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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

5

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46 *Miscanthus sinensis*

47

48 **Abstract**

49 Global soil carbon (C) stocks account for approximately three times that found in the  
50 atmosphere. In the Aso mountain region of southern Japan, semi-natural grasslands have  
51 been maintained by annual harvests and/or burning for more than 1,000 years.  
52 Quantification of soil C stocks and C sequestration rates in Aso mountain ecosystem is  
53 needed to make well-informed, land-use decisions to maximize C sinks while minimizing  
54 C emissions. Soil cores were collected from six sites within 200 km<sup>2</sup> (767-937 m asl.)  
55 from the surface down to the k-Ah layer established 7,300 years ago by a volcanic  
56 eruption. The biological sources of the C stored in the Aso mountain ecosystem was  
57 investigated by combining C content at a number of sampling depths with age (using <sup>14</sup>C  
58 dating) and δ<sup>13</sup>C isotopic fractionation. Quantification of plant phytoliths at several  
59 depths was used to make basic reconstructions of past vegetation and was linked with C-  
60 sequestration rates. The mean total C stock of all six sites was 232 Mg C ha<sup>-1</sup> (28-417 Mg  
61 C ha<sup>-1</sup>), which equates to a soil C sequestration rate of 32 kg C ha<sup>-1</sup> yr<sup>-1</sup> over 7,300 years.  
62 Mean soil C sequestration rates over 34, 50 and 100 years were estimated by an equation  
63 regressing soil C sequestration rate against soil C accumulation interval, which was  
64 modeled to be 618, 483 and 332 kg C ha<sup>-1</sup> yr<sup>-1</sup>, respectively. Such data allows for a deeper  
65 understanding in how much C could be sequestered in *Miscanthus* grasslands at different  
66 time scales. In Aso, tribe Andropogoneae (especially *Miscanthus* and *Schizoaegyrium*  
67 genera) and tribe Paniceae contributed between 64 and 100% of soil C based on δ<sup>13</sup>C  
68 abundance. We conclude that the semi-natural, C<sub>4</sub>-dominated grassland system serves as  
69 an important C sink, and worthy of future conservation.

70 **Introduction**

71 Climate change and increasing atmospheric CO<sub>2</sub> are inextricably linked (IPCC, 2007).  
72 Estimates of global soil carbon (C) stocks are 2,500 Pg (organic C, 1,550 Pg; inorganic C,  
73 950 Pg), which is 3.3 times higher than that of atmospheric C stocks (760 Pg) (Lal, 2004,  
74 2008).

75 Grassland ecosystems can contribute to C mitigation through biomass feedstock  
76 production (substituting fossil fuels) and through C sequestration in the soil (Carpenter-  
77 Boggs *et al.*, 2003; Arshad *et al.*, 2004; Bronson *et al.*, 2004). Guo & Gifford (2002)  
78 reported that land-use change from cropland or forest to grassland can increase soil C  
79 stocks. Grassland ecosystems comprise approximately 41% of the terrestrial land area,  
80 except for areas of permanent ice cover (Adams *et al.*, 1990; White *et al.*, 2000).  
81 Moreover, soil organic matter (SOM) in temperate grasslands has been estimated to  
82 account for 331 Mg ha<sup>-1</sup>, and grasslands contain 12% of global SOM (Conant *et al.* 2001;  
83 Schlesinger, 1977). Natural and semi-natural grasslands, which are comprised of several  
84 graminoid and forb species, including *Miscanthus sinensis*, account for 24% of grasslands  
85 (National Parks Association of Japan, 1996) and, in recent years, 4% (1,512 Mha) of  
86 Japan (Himiyama *et al.*, 1995). Many Japanese grasslands have been managed by annual  
87 harvesting for fuel and fodder and/or burning (Otaki, 1999; Stewart *et al.*, 2009). In the  
88 Aso region of southern Japan, the grassland vegetation is dominated mainly by  
89 *Miscanthus sinensis*, *Pleioblastus variegatus*, *Spodiopogon sibiricus*, *Arundinella hirta*,  
90 and *Imperata cylindrica* (Koyama & Ogawa, 1993; Yamamoto *et al.*, 2002). In this region,  
91 Andisols with high organic C content are widely distributed (Matsuyama & Saigusa,  
92 1994; Toma *et al.*, 2011).

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

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

124

125

126

127 **Materials and Methods**

128 *Site description*

129 The study was conducted in a semi-natural grassland on Andisols located on the northern  
130 rim and center of the Mt. Aso caldera in Kumamoto Prefecture, southern Japan, which  
131 was within an area of 225 km<sup>2</sup> (15 km × 15 km) (Fig. 1). In the region, *M. sinensis*  
132 vegetation is widely distributed (Fig. 2). The location of the sample sites, which were  
133 named site-A, site-B, site-C, site-D, site-E, and site-F, are shown in Fig. 1. Current land  
134 use in sites A, C, D, and F were meadows; site-B was grassland that had not been  
135 harvested for more than 40 years, and site E was a grazed pasture. The common  
136 management applied in each site was an annual burning event in each March. Mean  
137 annual precipitation and air temperature over a 30-year period (1971-2000) around the  
138 Aso area were 3,250 mm and 9.6°C, respectively. In the Aso region, the K-Ah horizon is  
139 a visually distinct volcanic ash layer deposited 7,300 years ago (Miyabuchi & Watanabe,  
140 1997).

