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Author(s)	Toma, Yo; Armstrong, Kevin; Ryan Stewart, J.; Yamada, Toshihiko; Nishiwaki, Aya; Fernández, Fabián G.
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Carbon sequestration in soil in a semi-natural *Miscanthus sinensis* grassland and
Cryptomeria japonica forest plantation in Aso, Kumamoto, Japan

Running title: Soil C sequestration in grassland and forest

Yo Toma^{1,2}, Kevin Armstrong³, J. Ryan Stewart³, Toshihiko Yamada¹, Aya Nishiwaki⁴,
Fabián G. Fernández^{3*}

¹Field Science Center for Northern Biosphere, Hokkaido University, Kita 11 Nishi 10,
Kita-ku, Sapporo, Hokkaido 060-0811, Japan

²Energy Biosciences Institute, University of Illinois, 1206 W Gregory Dr, Urbana, IL
61801, USA

³Department of Crop Sciences, University of Illinois, 1102 S. Goodwin Ave, Urbana, IL
61801, USA

⁴Field Science Center, Faculty of Agriculture, University of Miyazaki, Miyazaki-shi,
Miyazaki-ken, 889-2192, Japan

*Corresponding author: tel: +1- 217-333-4426; fax: +1- 217-333-5299; e-mail:
fernande@illinois.edu

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 $\delta^{15}\text{N}$

Abstract

Although *Miscanthus sinensis* grasslands (Misc-GL) and *Cryptomeria japonica* forest plantations (Cryp-FP) are proposed bioenergy feedstock systems, their relative capacity to sequester C may be an important factor in determining their potential for sustainable bioenergy production. Therefore, our objective was to quantify changes in C accumulation rates 47 years after a Misc-GL was converted to a Cryp-FP. The study was conducted on adjacent Misc-GL and Cryp-FP located on Mt. Aso, Kumamoto, Japan. After Cryp-FP establishment, only the Misc-GL continued to be managed by annual burning. Mass C and N, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$ at 0 to 30 cm depth were measured in 5 cm increments. Carbon and N concentrations, C:N ratio, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$ were measured in litter and/or ash, and rhizomes or roots. Although C input in Misc-GL by *M. sinensis* was approximately 36% of that in Cryp-FP by *C. japonica*, C accumulation rate in soil in Misc-GL ($503 \text{ kg C ha}^{-1} \text{ yr}^{-1}$) was higher than that in Cryp-FP ($284 \text{ kg C ha}^{-1} \text{ yr}^{-1}$). This was likely the result of larger C input from litter to soil, C quality (C:N ratio and lignin concentration in litter) and possibly more recalcitrant C (charcoal) inputs by annual burning. The difference in soil $\delta^{15}\text{N}$ between sites indicated that N was better preserved and had greater cycling between plant, microbes, and soil in Misc-GL than in Cryp-FP. Our data indicate that in terms of soil C sequestration, biomass production with Misc-GL may be more advantageous than with Cryp-FP in Aso, Japan.

Introduction

Increasing atmospheric carbon dioxide (CO_2) levels are the product of anthropogenic activities according to the Intergovernmental Panel on Climate Change (2007).

Replacement of fossil fuel by renewable sources of bioenergy provides a viable option to achieve net reductions in CO_2 emissions (Clifton-Brown *et al.*, 2007). The combustion of chemical energy stored in bioenergy feedstock is considered C neutral given that C in the plant was originally fixed from the atmosphere.

Large amounts of C sequestration could occur through production of several dedicated bioenergy crops, including herbaceous plants, woody plants, and even unharvested forest residue (Fischer & Schrattenholzer, 2001). In particular, the *Miscanthus* genus, which is comprised of several highly productive perennial grass species, has significant potential as a bioenergy crop due to its relatively low nutrient requirements (Lewandowski *et al.*, 2003; Heaton *et al.*, 2004), high water-use efficiencies (Clifton-Brown *et al.*, 2002), and high productivity ranging from 1.0 to 40 Mg $\text{ha}^{-1} \text{ yr}^{-1}$ (Jones & Walsh, 2001; Stewart *et al.*, 2009). Although *Miscanthus* can be very productive, other species should also be considered for biofuel production. Henry (2010) reported the biomass yield potential of nearly 15 Mg $\text{ha}^{-1} \text{ yr}^{-1}$ of woody species such as those in the *Populus* and *Salix* genera. Also, forest wood-residues can be used for wood chips and pellets; biogas; bio-ethanol; and other bioenergy products (Parikka, 2004; The Japan Institute of Energy, 2005; Kojima, 2009).

In Europe and the U.S., considerable effort has been devoted for the past 20 years to understand C sequestration in commercial fields of *Miscanthus x giganteus*, which is a sterile allopolyploid hybrid of *M. sinensis* and *Miscanthus sacchariflorus* (Hansen *et al.*,

2004; Clifton-Brown *et al.*, 2007). However, such studies were conducted in relatively short-term research plots (i.e., 16 years or less) and may not represent long-term conditions. In contrast, *M. sinensis* grasslands in Japan have been actively managed, mostly through burning and mowing, for decades to hundreds of years (Otaki, 1999; Stewart *et al.*, 2009). In Kumamoto Prefecture, Japan, *M. sinensis* grasslands have persisted for more than a thousand years due to active management by burning (Otaki, 1999). *Cryptomeria japonica* (Japanese cedar) forest plantations are also distributed throughout the grasslands in Kumamoto and may represent a system for facilitating large amounts of C sequestration. Despite the fact that woody plants generally produce a greater amount of more recalcitrant materials than grasses, 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 (Post & Kwon, 2000; Guo & Gifford, 2002). Nonetheless, the difference in C accumulation rate in soil and the impacts of land-use change from *M. sinensis* and *C. japonica* is not well understood.

