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Carbon sequestration under *Miscanthus* in sandy and loamy soils estimated by natural ^{13}C abundance

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Summary

Most studies of soil organic-carbon (SOC) dynamics using ^{13}C natural abundance have been conducted with maize. Here, we present data about the sequestration of C derived from a perennial C_4 plant, *Miscanthus* × *giganteus* (Greef et Deu.) grown on loamy and sandy soils for 9 and 12 y, respectively. We expected a higher contribution of *Miscanthus*-derived C to SOC formation compared to maize because of (1) higher net biomass production by *Miscanthus*, (2) lower shoot-to-root ratio, (3) deeper roots, and (4) the absence of plowing. In both soils, there was a significant contribution of *Miscanthus*-derived C down to 1 m soil depth. The maximal contents of 3.0 g C_4-C (kg soil)$^{-1}$ and 2.4 g C_4-C (kg soil)$^{-1}$ for loamy and sandy soil, respectively, were observed for the upper 0–10 cm layer. The decline in the amount of *Miscanthus*-derived C with soil depth was significant for both soils, but without significant differences between the differently textured soils except the depth of 0–10 cm. The total SOC was similar under *Miscanthus* and under reference grassland in the sandy soil (both 6.4 kg C m^{-2} down to 1 m soil depth). Amounts of SOC were slightly higher under grassland at the loamy site (12.1 kg C m^{-2} compared with 11.2 kg C m^{-2}). So, C accumulation under *Miscanthus* was similar to that under perennial grasses. After 9 and 12 y, respectively, the yearly incorporation of *Miscanthus*-C in SOC of the upper 0–30 cm was 0.23 g C_4-C (kg soil)$^{-1}$ y^{-1} in the loamy and 0.11 g C_4-C (kg soil)$^{-1}$ y^{-1} in the sandy soil. This C_4-C incorporation in loamy soil under *Miscanthus* was 1.6–1.8 times higher than results reported for maize C_4-C incorporation in SOC grown under similar climatic conditions. In the sandy soil, the C_4-C incorporation under *Miscanthus* was nearly the same as under maize. The fraction of 22% of the *Miscanthus* residues remaining in SOC was similar to that one of maize residues in loamy soil. In sandy soil, only a small fraction of 9% of the *Miscanthus* residues was incorporated in SOC.

Key words: C sequestration / mean residence time / *Miscanthus* × *giganteus* / natural ^{13}C abundance / soil organic carbon / C turnover

1 Introduction

In carbon (C)-sequestration studies, knowledge of the annual contribution of "new" organic carbon to soil organic carbon (SOC) is very important. Apart from methods using humification of artificially ^{14}C- or ^{13}C-labeled plants or plant residues, natural differences in the abundance of ^{13}C between C_4 and C_3 plants are frequently used to distinguish between old SOC and new plant-derived C (*e.g.*, *Volkhoff* and *Cerri*, 1987; *Balesdent* and *Mariotti*, 1987; *Kristiansen* et al., 2005). The discrimination against ^{13}C is higher at C_3 photosynthesis, making the $\delta^{13}C$ values of C_3 plants smaller (ca. –27‰) than those of C_4 plants (ca. –12‰) (*Ehleringer* and *Cerling*, 2002). Therefore, by growing a C_4 plant on soils with former C_3 vegetation, the amount of C_4 plant–derived C can be estimated on the basis of the changing $\delta^{13}C$ values of SOC. A reference location with similar soil properties and land-use history is required to represent a soil with unchanged vegetation as well as to consider isotopic effects during humification and microbial utilization of plant residues (*Balesdent* and *Mariotti*, 1996). Nearly all investigations using ^{13}C natural abundance have been conducted with maize (*e.g.*, *Balesdent* and *Balabane*, 1996; *Ludwig* et al., 2003). Focussing solely on one of the various C_4 plants cultivated in temperate climates may yield biased information about C dynamic in soils. Nevertheless, a limited number of investigations with other C_4 crops is available. *Garten* and *Wullschleger* (2000) studied switchgrass (*Panicum virgatum* L.) and found extremely high portions of switchgrass-derived C (19%–31%) in the upper 40 cm of soil after only 5 y of cultivation. As far as we know, only two investigations of C dynamics using natural ^{13}C of *Miscanthus* cultivated under European conditions are available from a sandy soil in Denmark (*Hansen* et al., 2004; *Foreid* et al., 2004). Therefore, additional studies with other C_4 plants growing on various soils are necessary.