141

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

143 Soil samples were collected for calculating bulk density at 5-cm increments (79 cm<sup>3</sup>) in  
144 sites A-E and 10-cm increments (159 cm<sup>3</sup>) at site-F from the soil surface to the bottom of  
145 the K-Ah layer on 27 November 2009. Also, soil samples for measuring soil C content,  
146 soil  $\delta^{13}\text{C}$ , soil  $^{14}\text{C}$  dating, and plant phytoliths were collected at the same depths.

147 Bulk density of each 5-cm or 10-cm increment was calculated by the weight of the  
148 samples dried in an oven at 105°C for 48 hr. Air-dried soil samples were sieved through a  
149 2-mm mesh and crushed finely to powder. The total soil C content of powdered soil



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

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

$$158 \quad \delta^{13}\text{C} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000,$$

159 where  $R_{\text{sample}}$  is the isotope ratio  $^{13}\text{C}/^{12}\text{C}$  of the sample and  $R_{\text{standard}}$  is the  $^{13}\text{C}/^{12}\text{C}$  of the  
160 PDB standard (Balesdent *et al.*, 1987). Proportion of C ( $X_{\text{C4}}$ ) of  $\text{C}_4$  plant species-derived  
161 C in the soil was calculated as follows:

$$162 \quad X_{\text{C4}} = (\delta^{13}\text{C}_{\text{soil}} - \delta^{13}\text{C}_{\text{C3}}) / (\delta^{13}\text{C}_{\text{C4}} - \delta^{13}\text{C}_{\text{C3}}) \times 100,$$

163 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  $\text{C}_3$  plant species,  
164 and  $\delta^{13}\text{C}_{\text{C4}}$  is  $\delta^{13}\text{C}$  of C derived from  $\text{C}_4$  plant species. Values of  $\delta^{13}\text{C}_{\text{C3}}$  and  $\delta^{13}\text{C}_{\text{C4}}$  were  
165 cited from reported values of mean  $\delta^{13}\text{C}$  of  $\text{C}_3$  plant species (-28 ‰, Yoneyama, 2008) or  
166 rhizome of *M. sinensis* (-14.7 ‰, Toma *et al.*, 2012). Because *M. sinensis* was the  
167 dominant plant species relative to soil C accumulation in this study, its value of  $\delta^{13}\text{C}$  for  
168 was used to represent that of  $\text{C}_4$  plant species (shown in Results).

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

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

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

$$182 \quad Y_t = C_1 \exp(-k_1 t) + C_2 \exp(-k_2 t) \quad \text{equation 1}$$

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

$$187 \quad R_T = (\sum_{t=1}^T Y_t) / T \quad \text{equation 2}$$

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

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

196 bath (300 W, 42 kHz) for 10 minutes. Particles coarser than 20  $\mu\text{m}$  were extracted by a  
197 precipitation method (Miyabuchi *et al.*, 2011). Identification and counting of plant  
198 phytoliths were performed under a polarizing microscope at 400 $\times$  magnification and  
199 continued until more than 400 glass beads were counted. Plant phytolith content was  
200 calculated by the following equation:

201 
$$\text{Phytolith content} = (Gg \times Pc) / Gc,$$

202 where  $Gg$  is the total number of glass beads in the sample equivalent to 1 g,  $Pc$  is the  
203 number of grains of one phytolith morphotype counted in the scan, and  $Gc$  is the number  
204 of glass beads counted in the scan. Identification of phytolith morphotypes was based on  
205 Fujiwara (1976), Fujiwara & Sasaki (1978), Kondo & Sase (1986), Sugiyama & Fujiwara  
206 (1986), Sugiyama *et al.* (1988), and Sugiyama (1999, 2001). With this technique, tribes  
207 Andropogoneae and Paniceae were identified. In addition, the *Miscanthus* was the only  
208 genus that could be identified in tribe Andropogoneae. The classification of Suzuki  
209 (1996) was used for subfamily Bambusoideae. Aboveground biomass production of  
210 identified plant species in collected soil layers (5-cm or 10-cm thickness) was calculated  
211 with the following equation:

212 
$$\text{Aboveground biomass production (kg m}^{-2} \text{ cm depth}^{-1}) = \text{number of phytolith per 1g}$$
  
213 
$$\text{soil} \times F \text{ (gram of dry plant biomass per 1 phytolith)} \times \text{bulk density (kg m}^{-2} \text{ cm depth}^{-1}) \times$$
  