Natural abundance of isotopic C in the soil profile beneath long-established C₄ and C₃ plants, such as *M. sinensis* and *C. japonica*, can provide clues regarding the relative contribution to soil C derived from these species since establishment (Hansen *et al.*, 2004; Clifton-Brown *et al.*, 2007). It follows that adjacent well-established sites with similar edaphic and climatic conditions represent a valuable opportunity to estimate the relative long-term C accumulation rate and capacity for C sequestration of *M. sinensis* and *C. japonica*. Thus, our objective was to characterize C accumulation in soil for both managed ecosystems and determine which one provides a superior alternative, in terms

of soil C sequestration, for the sustainable production of biofuel feedstock.

Materials and methods

Site description

The study was conducted in a semi-natural *M. sinensis*-dominated grassland (Misc-GL) and *Cryptomeria japonica* forest plantation (Cryp-FP) located on the northern rim of the Mt. Aso caldera in Kumamoto Prefecture (33°01.58'N, 131°03.89'E, 794 m above sea level) on a fine-loamy, mixed, mesic thaptic Melanudans (USDA, 2010). Mean annual precipitation and air temperature over a 30-year period (1971-2000) were 3,250 mm and 9.6°C, respectively. The Misc-GL (0.4 ha) and Cryp-FP (0.3 ha) sites were adjacent to each other. In Misc-GL, plant species with high relative dominance, calculated by canopy coverage (JSGS, 2004), were *M. sinensis* (47.1%), *Arundinella hirta* (9.9%), *Pleioblastus argenteostriatus* (5.5%), *Amphicarpa bracteata* (6.0%), *Artemisia indica* (3.6%), *Lespedeza bicolor* (3.0%), *Pteridium aquilinum* subsp. *japonicum* (2.8%), and *Lespedeza cuneata* (2.0%). Cryp-FP was established with *C. japonica* seedlings in 1962. Prior to seedling establishment, *M. sinensis* was abundant in the Cryp-FP area. Due to dense canopy coverage and leaf litter accumulation, there were no understory species present in the Cryp-FP at the time of this study. Both sites were burned each year in March, but after 1961, only the Misc-GL continued to be burned each year.

Selected physical and chemical properties of the soils are listed in Table 1. A distinct feature of the soil profile was the soil layer from 64 to 75 cm depth in Misc-GL and below 80 cm depth in Cryp-FP, commonly known in Japan as K-Ah, which is chemically and visually distinct and was deposited after an eruption that occurred approximately

7,300 years ago from the Mount Kikai volcano (Miyabuchi & Watanabe, 1997). Soil pH in both sites varied from 5 to 5.7. Total C ranged from 196 to 228 g C kg⁻¹ in Misc-GL and Cryp-FP for the top 49 and 80 cm of soil depth, respectively. Soil C:N ratio in the top 24 cm in Misc-GL and the 0 to 15 cm depth in Cryp-FP was lower relative to deeper soil horizons.

Soil and plant sampling

Undisturbed 100 cm³ soil sample cores and bulk samples were collected every 5 cm increments down to 30 cm below the soil surface at both sites from four randomly chosen points on 26 February 2008. A stainless steel core (diameter; 50 mm) and supplemental soil sampler (DIK-1630, Daiki Rika Kogyo Co., Ltd., Saitama, Japan) were used for collecting undisturbed soil samples to avoid soil compaction. Each 5 cm increment was extruded before sampling the next layer. After measuring the fresh weight of core soil samples, half of them were oven dried for 24 hours at 105°C to determine the dry/wet ratio of the soil. Bulk density of each 5 cm increment was calculated by multiplying total weight of fresh soil samples by the dry/wet ratio. The bulk soil samples were air dried and sieved through a 2-mm mesh. Sieved soil samples were milled with an agate mortar and then sieved through a 0.2-mm mesh.

In a related study, aboveground biomass of *M. sinensis* in Misc-GL contributed 98.6% of the total aboveground biomass (Toma *et al.*, 2010a). Therefore, *M. sinensis* was assumed to be the primary supplier of C in Misc-GL. Rhizomes and aboveground biomass of *M. sinensis* in Misc-GL and roots of *C. japonica* were collected from four randomly chosen areas (1 m x 1 m x 0.3m soil depth) within each site on 26 February

2008. In Misc-GL, each quadrat included at least two crowns of *M. sinensis*. After the grassland was burned on 21 March 2008, leaf litter and ash in Misc-GL was collected from four randomly chosen areas (1 m x 1 m). Samples were oven dried at 70°C for 48 hours and milled with an ultra-centrifugal mill (ZM 200, Retsch, Germany) with a 0.12-mm sieve. Density of *M. sinensis* rhizomes within the top 30 cm was calculated from dried mass of collected rhizomes.

Soil carbon and nitrogen measurements

Soil and plant samples were analyzed for C and N concentration, $^{13}\text{C}/^{12}\text{C}$, and $^{15}\text{N}/^{14}\text{N}$ with an elemental analyzer (ECS 4010 CHNSO Analyzer, Costech Analytical Technologies Inc., CA, USA) and an isotope ratio mass spectrometer (Delta V Advantage, Thermo Fisher Scientific Inc., MA, USA). Because the soil was not calcareous, measured total soil C can be regarded as soil organic C (Nelson & Sommers, 1996). The $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ data were measured relative to the international Pee Dee Belemnite (PDB) and atmospheric N₂ standards, respectively.

Values were reported in standard δ notation (‰) as follows:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000,$$

where R_{sample} is the isotope ratio $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ of the sample and R_{standard} is the $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ ratio of the PDB or atmospheric N₂ standards, respectively.