In Europe, *Miscanthus* × *giganteus* (Greef et Deu.) is used as bio-energy crop with high aboveground biomass–yield potential (*e.g.*, *Beuch*, 2000). It can be cultivated for 15 up to 25 y without replanting and is harvested yearly, often in the following spring to reduce ash contents. Based on the results of *Hansen* et al. (2004) and *Garten* and *Wullschleger* (2000), but especially considering the physiological properties and growing methods of *Miscanthus*, the C dynamics in soils under *Miscanthus* are expected to differ from those under maize. The following features of *Miscanthus*' physiology and cultivation lead us to expect that the contribution of *Miscanthus*-C to SOC could be higher than found for maize: (1) perennial plants such as *Miscanthus* displace high portions of the assimilated C belowground as a C reservoir for growth in spring (*Kuzyakov* and *Domanski*, 2000), (2) *Miscanthus* has a very deep and well-developed root system (*Miridokawa* et al., 1975; *Neukirchen*, 1999), (3) the absence of soil tillage means less aeration, lower plant residues–de-

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composition rates, and better C stabilization for longer periods, (4) a high input of aboveground harvest residues, because harvesting in late winter or early spring leads to an accumulation of stubbles and leaves on the soil surface as pre-harvest losses (*e.g.*, *Beuch*, 1999), and (5) slower decomposition of plant residues (stubbles, leaves, and roots) because the absence or reduction of N fertilization lead to a larger C : N ratio. Thus, our hypothesis was a higher annual contribution of C_4-derived C in soils under *Miscanthus* and a different depth distribution of C_4-derived C compared to those observed in soils under maize.

Different stabilization of SOC as well as different aeration frequently results in different turnover rates in differently textured soils (*Huggins* et al., 1998; *Wang* and *Hsieh*, 2002). We expected higher turnover rates in a coarser soil and therefore compared a loamy and a sandy soil with similar cultivation periods of *Miscanthus* in Germany.

2 Material and methods

One of the two fields under *Miscanthus* was located in Stuttgart-Hohenheim, Baden-Württemberg, Germany (48°43′ N, 9°13′ E), on a loamy Gleyic Cambisol (*WRB*, 1998). Mean annual temperature is 8.7°C and average annual rainfall 679 mm (1961–1990, meteorological station Stuttgart-Hohenheim). Soil texture was silty loam without any significant textural change in soil profile. *Miscanthus* was planted in May 1994 on a former grassland plot, and aboveground standing biomass was harvested annually in February or March. *Miscanthus* yields at the loamy site were 0.95 kg C m^{-2} y^{-1} on average. Soil and plant samples for SOC and $\delta^{13}C$ analysis were taken in April 2003. The cultivation period at the sampling time was around 9 y. The second site was in Großbeeren, 10 km S of Berlin (52°21′ N, 13°19′ E), on a sandy Gleyic Cambisol (*WRB*, 1998). Mean annual temperature is 8.7°C (1961–1990, meteorological station Potsdam), average annual rainfall 548 mm (1961–1990, meteorological station Großbeeren). Soil texture was loamy sand without any significant textural change in soil profile. *Miscanthus* was cultivated for 12 y at sampling in September 2003 and was established on a former fallow. *Miscanthus* yields at the sandy site were 0.64 kg C m^{-2} y^{-1} after 4 y of cultivation in 1995. The field was not harvested during the last 7 y. Grassland plots adjacent to the *Miscanthus* fields were used as references. Soil profiles both from the grassland and *Miscanthus* site were prepared to obtain volume samples in order to determine bulk densities and general soil characteristics. For $\delta^{13}C$ analyses, soil samples were taken with a soil auger in steps of 10 cm to a depth of 100 cm. The distance between the replications was about 15 m. In Hohenheim, three field replicates were taken from *Miscanthus* soil, and two analytical replicates were measured from one sample of every depth layer for soil under grassland. In Großbeeren, five replicates were available from the *Miscanthus* plot and two from the reference plot. The soil samples were air-dried at room temperature and sieved (2 mm mesh size). Afterwards, in a subsample, all visible root and plant remains were removed with tweezers, and the soil was ball-milled. Plant samples (shoots, roots, and rhizomes) were dried at 60°C and ground. Amounts of 25–30 mg of ground soil samples and 4–6 mg of ground plant material were weighed into tin capsules for $\delta^{13}C$ analyses. $\delta^{13}C$ was measured on an isotope-ratio mass spectrometer (IRMS 20–20, PDZ Europe) coupled with a C/N-Analyzer (Carlo Erba) (0.1% precision). The portion of *Miscanthus*-derived C in SOC was calculated according to *Balesdent* and *Mariotti* (1996):

