phytoliths, Poaceae taxa were dominant in the total number of plant phytoliths. In addition, soil mass C was positively correlated with estimated biomass production of Poaceae (Fig. 9). These findings are supported by

Sugiyama *et al.* (2002), who reported that the origin of organic matter in Andisols in southern Kyushu in Japan derived from *Miscanthus* and/or *Pleioblastus* sect. *Nezasa* in the Holocene epoch (~10,000 years ago). This suggests that the belowground roots and rhizomes of *M. sinensis* are the main contributors to soil C sequestration in these semi-natural grasslands. Charcoal C supplied by biomass burning, however, may still be an important soil C source. However, quantitative analysis of C supply from belowground biomass and charcoal remains an open research question to better understand the mechanisms of soil C sequestration.

Comparison of soil C sequestration rate and soil C stock with other land-use type

Soil C sequestration rate over a 15-year period (1,018-1,885 kg C ha⁻¹ yr⁻¹, Fig. 4) in semi-natural grassland in Aso showed similar potential with reported C sequestration values in *M. × giganteus* fields (Table 1), but the values in our study are difficult to compare with those studies because of differences in depths of soil layers studied. Hansen *et al.* (2004) and Clifton-Brown *et al.* (2007) reported soil C accumulation rates of 780-1,120 (within 0-100-cm depth of soil) and 590 kg C ha⁻¹ yr⁻¹ within the top 30-cm depth, respectively (Table 1). Lee *et al.* (2007) reported that the soil C sequestration rate (within 0-90-cm depth of soil) was 2,400 kg C ha⁻¹ yr⁻¹ in a fertilized switchgrass (*Panicum virgatum* L.) cultivation field. Bioenergy crop models estimate a soil C sequestration potential of 620 kg C ha⁻¹ yr⁻¹ (Smith, 2004). Hence, the potential annual soil C sequestration rate of the semi-natural grasslands was within a higher and similar range to *Miscanthus* and switchgrass planted for bioenergy in other parts of the world. In comparison with grassland consisting of *Lolium perenne*, in which nitrate-ammonium or

urea were applied, mean soil C sequestration calculated by change of soil C content were 29-619 kg C ha⁻¹ yr⁻¹ (Jones *et al.*, 2006). Toma *et al.* (2012) reported soil C sequestration rate in *M. sinensis* in site-B (503 kg C ha⁻¹ yr⁻¹) was higher than *Cryptomeria japonica* forest plantation over a 47-yr period (284 kg C ha⁻¹ yr⁻¹, Table 1). Schlesinger (1990) reported soil C sequestration rate in various climates and ecosystems. In a temperate forest ecosystem, soil C sequestration rate ranged from 17 kg C ha⁻¹ yr⁻¹ to 120 kg C ha⁻¹ yr⁻¹ over a 1,200-6,500 yr interval. Because the calculated soil C accumulation rate in our study over a 1,200-6,500 yr interval ranged from 31 kg C ha⁻¹ yr⁻¹ to 124 kg C ha⁻¹ yr⁻¹ (Table 1), soil C sequestration in the Aso region was relatively larger than in many forest ecosystems. Generally, plant succession climaxes to woody species in Japan (Yamamoto *et al.*, 1997; Molles, 2008), and change in land use from grassland to forest generally causes declines in soil C stock due to smaller annual turnover of organic matter from dying tree roots compared with that of grass roots (Guo & Gifford, 2002; Post & Kwon, 2000). Therefore, abandoned semi-natural grassland without human activity could decrease soil C accumulation. In the studied semi-natural grassland, grass biomass has been utilized as a feed and material for organic fertilizer and biofuel for more than 1,000 years where soil C has accumulated. Thus, semi-natural grassland in Aso demonstrated the sustainable use of grassland for C accumulation in soil.

Conclusion

We conclude that soil C sequestration rate in semi-natural grassland in Aso was estimated to be 1885, 331, and 28 kg C ha⁻¹ yr⁻¹ from 1, 100, and 7,300 years interval, and grassland dominated C₄ plant species with annual burning and/or harvest exhibits potential as a stable C sink. Therefore, the semi-natural grasslands in Aso potentially acts as an important C sink in Japan because of their ability to sequester large amounts of atmospheric C. The coupled natural and human system of the semi-natural grassland in Aso acts as a model system in terms of demonstrating the sustainable use of grassland for animal and renewable energy production as they relate to C accumulation in soil.

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Figure captions

Fig. 1. Location and altitude of the soil sampling sites (A to F) of semi-natural grasslands in Aso, Japan.

Fig. 2. Semi-natural *Miscanthus sinensis* dominated grassland on Mt. Aso caldera in Kumamoto Prefecture, Japan.

Fig. 3. Carbon (C) content, bulk density, $\delta^{13}\text{C}$, and mass C in soil profiles in site-A (a), site-B (b), site-C (c), site-D (d), site-E (e), and site-F (f) in Aso, Japan. Black color in soil C stock represents C from C_4 plant. Numbers under dotted line in the columns of $\delta^{13}\text{C}$ and mass C represent ^{14}C date and accumulation of soil C from soil surface to the layer, respectively.