Proportion of C (X_C) of *C. japonica*-derived C in the soil was calculated as follows using the method by Hansen *et al.* (2004) and Clifton-Brown *et al.* (2007):

$$X_C = (\delta^{13}\text{C}_{\text{new-cryp}} - \delta^{13}\text{C}_{\text{old}}) / (\delta^{13}\text{C}_{\text{cryp}} - \delta^{13}\text{C}_{\text{old}}) \text{ (equation 1),}$$

where $\delta^{13}\text{C}_{\text{new-cryp}}$ is $\delta^{13}\text{C}$ of soil in Cryp-FP and $\delta^{13}\text{C}_{\text{old}}$ is $\delta^{13}\text{C}$ before *C. japonica* was

established. Because vegetation and management before 1962 at both sites was the same, average $\delta^{13}\text{C}_{\text{new-misc}}$, which was the average $\delta^{13}\text{C}$ of 0 to 30 cm soil depth increment in Misc-GL, was used for $\delta^{13}\text{C}_{\text{old}}$. $\delta^{13}\text{C}_{\text{cryp}}$ is $\delta^{13}\text{C}$ of litter in Cryp-FP or roots of *C. japonica*. When significant differences in soil $\delta^{13}\text{C}$ between Misc-GL and Cryp-FP occurred, mass C in soil derived from *C. japonica* (soil C_{cryp}) and from *M. sinensis* (soil C_{misc}) was calculated as follows:

$$\text{Soil } \text{C}_{\text{cryp}} = \text{mass C in soil in Cryp-FP} \times X_C \text{ (equation 2)}$$

$$\text{Soil } \text{C}_{\text{misc}} = \text{mass C in soil in Misc-GL} - (\text{mass C in soil in Cryp-FP} - \text{soil } \text{C}_{\text{cryp}}) \text{ (equation 3)}$$

Accumulation rate of C was calculated with soil C_{cryp} and soil C_{misc} each divided by the years since the *C. japonica* forest plantation was established (i.e., 47 years). To calculate the trophic level of soil N, an empirical model by Ogawa *et al.* (1997) was used as follows:

$$\bar{\delta}^{15}\text{N}_{\text{TL=a}} = 3.3(n - 1) + \delta^{15}\text{N}_{\text{TL=1}}$$

where n is trophic level (e.g., n of plant is 1 and herbivore is 2), $\bar{\delta}^{15}\text{N}_{\text{TL=a}}$ is the $\delta^{15}\text{N}$ in soil samples, and $\delta^{15}\text{N}_{\text{TL=1}}$ is the $\delta^{15}\text{N}$ in the plant. $\delta^{15}\text{N}_{\text{TL=1}}$ in Misc-GL and Cryp-FP is $\delta^{15}\text{N}$ of litter and ash of the *M. sinensis* grassland and *C. japonica* litter, respectively. $\delta^{15}\text{N}_{\text{TL=a}}$ in both Misc-GL and Cryp-FP was $\delta^{15}\text{N}$ of the 0 to 5 cm soil depth in the respective sites.

*Estimation of total carbon input into soil in *Miscanthus sinensis* grassland and *Cryptomeria japonica* forest plantation*

Primary sources of C flux into soil are generally organic matter of aboveground plant

tissue and dead biomass of belowground organs (Emanuel *et al.*, 1984; Shibata *et al.*, 2005). We assumed that growth and death of belowground organs were likely equal due to a leveling off of growth 5 and 20 years after establishment for *M. sinensis* and *C. japonica*, respectively (Matumura, 1998; National Institute for Environmental Studies, 2010). Thus, C supply by belowground organs of *M. sinensis* were equal to belowground net primary production (BNPP). In Misc-GL, C inputs from *M. sinensis* biomass into the soil consist of BNPP and litter and ash after burning. Numata (1976) reported BNPP of *M. sinensis* to be 25-30% (mean equal to 27.5%) of aboveground net primary production (ANPP). In Misc-GL, aboveground biomass C before burning is considered ANPP. For this study, BNPP in Misc-GL was calculated as 27.5% of ANPP. Since the Cryp-FP had no ash from burning, C inputs in this system consisted of litter and BNPP. For *C. japonica*, we used a mean annual litter C input of $3,390 \text{ kg C ha}^{-1} \text{ yr}^{-1}$ as mentioned by several authors (Andow, 1970; Saito, 1981; Nakane, 1995) and BNPP was calculated as the product of measured C concentration of roots and the average root growth of 35-year-old *C. japonica* trees ($4,600 \text{ kg ha}^{-1} \text{ yr}^{-1}$) as reported by Karizumi (1985).

Statistical analysis

All data were analyzed using SAS software (SAS Institute, 2009). The MIXED procedure was used to detect differences between Misc-GL and Cryp-FP for C concentration, N concentration, C:N ratio, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$. Models were constructed to include soil depth (soil model) and to include plant material (plant model). The soil model was used to test the effect of vegetation cover (Misc-GL or Cryp-FP), soil depth

(5 cm increments for the top 30 cm) and their interaction on the above variables. Soil depth was analyzed as a repeated measurement. The plant model was used to test the effect of the interaction between vegetation cover (*M. sinensis* or *C. japonica*) and plant material (litter or rooting structure) on the above variables. The rooting structures are defined as *M. sinensis* rhizomes or *C. japonica* roots. The Type 3 test of fixed effects was used to determine significance at $P < 0.05$, and significant main effects were investigated using the LSMEANS statement. Significant interactions were explored using the SLICE option in MIXED for soil depths and rooting structures (Littell *et al.*, 2006).

Results

Nitrogen concentration of litter and ash in Misc-GL was 40% lower compared to litter in Cryp-FP ($P < 0.01$) (Table 2). Also, the C:N ratio was 22% lower for *M. sinensis* rhizomes compared to *C. japonica* roots ($P < 0.05$). However, there were no differences in C concentration, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$ between litter and ash in Misc-GL and litter in Cryp-FP or rhizomes of *M. sinensis* and roots of *C. japonica*. Although not statistically significant, mean values of Misc-GL $\delta^{13}\text{C}$ of litter and ash and rhizomes were higher than litter and roots in Cryp-FP, respectively (Table 2).