$$\%C_{Miscanthus} = \frac{\delta^{13}C_t - \delta^{13}C_3}{\delta^{13}C_4 - \delta^{13}C_3} \cdot 100, \tag{1}$$

where $\delta^{13}C_t$ is the $\delta^{13}C$ value of the soil with *Miscanthus,* $\delta^{13}C_3$ is the $\delta^{13}C$ value of the corresponding layer of reference soil with continuous C_3 vegetation, $\delta^{13}C_4$ is the $\delta^{13}C$ value of a poor C_4 soil under *Miscanthus.* It was calculated based on the $\delta^{13}C$ value of the *Miscanthus* (mean of root, shoot, and leaves) and corrected for isotopic fractionation during humification by subtraction of the differences between $\delta^{13}C$ of C_3 vegetation and $\delta^{13}C$ of SOC of the corresponding soil layer of the C_3 soil. This approach assumes equal isotopic fractionation for humification of C_3 plants and C_4 plants and considers different fractionation in different soil depths.

Total input of *Miscanthus*-derived residues during cultivation period was calculated under the assumption that (1) pre-harvest losses were 30% of total aboveground-biomass production (*Boelcke* et al., 1998), (2) direct harvest losses (*e.g.*, stubbles) were 10% (*Beuch*, 1995), and (3) belowground biomass was 50% of aboveground biomass (*Neukirchen*, 1999) and 4% of the belowground root C were mineralized yearly (*Boelcke* et al., 1998). During the 3 y establishment phase of *Miscanthus*, yield was fixed to 50% of the average biomass yield of the established *Miscanthus* stock (*Clifton-Brown* et al., 2001). Total aboveground-biomass production was first calculated based on the *Miscanthus* yield and the information about pre-harvest and direct harvest losses. Afterwards, yearly *Miscanthus*-C input as pre-harvest losses, direct harvest losses, and dying root biomass was calculated and added up for the whole vegetation time.

Turnover rate of C_3-derived SOC was calculated by using an exponential approach according to the difference in the amount of C_3-derived C in *Miscanthus* soil and the amount of C_3-derived C in grassland soil (*Gregorich* et al., 1995). The mean residence times (MRTs) were calculated as reciprocal to turnover rates. Standard deviation of *Miscanthus*-derived C was calculated according to *John* et al. (2003). The significance of differences between sandy and loamy soils were examined using one-way analysis of variance (ANOVA, α = 0.05). Standard errors of means are presented on the figures as variability parameter.

3 Results and discussion

3.1 Total organic-C content

In the upper 30 cm of the loamy soil, the SOC content under *Miscanthus* (11–14 g C kg^{-1}) was lower than under grassland (13–16 g C kg^{-1}). Soil organic-C content declined clearly with soil depth (Fig. 1). The absolute SOC amounts in the loamy soil were slightly lower under *Miscanthus* compared to grassland, both in the upper 30 cm (5.4 *vs.* 7.0 kg C m^{-2}) and at

0–100 cm (11.2 *vs.* 12.1 kg C m^{-2}). For the sandy soil, absolute SOC amounts at 0–100 cm were slightly higher under grassland than under *Miscanthus* (7.0 kg SOC m^{-2} *vs.* 6.4 kg SOC m^{-2}). More SOC was found under *Miscanthus* for the upper 30 cm (4.6 kg C m^{-2} *vs.* 4 kg C m^{-2}). Soil organic-C amounts were higher in both loamy plots than in both sandy plots (Fig. 1, Fig. 2). This is explained by slower decomposition of plant residues in loamy soil due to less aeration, as well as by higher protection of SOC by clay particles. In opposite to *Boelcke* et al. (1998) who found a SOC content increase by 0.12% (silty loam) and 0.3% (sand) after 6–9 y of *Miscanthus* cultivation, we found no significant differences to the SOC between *Miscanthus* and grassland plots. These results suggested that C accumulation under *Miscanthus* is similar to that under perennial C_3 grasses.