Fig. 4. Relationship between soil carbon (C) sequestration rate and soil C accumulation interval, and fitting curve of this relationship in a semi-natural grasslands in Aso, Japan.

Fig. 5. Phytolith morphotypes of genus *Miscanthus* (a), (b), tribe Andropogoneae (c), and tribe Paniceae (d) recovered from the soil in semi-natural grasslands in Aso, Japan.

Fig. 6. Contribution of vegetation types identified by phytoliths recovered at six sites in semi-natural grasslands in Aso, Japan. Filled, grayed, and open bars represent vegetation of grasses, trees, and unknown, respectively.

Fig. 7. Contribution of plant species to biomass derived from three dominant types of C₄ plants in semi-natural grasslands in Aso, Japan. Filled, grayed, and open bars represent tribe Andropogoneae, tribe Paniceae, and genus *Miscanthus*.

Fig. 8. Relationship between soil carbon (C) content and total number of plant phytolith in soil in semi-natural grasslands in Aso, Japan.

Fig. 9. Relationship between calculated biomass production by plant of Poaceae and soil mass C in semi-natural grasslands in Aso, Japan.

725

726 Table

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728

729 Table 1. Soil carbon (C) sequestration rates in several ecosystems.

Location	GPS coordinates	Elevation (m)	Species	Soil type	Period (year)	Soil C sequestration rate (kg C ha ⁻¹ yr ⁻¹)	References
Hornum, Denmark	56°50'N, 09°26' E	32	<i>M. ×giganteus</i>	Typic Haplumbrept (USDA soil taxonomy)	9	780	Hansen <i>et al.</i> (2004)
Hornum, Denmark	56°50'N, 09°26' E	32	<i>M. ×giganteus</i>	Typic Haplumbrept (USDA soil taxonomy)	16	1,120	Hansen <i>et al.</i> (2004)
Ireland	52°39'N, 07°50' W	80	<i>M. ×giganteus</i>	Mollic Gleysol (FAO)	15	590	Clifton-Brown <i>et al.</i> (2007)
Scotland	55°52'N, 03°52' E	200	<i>Lolium perenne</i>	Gleysol (FAO)	6	29-619	Jones <i>et al.</i> (2006)
Aso, Japan	33°01.58'N, 131°03.89' E	794	<i>M. sinensis</i>	Typic Melanudans (USDA soil taxonomy)	47	503	Toma <i>et al.</i> (2012)
			<i>Cryptomeria japonica</i>	Typic Melanudans (USDA soil taxonomy)	47	284	Toma <i>et al.</i> (2012)
Reviewed data			Temperate forest		1,200-6,500	17-120	Schlesinger (1990)
Aso, Japan	32°54.77' - 33°01.56' N 131°00.73' - 131°09.40' E	767-937	<i>M. sinensis</i>	Typic Melanudans (USDA soil taxonomy)	1-15	1,018-1,884	This study
			<i>M. sinensis</i>	Andisons (USDA soil taxonomy)	1,200-6,500	31-124	This study

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Fig. 1 Location and altitude of the soil sampling sites (A to F) of semi-natural grasslands in Aso, Japan.

Fig. 2 Semi-natural *Miscanthus sinensis* dominated grassland on Mt. Aso caldera in Kumamoto Prefecture, Japan.

Fig. 3 Carbon (C) content, bulk density, $\delta^{13}\text{C}$, and mass C in soil profiles in site-A (a), site-B (b), site-C (c), site-D (d), site-E (e), and site-F (f) in Aso, Japan. Black color in soil C stock represents C from C₄ plant. Numbers under dotted line in the columns of $\delta^{13}\text{C}$ and mass C represent ¹⁴C date and accumulation of soil C from soil surface to the layer, respectively.

Fig. 4 Relationship between soil carbon (C) sequestration rate and soil C accumulation interval, and fitting curve of this relationship in a semi-natural grasslands in Aso, Japan.

Fig. 5 Phytolith morphotypes of Genus *Miscanthus* (a), (b), Tribe Andropogoneae (c), and Tribe Paniceae (d) recovered from the soil in semi-natural grasslands in Aso, Japan.

Fig. 6 Contribution of vegetation types identified by phytoliths recovered at six sites in semi-natural grasslands in Aso, Japan. Filled, grayed, and open bars represent vegetation of grasses, trees, and unknown, respectively.

Fig. 7 Contribution of plant species to biomass derived from three dominant types of C_4 plants in semi-natural grasslands in Aso, Japan. Filled, grayed, and open bars represent Tribe *Andropogoneae*, Tribe *Paniceae*, and Genus *Miscanthus*.

Fig. 8 Relationship between soil carbon (C) content and total number of plant phytolith in soil in semi-natural grasslands in Aso, Japan.

Fig. 9 Relationship between calculated biomass production by plant of *Poaceae* and soil mass C in semi-natural grasslands in Aso, Japan.