Mass C in soil under Misc-GL and Cryp-FP ranged from 3.62 to 4.60 and 3.19 to 4.05 kg C m⁻² 5 cm depth⁻¹, respectively (Table 3). Mass C in 0 to 5 cm and 25 to 30 cm depth of soil in Cryp-FP was 24.4% and 18.5% lower than in Misc-GL, respectively (Table 3). Mass N in Misc-GL and Cryp-FP ranged from 184 to 232 and 176 to 198 g N m⁻² 5 cm depth⁻¹, respectively (Table 3). While soil mass N in the 0 to 5 cm depth

increment was 55 g N m⁻² greater in Misc-GL compared to Cryp-FP, the difference was not significant (Table 3).

Soil $\delta^{13}\text{C}$ varied from -17.1 to -16.6 and -21 to -15.5‰ in Misc-GL and Cryp-FP, respectively (Figure 1). Averages of soil $\delta^{13}\text{C}$ in Misc-GL and Cryp-FP were -16.8‰ and -17.4‰, respectively. Soil $\delta^{13}\text{C}$ (-17‰) in the 0 to 5 cm depth of soil under Misc-GL was significantly higher than under Cryp-FP (-21‰), whereas in the depths of 20 to 25 cm and 25 to 30 cm in Misc-GL, $\delta^{13}\text{C}$ (-16.6‰) was significantly lower than in Cryp-FP (-15.5‰) (Figure 1). Soil $\delta^{15}\text{N}$ in Misc-GL and Cryp-FP varied from 5.98 to 8.50 and 3.01 to 7.32‰, respectively (Figure 2). Soil $\delta^{15}\text{N}$ in the top 15 cm depth of soil in Misc-GL was significantly higher than in Cryp-FP (Figure 2). Conversely, in the 25 to 30 cm depth, soil $\delta^{15}\text{N}$ in Misc-GL was lower than in Cryp-FP (Figure 2).

Using equation (1), it was determined that over a 47-yr period, 42% of the mass C in the top 5 cm of the soil was derived from *C. japonica*. This amounts to an accumulation on the top 5 cm of the soil of 1.33 kg C m⁻² (accumulation rate of 284 kg C ha⁻¹ yr⁻¹) derived from Cryp-FP. On the other hand, cumulative C over the 47-yr period for the top 5 cm of soil in Misc-GL was 236 kg C m⁻² (accumulation rate of 503 kg C ha⁻¹ yr⁻¹). It follows that C accumulation rate in the 0-to-5 cm soil depth in Misc-GL was 1.77 times higher than in Cryp-FP. Using the $\delta^{15}\text{N}$ of 0 to 5 cm soil and litter and ash in Misc-GL and those in Cryp-FP (Table 2 and Figure 2), the soil N trophic level values were 3.08 for Misc-GL and 2.14 for Cryp-FP.

Mass of *M. sinensis* rhizomes within the 0 to 30 cm depth was 2.06 kg m⁻². Annual C input in Misc-GL by litter was 62.5 kg C ha⁻¹ yr⁻¹ (Table 4). While not a complete measurement of belowground biomass, *M. sinensis* produced 2.1 kg of rhizomes m⁻²

within the top 30 cm of soil. The annual C input from belowground biomass was estimated at 1,962 kg C ha⁻¹ yr⁻¹ (27.5% of the 7,135 kg C ha⁻¹ present in aboveground biomass before burning). Thus, total C input in Misc-GL was 2,025 kg C ha⁻¹ yr⁻¹. On the other hand, total C input in Cryp-FP was estimated at 5,690 kg C ha⁻¹ yr⁻¹ (sum of 3,390 kg C ha⁻¹ yr⁻¹ from annual litter fall C, and 2,309 kg C ha⁻¹ yr⁻¹ from BNPP) (Table 4).

Discussion

Soil carbon dynamics

C₃ plants, such as *C. japonica*, use the enzyme ribulose-1,5-bisphosphate carboxylase to incorporate atmospheric CO₂. This enzyme discriminates more against the heavier ¹³CO₂ isotope than phosphoenolpyruvate carboxylase, which is used by C₄ plants, such as *M. sinensis*, to fix CO₂. Thus, C₃ plant material and the soil C derived from these plants have more negative ¹³C levels than C₄ plants. Carbon inputs derived from *C. japonica*, as measured by δ¹³C, appeared to influence only the top 5 cm of the soil (Fig. 1) likely because that soil layer received larger C input from litter and/or roots relative to deeper soil layers. Below the 5 cm soil depth, there was no strong evidence that C derived from *C. japonica* influenced soil δ¹³C. A trend for lower δ¹³C at the 5 to 15 cm depth increment in the Cryp-FP may be an indication that while roots are influencing soil C, the rate of change might be too slow to be detected 47 years after the forest was established. Since δ¹³C in roots and litter of *C. japonica* were lower, although not significant, than rhizomes and litter and ash of *M. sinensis* (Table 2), it would be expected that over time, C derived from *C. japonica* would reduce soil δ¹³C deeper in the soil. The change towards more positive soil δ¹³C in Cryp-FP at the 20 to 30 cm

depth relative to that of Misc-GL likely does not reflect changes induced by vegetation. Not only was the change in the opposite direction ($\delta^{13}\text{C}$ of Cryp-FP becoming more positive), but as explained above, 47 years seems to be a relatively short time to induce changes in deeper soil layers where deposition of organic materials and root and soil-fauna activity are generally lower.