214 
$$5 \text{ or } 10 \text{ (cm depth)}$$

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

219 *Nezasa*), 0.75 (*Sasa* sect. *Sasa*), and 0.3 (*Sasa* sect. *Crassinodi*) (Sugiyama, 2000;  
220 Sugiyama *et al.*, 2002; Inoue *et al.*, 2001; Watanabe *et al.*, 1996).  
221  
222 *Estimation of regional soil carbon storage and mean soil C sequestration rate in semi-*  
223 *natural grassland*  
224 Soil C stock in semi-natural grassland was calculated by regression between soil C  
225 sequestration rate and duration of soil C accumulation. Soil C stock was estimated for  
226 7,300 years, because similar vegetation in semi-natural grassland in the Aso region was  
227 reported to be retained for approximately 10, 000 years (Miyabuchi & Sugiyama, 2006;  
228 Miyabuchi *et al.*, 2010). Total soil C stock for 7,300 years and soil C sequestration rate in  
229 semi-natural grassland in the Aso region was estimated in the area of current semi-natural  
230 grassland (11,000 ha; Ohtaki, 1999). Total soil C sequestration rate in natural and semi-  
231 natural grassland in Japan (1,512 Mha; Himiyama *et al.*, 1995) was also calculated.  
232  
233 *Statistical analysis*  
234 Statistical analyses were performed using “R” (version 2.10.1, R Development Core  
235 Team, 2005). Regression analysis between C content and total number of plant phytoliths,  
236 or soil mass C and calculated biomass production were performed by the least squares  
237 method.  
238

## 239 Results

240 Soil C contents at six sites ranged from 5 to 252 g C kg<sup>-1</sup>, and generally decreased with  
241 depth (Fig. 3). In contrast with soil C, bulk densities increased with depth from 0.12 to  
242 0.43 g cm<sup>-3</sup>. Soil C stocks estimated from soil C and bulk densities above K-Ah ranged  
243 from 28.1 Mg C ha<sup>-1</sup> (site-E) to 417 Mg C ha<sup>-1</sup> (Site-B). Soil C sequestration rates  
244 throughout the soil profile estimated from <sup>14</sup>C dating showed that these significantly  
245 decreased with increasing soil C accumulation interval (Fig. 4). Toma *et al.* (2012)  
246 reported that mean soil sequestration rate during recent 47 years in site-B was calculated  
247 to 503 kg C ha<sup>-1</sup> yr<sup>-1</sup>. Including this, mean soil C sequestration rate was fitted into  
248 equation 1.  $C_1$  and  $C_2$  in equation 1 were 1910 and 193.4, respectively. The  
249 decomposition rate constant for labile ( $k_1$ ) and non-labile ( $k_2$ ) fractions were 0.123 and  
250 0.001, respectively. Coefficient of determination of the fitting curve was 0.984 (Fig. 4).  
251 This model showed the soil C sequestration rate for 1-, 34-, 50-, and 100-yr durations  
252 were 1,885, 617, 483, and 332 kg C ha<sup>-1</sup> yr<sup>-1</sup>, respectively. Soil-C sequestration rate for  
253 7,300 years in sites A-F were 38, 57, 32, 10, 4, and 50 kg C ha<sup>-1</sup> yr<sup>-1</sup>, respectively. Soil C  
254 sequestration rate for 7,300 years were less than 3 % compared with sequestered C for a  
255 recent 1-year period. Soil  $\delta^{13}\text{C}$  ranged from -14.0 to -21 ‰ (Average: -16.8 ‰, CV:  
256 7.76 %, Fig. 3). Soil C derived from C<sub>4</sub> plant species varied from 53 to 100 % (Average:  
257 84 %, Fig. 3).

258 Phytoliths were quantified in soil for family Poaceae for the C<sub>4</sub> *Miscanthus* (Fig. 5a,  
259 b) and other genera of Andropogoneae (Fig. 5b), Paniceae (Fig. 5c), and genus *Zoysia*,  
260 and for the C<sub>3</sub> genus *Oryza*, genus *Pleioblastus*, and genus *Sasa*. Phytolith concentrations  
261 from trees, such as genus *Fagus* and genus *Quercus* ranged from 0 to 4.8% (Fig. 6).

262 Phytoliths from Poaceae accounted for up to 75% of total plant phytoliths. Contribution  
263 of plant species to biomass derived from three dominant types of C<sub>4</sub> plants varied among  
264 sampling sites (Fig. 7). Paniceae was dominant 10-25 cm in site-A (69-81%), 0-55 cm in  
265 site-B (52-70%), 20-25 cm in site-C (56%), 30-35 cm in site-E (56%), and 0-5 cm in site-  
266 F (55%). In the other sites and soil depths, *Miscanthus* was dominant (53-100%), except  
267 for 30-35 cm in site-A. Phytoliths in site-A possibly included a morphotype that appeared  
268 to be that of *Miscanthus*, but not specific to the known *Miscanthus* morphotype. Soil C  
269 content and mass C increased with the total number of phytoliths in soil (Fig. 8) and  
270 calculated biomass production by Poaceae (Fig. 9).