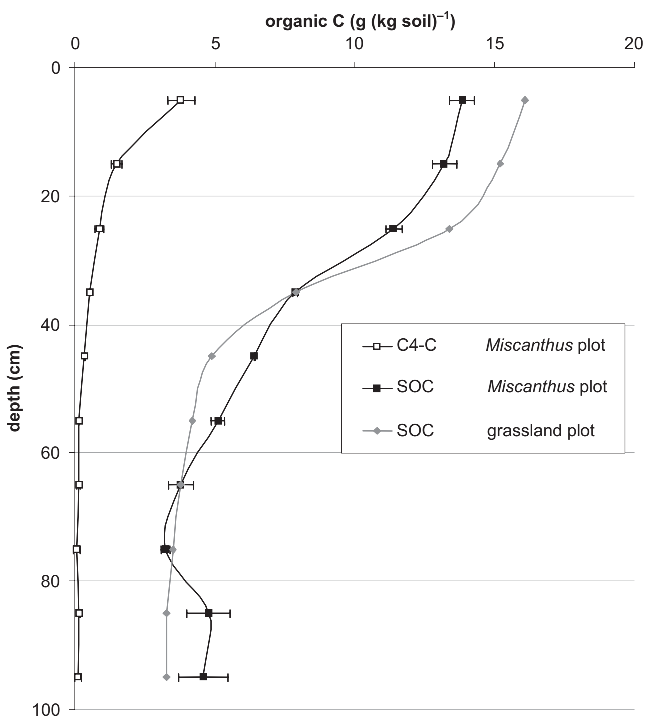

Figure 1: Total C and C_3- and C_4-derived C in loamy *Miscanthus* and grassland soil (± SE).

3.2 Contribution of *Miscanthus*-derived C to SOC

The $\delta^{13}C$ values slightly increased with depth in both grassland soils (Fig. 3). Similar trends were observed in several other studies (*e.g., Veldkamp*, 1994; *Gregorich* et al., 1995). In addition to ^{13}C discrimination during decomposition (*Ågren* et al., 1996), the decrease in $\delta^{13}C$ in of atmospheric CO_2 during the last century contributes to decrease of d^{13}C values in the upper soil horizon (*Gregorich* et al., 1995). The input of *Miscanthus*-derived C resulted in significantly greater $\delta^{13}C$ values of the SOC on the sandy and loamy sites compared to the soils under grassland. In the loamy topsoil, 3.8 g C_4-C (0–10 cm) to 0.8 g C_4-C kg^{-1} (20–30 cm) (kg soil)$^{-1}$ were *Miscanthus*-derived (Fig. 1). The content of *Miscanthus*-derived C in the sandy topsoil was lower than that in loamy soil (Fig. 1–2). However, the differences between the sandy and the loamy soils were significant only for 10–20 cm ($p < 0.05$).

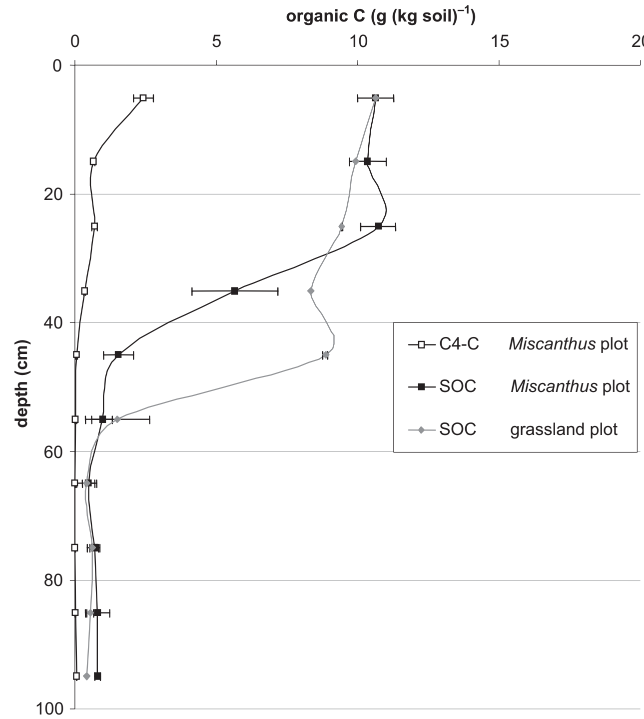

Figure 2: Total C and C_3- and C_4-derived C in sandy *Miscanthus* and grassland soil (± SE).