Because of burning in Misc-GL, the total amount of C deposited on the soil surface was much smaller than the amount deposited in litter in Cryp-FP (Table 4). However, ash and charcoal are typically much finer in size than litter and likely become rapidly incorporated in the soil by physical rather than biological decomposition processes. On the other hand, most of the C deposited on the soil surface in Cryp-FP remained as C in the litter and likely was not as rapidly incorporated in the soil, but rather was slowly decomposed by microorganisms that also released a portion of C in the litter as CO_2 to the atmosphere. In a study by Guo & Gifford (2002), a 10% decline in soil C sequestration occurs after pastures change to plantation forests because decomposition of woody plant materials deposited in the soil results in the formation of less soil organic matter relative to the pasture. Generally, organic matter decomposition increases with increasing temperature (Chapin *et al.*, 2002; Boone *et al.*, 1998; Shimizu *et al.*, 2009; Toma *et al.*, 2010c). While temperatures were not measured in this study, temperatures under coniferous forests tend to be less conducive to decomposition of organic materials than grasslands. A study by Kashiwagi (1991) in Nagano, Japan (36°31'N, 138°21'E, 1,315 m above sea level) indicated that effective cumulative air temperature (i.e., cumulative air temperature above 5°C at 6 cm above soil surface) of *M. sinensis* grassland was 2,067°C and for a secondary *Pinus densiflora* forest was 1,648°C.

Burning in Misc-GL might not only accelerate the process of C incorporation into the soil, but can be important in modifying the quality of the C source. Ash and charcoal produced during burning are more recalcitrant than litter (Seiler & Crutzen, 1980; Skjemstad *et al.*, 2002; Lehmann *et al.*, 2006), and thus represent a more stable form of soil C. It is well known that C:N ratios are important factors for C decomposition (Huang *et al.*, 2004; Toma & Hatano, 2007). Although C:N ratios of litter and ash in Misc-GL or litter in Cryp-FP were not statistically different in our study due to high variability, there was a 50% increase in the C:N ratio of litter and ash of Misc-GL compared to litter in Cryp-FP. While the level of recalcitrance of charcoal and ash materials is likely the most important C-quality factor influencing C accumulation in our study, lignin content may also have contributed to the difference in C accumulation rate between the two study sites. Increased lignin content in organic matter can contribute to a reduction in C decomposition rate (Wieder *et al.*, 2009; Austin & Ballaré, 2010). While lignin content was not measured in our study, others have reported lignin concentration in *C. japonica* litter of 33.7-41.0% (Inagaki *et al.*, 2004) compared to lignin content of aboveground organs of *M. sinensis* of 15.5-24.4% (Yoshida *et al.*, 2008; Osono, 2010; Serrano *et al.*, 2010). It is possible that greater lignin content in Cryp-FP may have resulted in a slower rate of degradation of forest litter into the soil organic C pool compared to Misc-GL in our study.

Finally, another factor that may have influenced a differential accumulation of soil C in Misc-GL and Cryp-FP was the difference in soil pH brought about by the change in vegetation. In our study, soil pH in Cryp-FP was relatively lower than that in Misc-GL (Table 1). Several studies have reported the relationship between soil organic C losses

and soil acidification (Davis & Lang, 1991; Parfitt *et al.*, 1997; Alfredsson *et al.*, 1998; Chen *et al.*, 2000; Iimura *et al.*, 2010). Iimura *et al.* (2010) reported decreases in soil C content and increases in fulvic acids:humic acids ratio in a Typic Melanudand soil with the succession of vegetation from *M. sinensis* grassland to *P. densiflora* secondary forest to coniferous tree forest dominated by *Quercus crispula*. Cerli *et al.* (2008) reported an increase in the relative quantity of fulvic acids with long-term afforestation on former agricultural land. This acidification resulted in the translocation of acidic and more soluble materials and likely induced podzolization. Iimura *et al.* (2010) also concluded that one of the causes of changes in the content of humic acids is the degree of podzolization caused by the fluxes of dissolved organic C. It is possible that in our study lower soil pH in Cryp-FP might have caused leaching of soil organic C and overall reduction of C accumulation relative to Misc-GL.

Soil nitrogen dynamics

Differing environmental conditions and quality of inputted organic matter into soil induced by change in land use from Misc-GL to Cryp-FP might change the structure of soil microbial communities. Dube *et al.* (2009) reported significantly higher soil microbial respiration in a *Pinus ponderosa* forest plantation at depths of 10 to 40 cm, compared to an adjacent natural grassland site on Typic Hapludands in Chile. Ectomycorrhizal fungi associated with tree roots have been reported to increase the mineralization of organic forms of N in soil via the production of extracellular hydrolase enzymes such as proteinase (Marschner & Dell, 1994; George & Marschner, 1996). Over a 30-yr period in Nagano, Japan, Iimura *et al.* (2010) suggested that C losses, disappearance of the

melanic epipedon, and changes in chemical properties of humic acids (aryl C moieties) could be due to microbial degradation occurring during ecological succession from a *M. sinensis* grassland to a secondary *P. densiflora* forest. Similar succession of microbial communities might have occurred in our study sites and may help explain the larger mass N and higher soil N trophic levels in Misc-GL than Cryp-FP. Generally, most of the underground organs (72-95%) are distributed within the top 30 cm of soil in *M. sinensis* grasslands (Yano & Kayama, 1978; Hayashi *et al.*, 1981). In our study site, the rhizome density per unit of soil volume was very high (2.06 kg m^{-2} 0.3-m depth $^{-1}$). This correlates well with the observed changes in $\delta^{15}\text{N}$ compared to Cryp-FP within the top 15 cm of the soil. It is likely that greater turnover of roots and rhizomes and greater exudation of organic materials from these organs along with higher activity of microorganisms within the top 15 cm of the soil in Misc-GL compared to Cryp-FP may be responsible for the observed changes in $\delta^{15}\text{N}$.