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

280

281 **Discussion**

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

283 Our estimation of mean soil C sequestration could be considered a representative value of  
284 this phenomenon in the Aso region because equation 2 for estimating soil C sequestration  
285 rate was established using all the data collected in sampling sites and the recent (47 years)  
286 mean soil C sequestration rate from Toma *et al.* (2012). Since mean soil C sequestration  
287 data at 47 years interval from Toma *et al.* (2012) was estimated in a comparison study of  
288 soil  $\delta^{13}\text{C}$  between *M. sinensis* grassland and *Cryptomeria japonica* forest plantation, the  
289 data of soil C sequestration in our study was not influenced by the nuclear testing, which  
290 confounds analysis of  $^{14}\text{C}$  data of soil-C sequestration. Estimated mean soil C  
291 sequestration using this regression model could significantly vary depending on the  
292 number of data points collected for mean soil C sequestration occurring over short  
293 timeframes (years to decades). Estimated mean soil C sequestration calculated by  
294 equation 1 and 2 was  $1,885 \text{ kg C ha}^{-1} \text{ yr}^{-1}$  over a 1-year soil C accumulation interval.  
295 However, including data, which were based on soil C accumulation in deeper soil layers  
296 (100-cm depth) in a cultivated *M. × giganteus* field (Hansen *et al.* 2004), into our data set  
297 (Table 1) resulted in 1-yr soil C sequestration estimates that varied from 881-5,026 kg C  
298  $\text{ha}^{-1} \text{ yr}^{-1}$ . This variation represented our estimation of mean soil C sequestration within  
299 recent years at short time scales. Coefficient of variation (CV) for estimates of mean soil  
300 C sequestration rates based on the regression model using data from our study and one  
301 including the data from Hansen *et al.* (2004) were more than 40% for 10-year projections.  
302 However, CV values decreased with increasing years of interval, and became less than  
303 5% for the estimate of soil-C sequestration rate over a 34-yr interval. This indicates that

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

313       Considering that aboveground biomass in semi-natural grassland in Aso has been  
314 harvested or burned every year for more than 1,000 years or possibly for more than  
315 10,000 years (Otaki 1999; Stewart *et al.* 2009), continuous use of semi-natural grassland  
316 by human and C sequestration in soil could be compatible. Soil C has been, thus,  
317 increased  $63 \text{ Mg C ha}^{-1}$  over a 100-year period, notwithstanding that soil C has been  
318 released due to heterotrophic respiration, wind and water erosion. Cultivation activity in  
319 agricultural fields, in which aboveground and/or belowground products were harvested  
320 and removed from fields, often causes soil C consumption (Coleman *et al.*, 1997; Yazaki  
321 *et al.*, 2004; Shimizu *et al.*, 2009). However, the semi-natural grasslands in Aso are  
322 potentially atmospheric C sink despite the continual harvesting aboveground biomass.

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



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

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

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

363

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

366 From the analysis of soil  $\delta^{13}\text{C}$ , average of soil C that originated from C<sub>4</sub> plants was 84%.  
367 Except for soil layer at 30-35-cm depth in Site-A (53 %), more than 72 % of total C came  
368 from C<sub>4</sub> plants, which suggests that the contribution of C by C<sub>4</sub> plants (Paniceae,  
369 Andropogoneae, *Miscanthus*, and *Zoysia*) was larger compared to that supplied by C<sub>3</sub>  
370 plants (*Oryza*, *Pleioblastus*, and *Sasa*), since the composition of dominant species has not  
371 changed greatly for more than 1,000 years due to annual burning practices (Ogura *et al.*,  
372 2002; Stewart *et al.*, 2009). Dominant species among identified C<sub>4</sub> plants by phytolith

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

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

396 large contributions of belowground biomass to soil C accumulation (Fig. 9). Most of the  
397 phytoliths, which are composed of silica and are synthesized in leaves, especially in  
398 epidermal cells of species in Poaceae (Kondo & Sase, 1986), originated from  
399 aboveground biomass. Kondo (1996) reported the amount of silica in roots and rhizomes  
400 in *Poaceae* was only 1% compared with that in aboveground organs. Silica content in  
401 root of *Sasa nipponica*, which was Poaceae, was only 7.1-26% of that in leaf (Fu *et al.*,  
402 2001). Positive correlations between above- and belowground biomass of plant have been  
403 observed in temperate bogs (Murphy & Moore, 2010), *Spartina alterniflora* (Gross *et al.*,  
404 1991), *Spartina maritima* (Castillo *et al.*, 2008), and loblolly pine (*Pinus taeda*) (Coyle *et*  
405 *al.*, 2008). Therefore, Fig. 9 shows how soil mass C increases with increasing total  
406 amount of biomass production (sum of above- and belowground biomass production). In  
407 our study site, however, most of aboveground biomass, except for fallen leaves, was  
408 removed to the out of grassland ecosystem due to burning or harvest. Thus, increasing of  
409 soil mass C depended on increasing belowground biomass production.

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

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

427

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

429 Soil C sequestration rate over a 15-year period (1,018-1,885 kg C ha<sup>-1</sup> yr<sup>-1</sup>, Fig. 4) in  
430 semi-natural grassland in Aso showed similar potential with reported C sequestration  
431 values in *M. × giganteus* fields (Table 1), but the values in our study are difficult to  
432 compare with those studies because of differences in depths of soil layers studied. Hansen  
433 *et al.* (2004) and Clifton-Brown *et al.* (2007) reported soil C accumulation rates of 780-  
434 1,120 (within 0-100-cm depth of soil) and 590 kg C ha<sup>-1</sup> yr<sup>-1</sup> within the top 30-cm depth,  
435 respectively (Table 1). Lee *et al.* (2007) reported that the soil C sequestration rate (within  
436 0-90-cm depth of soil) was 2,400 kg C ha<sup>-1</sup> yr<sup>-1</sup> in a fertilized switchgrass (*Panicum*  
437 *virgatum* L.) cultivation field. Bioenergy crop models estimate a soil C sequestration  
438 potential of 620 kg C ha<sup>-1</sup> yr<sup>-1</sup> (Smith, 2004). Hence, the potential annual soil C  
439 sequestration rate of the semi-natural grasslands was within a higher and similar range to  
440 *Miscanthus* and switchgrass planted for bioenergy in other parts of the world. In  
441 comparison with grassland consisting of *Lolium perenne*, in which nitrate-ammonium or