Total amounts of *Miscanthus*-derived C at 0–100 cm depth accounted for 1.1 kg C_4-C m^{-2} and were nearly twice as high as in the sandy soil (0.6 kg C m^{-2}). In a sandy soil in Denmark under *Miscanthus, Hansen* et al. (2004) found 0.7 kg C_4-C m^{-2} (0–100 cm) after 9 y.

About 85% of the total *Miscanthus*-derived C at 0–100 cm were concentrated in the upper 30 cm of the sandy soil and 76% in the upper 30 cm of the loamy soil. This corresponds to results of *Gregorich* et al. (1995), who found 88% of total maize-derived C in the Ap horizon (0–27 cm). Depth had a strong effect on the contribution of *Miscanthus*-derived C to SOC (Fig. 1–2). In contrast to our study and the study of *Hansen* et al. (2004) with *Miscanthus, Gregorich* et al. (1995) detected no maize-derived C below 60 cm. This supports our hypothesis of a higher C sequestration under *Miscanthus* especially in deeper soil layers compared to maize.

The annual incorporation of *Miscanthus*-derived C in the upper 30 cm was 0.23 g C kg^{-1} y^{-1} in the loamy and 0.11 g C kg^{-1} y^{-1} in the sandy soil. The average annual incorporation of maize-derived C into SOC of Ap horizons of selected soils with different textures was 0.13 g C kg^{-1} y^{-1} (*Kuzyakov* and *Schneckenberger*, 2004) with high variations (0.03–2.6 g C kg^{-1} y^{-1}, STD 0.053) caused by tillage, fertilization level, aboveground-biomass production, climatic conditions, and soil texture. The comparison of the annual incorporation of *Miscanthus*- and maize-derived C into SOC showed a higher incorporation of *Miscanthus*-C only for the loamy soil. For sandy soil exclusively, we could observe a slightly higher or similar incorporation of *Miscanthus*-derived C compared to maize-derived C. *Flessa* et al. (2000) found an incorporation

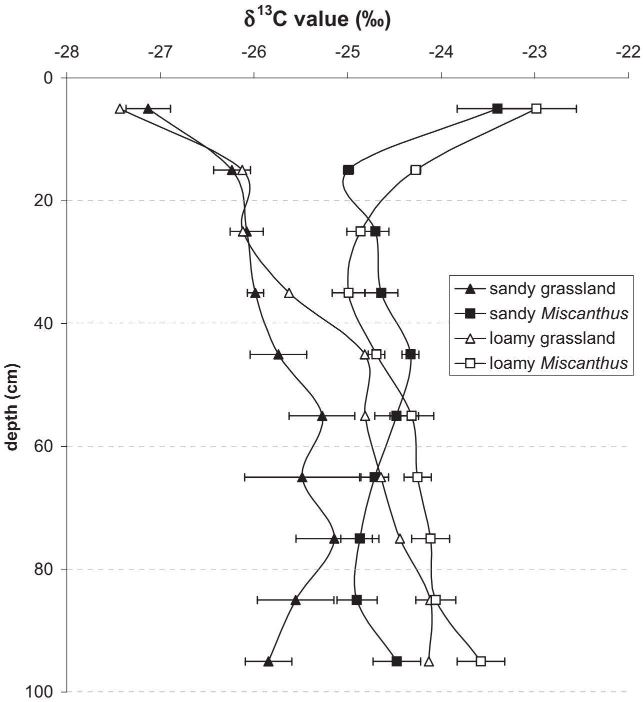

Figure 3: δ^{13}C values (± SE) of the SOC of the loamy and sandy soils after 9–12 y of *Miscanthus* cultivation and under reference C_3 grassland.