Comparison of carbon accumulation rate in soil in Misc-GL or Cryp-FP with other studies

Our study site does not constitute a true feedstock production system because aboveground biomass is removed by burning and part of the C in the form of charcoal and ash (along with litter) is cycled back to the system. Still, it is valuable to compare this system to other systems not only in terms of their potential for biomass production, but also in terms of their potential for C accumulation in the soil. Yazaki *et al.* (2004) reported a 2-yr mean C accumulation rate of $-750 \text{ kg C ha}^{-1} \text{ yr}^{-1}$ in a *M. sinensis* grassland established in an Andisol soil in Nagano, Japan. This value did not account

for belowground net primary production (BNPP), but a recent study by Toma *et al.* (2010b), in which BNPP was estimated, indicated that a *M. sinensis* dominated grassland in Hokkaido, Japan accumulated C at a rate of 620 kg C ha⁻¹ yr⁻¹ and may constitute a sink of atmospheric C. In an Inceptisols soil in Denmark, Hansen *et al.* (2004) reported soil C accumulation rates of 780 and 1,120 kg C ha⁻¹ yr⁻¹ within 0 to 100 cm of soil for 9- and 16-yr-old *M. x giganteus* cultivated fields, respectively. In addition, Clifton-Brown *et al.* (2007) reported 590 kg of soil C ha⁻¹ yr⁻¹ within the top 30 cm was derived from *M. x giganteus* production over a 15-yr period on Inceptisols in Ireland. While C accumulation rate in Misc-GL in our study was overall lower than that in *M. x giganteus*, it is possible that the discrepancy arises from the difference in sampling depth used to calculate C accumulation for the different studies, which makes it difficult to directly compare the data. Also, Heaton *et al.* (2004) reported the average annual yield of *M. x giganteus* was 22.4 Mg ha⁻¹ yr⁻¹ (biomass production range was 6-38 Mg ha⁻¹ yr⁻¹), whereas annual yield of *M. sinensis* in Japan varied from 1.8 to 12.5 Mg ha⁻¹ yr⁻¹ (Stewart *et al.*, 2009). In a related study we indicated that aboveground biomass of *M. sinensis* in our study site was approximately 16 Mg ha⁻¹ yr⁻¹ (Toma *et al.*, 2010a). The greater soil C accumulation in *M. x giganteus* may be a reflection of the greater biomass produced compared to *M. sinensis*. Comparison of these studies seem to indicate that the semi-natural *M. sinensis* grassland with annual burning in our study site has greater potential for soil C accumulation than for production of biomass relative to *M. x giganteus*. Possibly preserving semi-natural *M. sinensis* grasslands by annual burning and concentrating feedstock production in more productive regions can best accomplish the goal of increasing both feedstock production and C sequestration.

Over a 23-yr period, the average soil C accumulation rate for *C. japonica* was 229 kg C ha⁻¹ yr⁻¹ at the 0 to 30 cm depth in Andisols in Ibaraki, Japan (Sakai *et al.*, 2010). A similar study reported an accumulation rate of 190 kg C ha⁻¹ yr⁻¹ over a 33-yr period for the top 5 cm soil depth in Entisols in Yamanashi, Japan (Sakai *et al.*, 2003). The rates presented by these two studies are similar to our reported C accumulation rate of 284 kg soil C ha⁻¹ yr⁻¹ in Cryp-FP. Sakai *et al.* (2010) reported that C accumulation rate in a *Chamaecyparis obtusa* forest plantation was 211 kg C ha⁻¹ yr⁻¹ in 0 to 30 cm depth in Andisols in Ibaraki, Japan. Ono *et al.* (2009) reported the 10-yr average of C accumulation rate in forest soil was 420 kg C ha⁻¹ yr⁻¹ in a temperate deciduous forest dominated by beech (*Fagus crenata*) and oak (*Quercus crispula*) in Ibaraki, Japan. Carbon accumulation rate in soil in coniferous evergreen tree forest plantations might be relatively lower compared to forests mainly comprised of deciduous species. Overall, our results and those reported from similar studies indicate that *M. sinensis*-dominated grasslands or cultivated *M. x giganteus* fields may have greater capacity to be atmospheric C sinks compared with deciduous or coniferous evergreen tree forests. These studies also agree with the findings of our study and would indicate that for the purpose of C sequestration in soil, preservation of semi-natural Misc-GL grasslands by annual burning may be more beneficial than transformation of these grasslands into forest plantations.

Conclusions

Carbon accumulation rate in soil (0 to 5 cm depth) was higher in Misc-GL than in Cryp-FP possibly due to the presence of more recalcitrant C input and slower rate of decomposition of such inputs. In addition, higher soil N trophic level values of top 15 cm depth in Misc-GL than in Cryp-FP indicated that soil N retention time might be longer in Misc-GL than in Cryp-FP and that there is a greater degree of N cycling between plant, microbes, and soil in Misc-GL than Cryp-FP. Our study indicates that Misc-GL has greater capacity for soil C sequestration than Cryp-FP.

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Table captions

Table 1. Soil physical and chemical characteristics in a semi-natural *Miscanthus sinensis* grassland (Misc-GL) and *Cryptomeria japonica* forest plantation (Cryp-FP) in Aso, Kumamoto, Japan.

Table 2. Carbon (C) content, nitrogen (N) content, C:N ratio, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$ of litter and ash and rhizomes (top 30 cm of soil depth) of a semi-natural *M. sinensis* grassland (Misc-GL), and litter and roots (top 30 cm of soil depth) of a *Cryptomeria japonica* forest plantation (Cryp-FP), in Aso, Kumamoto, Japan.