442 urea were applied, mean soil C sequestration calculated by change of soil C content were  
443 29-619 kg C ha<sup>-1</sup> yr<sup>-1</sup> (Jones *et al.*, 2006). Toma *et al.* (2012) reported soil C  
444 sequestration rate in *M. sinensis* in site-B (503 kg C ha<sup>-1</sup> yr<sup>-1</sup>) was higher than  
445 *Cryptomeria japonica* forest plantation over a 47-yr period (284 kg C ha<sup>-1</sup> yr<sup>-1</sup>, Table 1).  
446 Schlesinger (1990) reported soil C sequestration rate in various climates and ecosystems.  
447 In a temperate forest ecosystem, soil C sequestration rate ranged from 17 kg C ha<sup>-1</sup> yr<sup>-1</sup> to  
448 120 kg C ha<sup>-1</sup> yr<sup>-1</sup> over a 1,200-6,500 yr interval. Because the calculated soil C  
449 accumulation rate in our study over a 1,200-6,500 yr interval ranged from 31 kg C ha<sup>-1</sup> yr<sup>-1</sup>  
450 <sup>1</sup> to 124 kg C ha<sup>-1</sup> yr<sup>-1</sup> (Table 1), soil C sequestration in the Aso region was relatively  
451 larger than in many forest ecosystems. Generally, plant succession climaxes to woody  
452 species in Japan (Yamamoto *et al.*, 1997; Molles, 2008), and change in land use from  
453 grassland to forest generally causes declines in soil C stock due to smaller annual  
454 turnover of organic matter from dying tree roots compared with that of grass roots (Guo  
455 & Gifford, 2002; Post & Kwon, 2000). Therefore, abandoned semi-natural grassland  
456 without human activity could decrease soil C accumulation. In the studied semi-natural  
457 grassland, grass biomass has been utilized as a feed and material for organic fertilizer and  
458 biofuel for more than 1,000 years where soil C has accumulated. Thus, semi-natural  
459 grassland in Aso demonstrated the sustainable use of grassland for C accumulation in soil.  
460

461 **Conclusion**

462 We conclude that soil C sequestration rate in semi-natural grassland in Aso was estimated  
463 to be 1885, 331, and 28 kg C ha<sup>-1</sup> yr<sup>-1</sup> from 1, 100, and 7,300 years interval, and grassland  
464 dominated C<sub>4</sub> plant species with annual burning and/or harvest exhibits potential as a  
465 stable C sink. Therefore, the semi-natural grasslands in Aso potentially acts as an  
466 important C sink in Japan because of their ability to sequester large amounts of  
467 atmospheric C. The coupled natural and human system of the semi-natural grassland in  
468 Aso acts as a model system in terms of demonstrating the sustainable use of grassland for  
469 animal and renewable energy production as they relate to C accumulation in soil.

470

471

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476



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688 **Figure captions**

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

691

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

694

695 Fig. 3. Carbon (C) content, bulk density,  $\delta^{13}\text{C}$ , and mass C in soil profiles in site-A (a),  
696 site-B (b), site-C (c), site-D (d), site-E (e), and site-F (f) in Aso, Japan. Black color  
697 in soil C stock represents C from  $\text{C}_4$  plant. Numbers under dotted line in the  
698 columns of  $\delta^{13}\text{C}$  and mass C represent  $^{14}\text{C}$  date and accumulation of soil C from  
699 soil surface to the layer, respectively.

700

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

704

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

707

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

711 Fig. 7. Contribution of plant species to biomass derived from three dominant types of C<sub>4</sub>  
712 plants in semi-natural grasslands in Aso, Japan. Filled, grayed, and open bars  
713 represent tribe Andropogoneae, tribe Paniceae, and genus *Miscanthus*.

714

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

717

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

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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 <sup>-1</sup> yr <sup>-1</sup> )	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)
Aso, Japan			<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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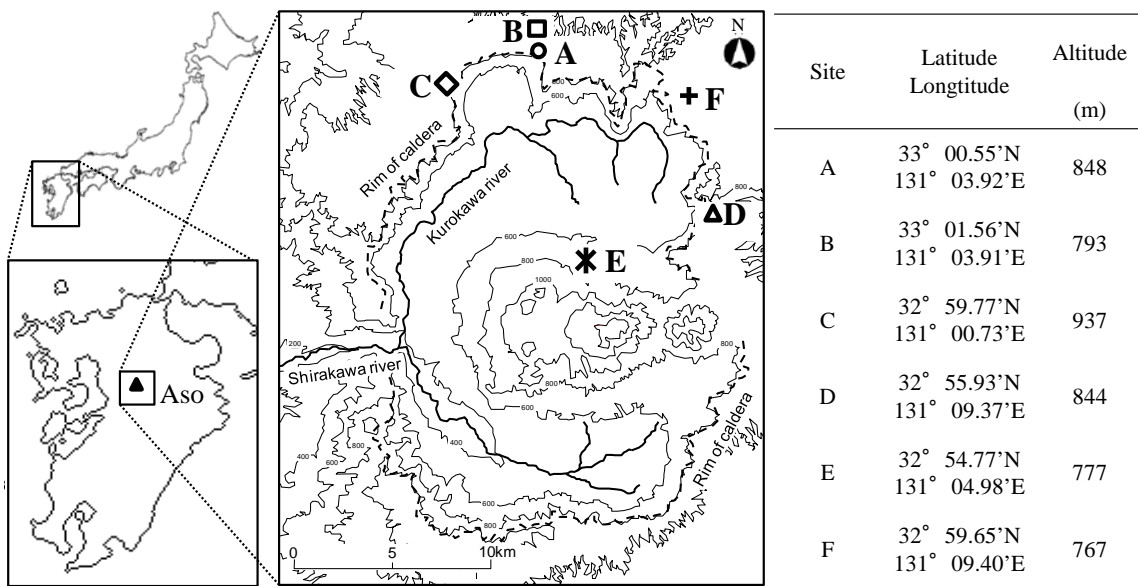