of 0.053 g C kg^{-1} y^{-1} of maize-derived C in SOC of a sandy soil. The annual incorporation of maize-derived C in the sandy soil of *Wanniarachchi* et al. (1999) was similar to our sandy *Miscanthus* soil. The average annual incorporation of maize-derived C in the Ap horizon of various soils with loamy texture was 0.17 g C kg^{-1} y^{-1} (*Kuzyakov* and *Schneckenberger*, 2004), somewhat less than the 0.23 g C kg^{-1} y^{-1} in the loamy soil under *Miscanthus.* The annual incorporation of *Miscanthus*-derived C in our sandy soil was lower than in the study of *Hansen* et al. (2004). They found an annual incorporation of 0.18 g C kg^{-1} *Miscanthus*-derived C in the top 20 cm of a sandy soil, while we found an annual incorporation of only 0.13 g C kg^{-1} (0–20 cm). Switchgrass, another perennial C_4 energy crop, showed much higher C-incorporation rates (*Garten* and *Wullschleger*, 2000) than those observed for *Miscanthus* and maize (*e.g.*, *Hansen* et al., 2004; *Collins* et al., 1999). Although the aboveground biomass of switchgrass was removed, the authors found a mean annual incorporation of switchgrass-derived C of 0.33 g C kg^{-1} in the upper 40 cm. However, the results of *Garten* and *Wullschleger* (2000) must be interpreted with caution because very low SOC contents (0.6%–0.8%) prior to switchgrass cropping may have led to a greater total C accumulation.

The part of the total input of *Miscanthus* residues retained in SOC was calculated by estimating the total C input to the soil during the cultivation period in comparison with the total amount of C_4-C remained in the SOC. Within 9 y, 22% of the C derived from the *Miscanthus* residues (5 kg C m^{-2}) were retained as SOC in the loamy soil (100 cm depth). Only 9% of *Miscanthus* residues (6.8 kg C m^{-2}) were incorporated in SOC of the sandy soil. This is lower than the accumulation of 26% of the *Miscanthus* residues in the SOC of the sandy soil reported by *Hansen* et al. (2004) where aboveground biomass was harvested. On our sandy site, the aboveground *Miscanthus* biomass was harvested only during the first 5 y. Therefore, most of the *Miscanthus*-shoot residues were decomposed aboveground. According to *Flessa* et al. (2000) and *Balesdent* and *Balabane* (1996), the accumulation of root residues in soil is higher than the accumulation of aboveground plant residues. Therefore, *Flessa* et al. (2000) found a much higher portion of silage-maize residues remained in the SOC compared to our loamy soil (31% at 0–70 cm *vs.* 22% at 0–100 cm). There should remain only direct harvest losses of 10% of the aboveground-biomass production on field (*Angers* et al., 1995) compared to 40% of the aboveground-biomass production of *Miscanthus* even on loamy site.

We calculated the MRT of the C_3-C in the upper soil horizon of the loamy soil using an exponential approach on the basis of the amount of C_3-C (kg C_3-C m^{-2}) in the *Miscanthus* field after 9 y of *Miscanthus* cultivation and the amount of C_3-C in the reference soil. We assumed that the C_3-C amount in the reference soil reflects the amount of C_3-C in the *Miscanthus* field prior to *Miscanthus* cultivation. In the loamy soil, the MRT of the C_3-C was 11 y for 0–10 cm, 34 y for 10–20 cm, and 39 y for 20–30 cm. *Collins* et al. (1999) found MRTs of 40–96 y for the top layers (0–20 cm) of variously textured soils. An MRT of 55 y was estimated for the C_3-C by *Gregorich* et al. (2001). While the MRTs of *Collins* et al. (1999) and *Gregorich* et al. (2001) are longer than the MRT of the C_3-C in our loamy soil, *Gregorich* et al. (1995) and *Angers* et al. (1995) found similar MRTs under maize in Canada and USA.

4 Conclusions

Continuous *Miscanthus* cropping on a loamy and a sandy soil during 9 and 12 y, respectively, led to C sequestration similar to that of perennial grassland. The C amount of *Miscanthus*-derived C_4-C incorporated annually into SOC of loamy soil was higher than that found in other investigations on maize. This is evident both in the total amounts of *Miscanthus* residues remaining in the soil after the cultivation and in the annually increase of the *Miscanthus*-derived C content. However, our expectation of a higher contribution of perennial *Miscanthus* compared to annual maize was supported only for the loamy soil. A higher contribution of *Miscanthus*-derived C to SOC in deeper soil layers compared to maize points to the possibility of belowground C sequestration by planting perennial C_4 grasses.

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