Table 3. Mass carbon (C) and mass nitrogen (N) in soil for 5 cm increments to a depth of 30 cm in a semi-natural *Miscanthus sinensis* grassland (Misc-GL) and *Cryptomeria japonica* forest plantation (Cryp-FP) in Aso, Kumamoto, Japan.

Table 4. Carbon input from litter and ash in a semi-natural *M. sinensis* grassland (Misc-GL), litter in *Cryptomeria japonica* forest plantation (Cryp-FP), and belowground net primary production (BNPP) in both sites in Aso, Kumamoto, Japan.

Figure captions

Figure 1. $\delta^{13}\text{C}$ in each 5 cm soil increment from 0 to 30 cm depth of soil in a semi-natural *M. sinensis* grassland (Misc-GL) and *Cryptomeria japonica* forest plantation (Cryp-FP) in Aso, Kumamoto, Japan. Filled and open bars are $\delta^{13}\text{C}$ values in Misc-GL and Cryp-FP, respectively. *Error bars* represent standard deviations ($n = 4$). **, * indicate difference between vegetation within the corresponding soil depth $P < 0.01$ and 0.05 , respectively.

Figure 2. $\delta^{15}\text{N}$ in each 5 cm soil increments from 0 to 30 cm depth of soil in a semi-natural *M. sinensis* grassland (Misc-GL) and *Cryptomeria japonica* forest plantation (Cryp-FP) in Aso, Kumamoto, Japan. Filled and open bars are $\delta^{15}\text{N}$ values in Misc-GL and Cryp-FP, respectively. *Error bars* represent standard deviations ($n = 4$). ** indicate difference between vegetation within the corresponding soil depth ($P < 0.01$).

Table 1. Soil physical and chemical characteristics in a semi-natural *Miscanthus sinensis* grassland (Misc-GL) and *Cryptomeria japonica* forest plantation (Cryp-FP) in Aso, Kumamoto, Japan.

Site	Horizon	Depth (cm)	Total C		Total N		Texture			L
			pH	content (g C kg ⁻¹)	content (g N kg ⁻¹)	C:N ratio	Sand (%)	Silt (%)	Clay (%)	
Misc-GL	Ah1	0-24	5.31	221	0.90	18.7	40.6	39.9	19.5	SL
	Ah2	24-49	5.33	196	0.57	25.0	62.1	30.2	7.67	
	Ah3	49-64	5.48	154	0.40	27.3	68.8	24.4	6.77	
	2AB	64-75	5.48	53	0.15	26.0	68.1	23.7	8.19	
	3AB	+75	5.73	114	0.30	28.7	60.9	25.8	13.3	
Cryp-FP	Ah1	0-15	5.02	222	0.93	20.1	40.0	42.5	17.5	L
	Ah2	15-28	5.13	228	0.76	24.1	31.5	43.2	25.3	
	Ah3	28-80	5.24	205	0.66	23.9	50.2	32.0	17.8	
	2AB	+80	5.54	106	0.32	25.3	63.7	26.2	10.1	

Table 2. Carbon (C) content, nitrogen (N) content, C:N ratio, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$ of litter and ash and rhizomes (top 30 cm of soil depth) of a semi-natural *M. sinensis* grassland (Misc-GL), and litter and roots (top 30 cm of soil depth) of a *Cryptomeria japonica* forest plantation (Cryp-FP), in Aso, Kumamoto, Japan.

	C concentration (g C kg ⁻¹)	N concentration (g N kg ⁻¹)	C:N ratio		$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
Litter and ash in Misc-GL	405 (3.85)	8.89 (0.68)b†	45.6 (7.18)		-18.5 (3.24)	-0.89 (0.75)
Litter in Cryp-FP	444 (28.4)	14.7 (1.23)a	30.4 (4.27)		-26.9 (0.3)	-0.76 (0.33)
Rhizomes of <i>M. sinensis</i>	447 (7.18)	7.51 (2.25)	64.1 (21.3)b		-14.7 (2.63)	-0.96 (0.66)
Roots of <i>C. japonica</i>	502 (2.32)	6.18 (0.68)	82.0 (9.14)a		-25.4 (1.09)	-1.02 (0.72)

Values between parentheses are standard deviations ($n = 4$).

†Within column, values followed by the same letter are not significantly different ($P > 0.01$).

Table 3. Mass carbon (C) and mass nitrogen (N) in soil for 5 cm increments to a depth of 30 cm in a semi-natural *Miscanthus sinensis* grassland (Misc-GL) and *Cryptomeria japonica* forest plantation (Cryp-FP) in Aso, Kumamoto, Japan.

Depth (cm)	Mass C		Mass N	
	Misc-GL	(kg C m ⁻²)	Misc-GL	(g N m ⁻²)
0-5	4.22 (0.51) ab†	3.19 (0.79) c**	232 (28.1) a	177 (52.9) a
5-10	3.62 (0.41) b	3.30 (0.33) b	193 (18.7) b	186 (19.7) a
10-15	3.76 (0.18) b	3.66 (0.45) b	203 (5.95) b	192 (24.4) a
15-20	3.71 (0.48) b	3.80 (0.39) a	199 (26.2) b	198 (24.6) a
20-25	3.87 (0.22) b	4.05 (0.23) a	199 (12.2) b	192 (19.2) a
25-30	4.60 (0.55) a	3.75 (0.23) ab*	184 (7.78) b	176 (10.8) a

Values between parentheses are standard deviations ($n = 4$).

†Within column, values followed by the same letter are not significantly different ($P > 0.05$).

**, * indicate difference between vegetation within the corresponding soil depth $P < 0.01$ and 0.05 , respectively.

Table 4. Carbon input from litter and ash in a semi-natural *M. sinensis* grassland (Misc-GL), litter in *Cryptomeria japonica* forest plantation (Cryp-FP), and belowground net primary production (BNPP) in both sites in Aso, Kumamoto, Japan.