Fig. 1 Location and altitude of the soil sampling sites (A to F) of semi-natural grasslands in Aso, Japan.



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

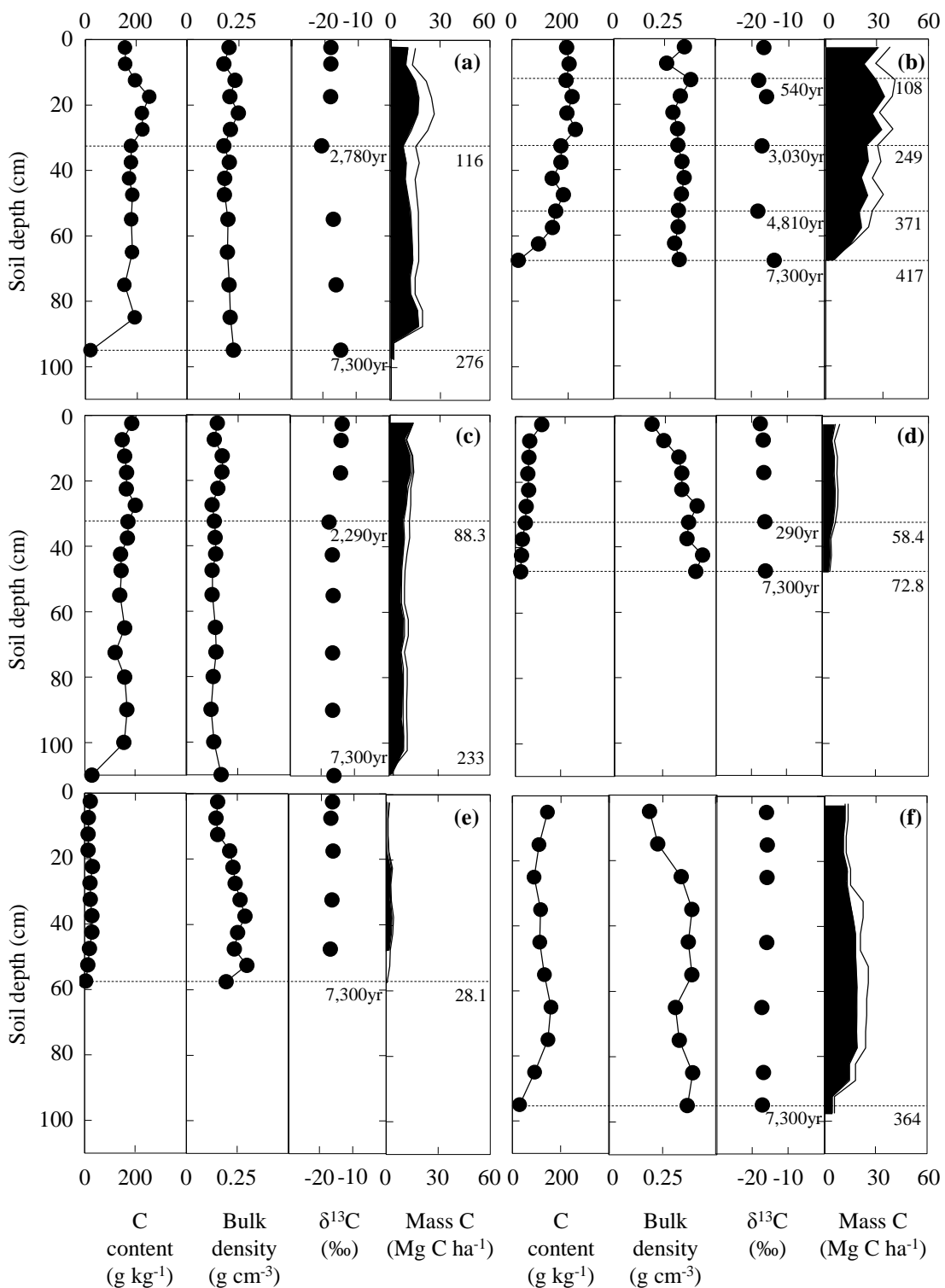


Fig. 3 Carbon (C) content, bulk density,  $\delta^{13}\text{C}$ , and mass C in soil profiles in site-A (a), site-B (b), site-C (c), site-D (d), site-E (e), and site-F (f) in Aso, Japan. Black color in soil C stock represents C from  $\text{C}_4$  plant. Numbers under dotted line in the columns of  $\delta^{13}\text{C}$  and mass C represent  $^{14}\text{C}$  date and accumulation of soil C from soil surface to the layer, respectively.



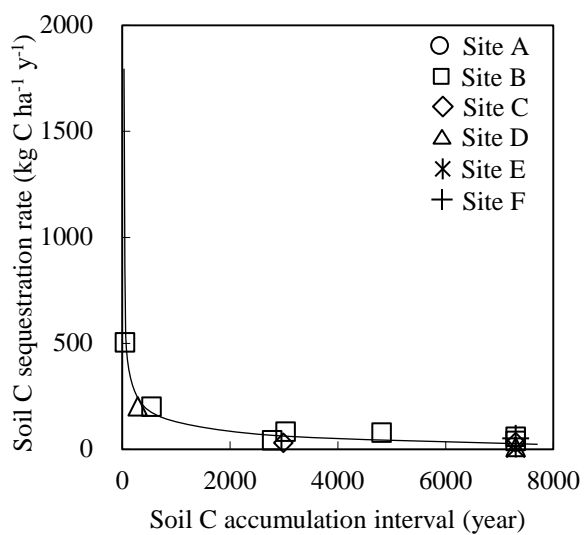


Fig. 4 Relationship between soil carbon (C) sequestration rate and soil C accumulation interval, and fitting curve of this relationship in a semi-natural grasslands in Aso, Japan.

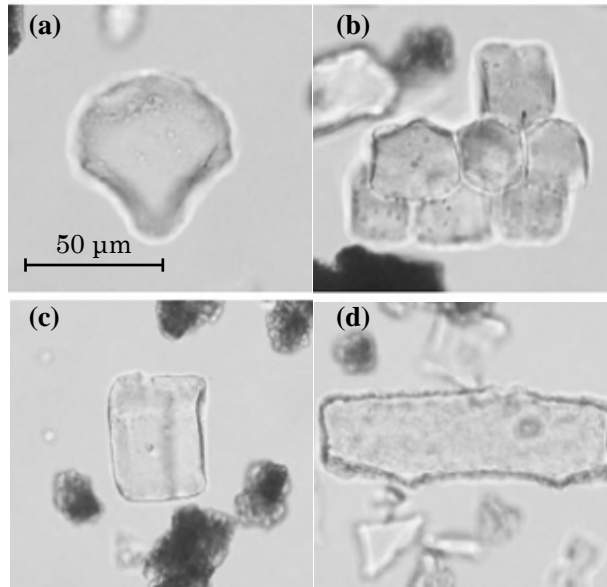


Fig. 5 Phytolith morphotypes of Genus *Miscanthus* (a), (b), Tribe Andropogoneae (c), and Tribe Paniceae (d) recovered from the soil in semi-natural grasslands in Aso, Japan.

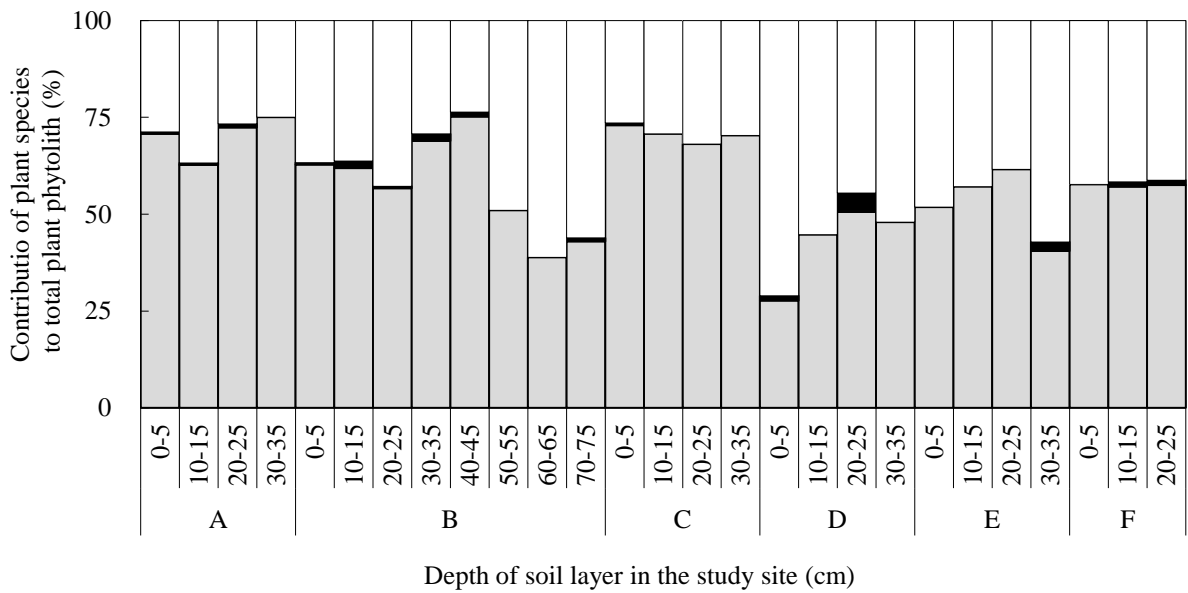


Fig. 6 Contribution of vegetation types identified by phytoliths recovered at six sites in semi-natural grasslands in Aso, Japan. Filled, grayed, and open bars represent vegetation of grasses, trees, and unknown, respectively.