	C input rate (kg C ha ⁻¹ yr ⁻¹)
Litter and ash in Misc-GL	62.5
BNPP in Misc-GL	1,962
Litter in Cryp-FP	3,390
BNPP in Cryp-FP	2,309

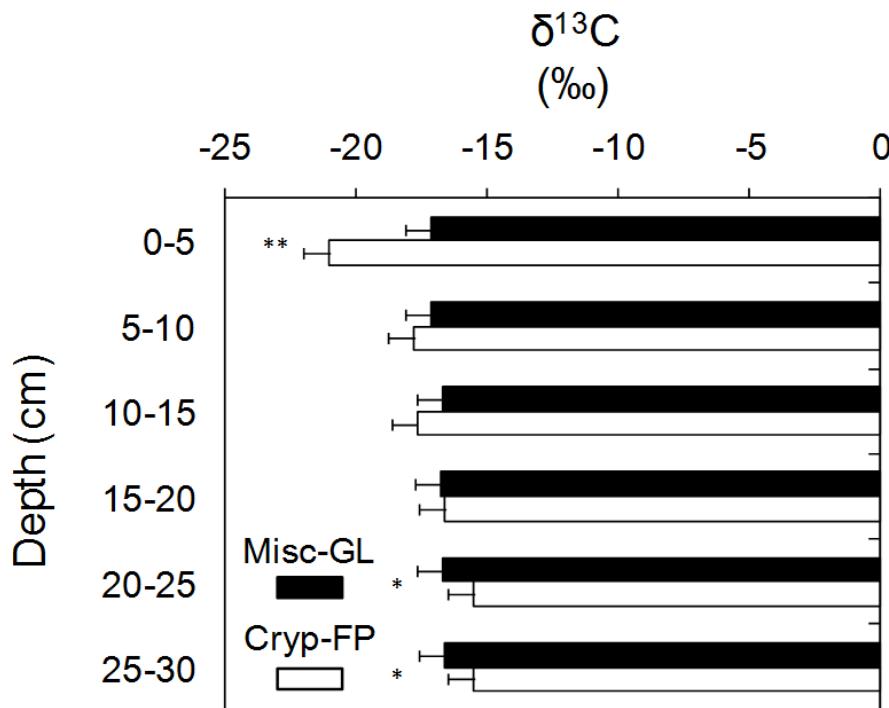


Fig 1 $\delta^{13}\text{C}$ in each 5 cm soil increment from 0 to 30 cm depth of soil in a semi-natural *M. sinensis* grassland (Misc-GL) and *Cryptomeria japonica* forest plantation (Cryp-FP) in Aso, Kumamoto, Japan. Filled and open bars are $\delta^{13}\text{C}$ values in Misc-GL and Cryp-FP, respectively. Error bars represent standard deviations ($n = 4$). **, * indicate difference between vegetation within the corresponding soil depth $P < 0.01$ and 0.05, respectively.

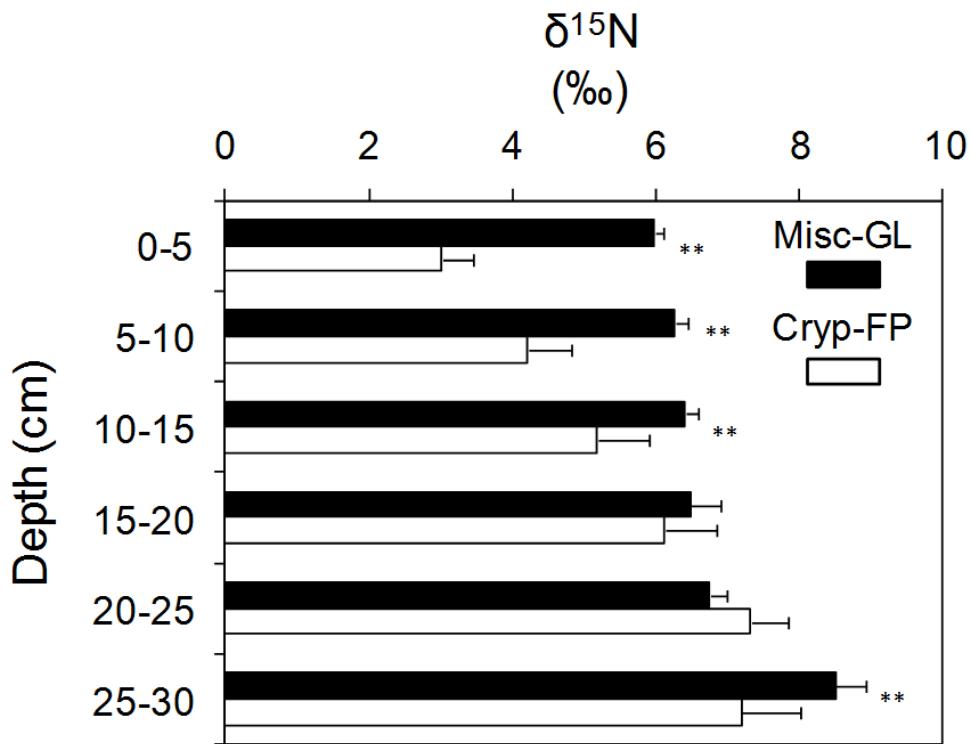


Fig. 2 $\delta^{15}\text{N}$ in each 5 cm soil increments from 0 to 30 cm depth of soil in a semi-natural *M. sinensis* grassland (Misc-GL) and *Cryptomeria japonica* forest plantation (Cryp-FP) in Aso, Kumamoto, Japan. Filled and open bars are $\delta^{15}\text{N}$ values in Misc-GL and Cryp-FP, respectively. Error bars represent standard deviations ($n = 4$). ** indicate difference between vegetation within the corresponding soil depth ($P < 0.01$).