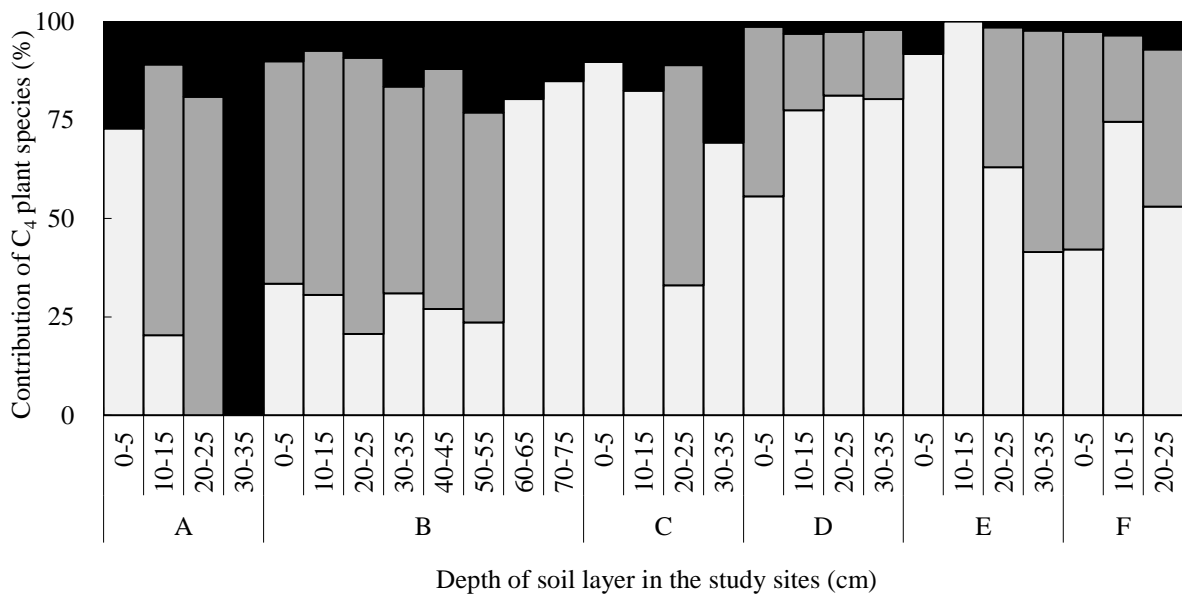


Fig. 7 Contribution of plant species to biomass derived from three dominant types of C<sub>4</sub> plants in semi-natural grasslands in Aso, Japan. Filled, grayed, and open bars represent Tribe *Andropogoneae*, Tribe *Paniceae*, and Genus *Miscanthus*.

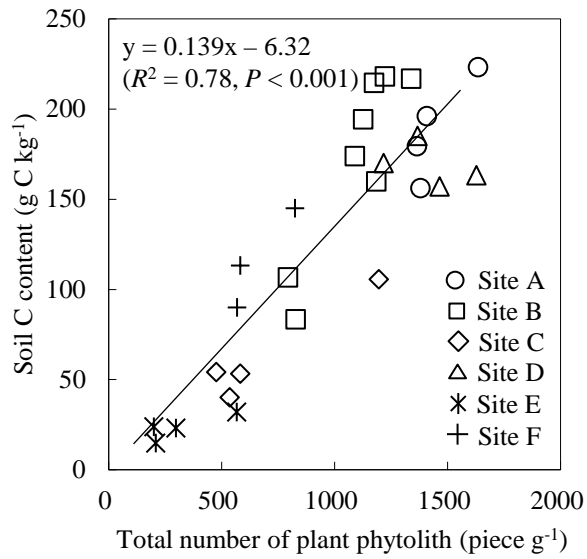


Fig. 8 Relationship between soil carbon (C) content and total number of plant phytolith in soil in semi-natural grasslands in Aso, Japan.

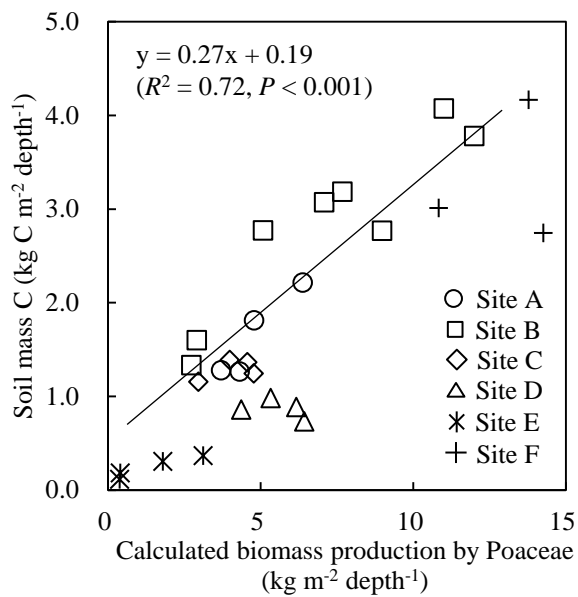


Fig. 9 Relationship between calculated biomass production by plant of *Poaceae* and soil mass C in semi-natural grasslands in Aso, Japan.