Carbon sequestration and turnover in soil under the energy crop Miscanthus: repeated 13C natural abundance approach and literature synthesis

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Abstract

The stability and turnover of soil organic matter (SOM) are a very important but poorly understood part of carbon (C) cycling. Conversion of C3 grassland to the C4 energy crop Miscanthus provides an ideal opportunity to quantify medium-term SOM dynamics without disturbance (e.g., plowing), due to the natural shift in the Δ13C signature of soil C. For the first time, we used a repeated 13C natural abundance approach to measure C turnover in a loamy Gleyic Cambisol after 9 and 21 years of Miscanthus cultivation. This is the longest C3-C4 vegetation change study on C turnover in soil under energy crops. SOM stocks under Miscanthus and reference grassland were similar down to 1 m depth. However, both increased between 9 and 21 years from 105 to 140 mg C ha−1 (P < 0.05), indicating nonsteady state of SOM. This calls for caution when estimating SOM turnover based on a single sampling. The mean residence time (MRT) of old C (>9 years) increased with depth from 19 years (0–10 cm) to 30–152 years (10–50 cm), and remained stable below 50 cm. From 41 literature observations, the average SOM increase after conversion from cropland or grassland to Miscanthus was 6.4 and 0.4 mg C ha−1, respectively. The MRT of total C in topsoil under Miscanthus remained stable at ~60 years, independent of plantation age, corroborating the idea that C dynamics are dominated by recycling processes rather than by C stabilization. In conclusion, growing Miscanthus on C-poor arable soils caused immediate C sequestration because of higher C input and decreased SOM decomposition. However, after replacing grasslands with Miscanthus, SOM stocks remained stable and the MRT of old C3-C increased strongly with depth.

Keywords: 13C natural abundance, C3-C4 vegetation change, carbon sequestration, energy crop, mean residence time, soil organic matter

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Introduction

Globally, soil organic matter (SOM) contains more than three times as much carbon (C) as the atmosphere (Fischlin et al., 2007). Even small changes to the soil C pool (e.g., altered stability, turnover) will have strong effects on the atmospheric CO2 concentration and the global C budget (Heimann & Reichstein, 2008). The total C stock changes depending on the balance between input and output, which are affected by land use, vegetation type, field management, nutrient availability, climate, etc. SOM pools are dynamic, even under steady state without total C stock changes, with continuous input of fresh C into various pools and their concurrent decomposition (von Lützow et al., 2007; Novara et al., 2013). Therefore, SOM turnover and stabilization are as important as C stocks, especially when considering ecosystem functions (Six & Jastrow, 2002).

SOM turnover can be estimated by 14C radiocarbon dating, 14C or 13C labeling (e.g., bomb 14C, Δ13C after free-air CO2 enrichment (FACE), Δ13C after C3-C4 vegetation changes, tracer application), budget approaches, changes of SOM pools after land-use conversion, and...
modeling (Six & Jastrow, 2002; Kuzyakov, 2011; Zang et al., 2017). Among these approaches, $^{13}$C natural abundance is powerful for evaluating SOM medium-term turnover after a C$_3$–C$_4$ vegetation change (Derrien & Amelung, 2011). The C derived from original (C$_3$) and from new (C$_4$) vegetation can be distinguished based on changes in the $\delta^{13}$C signature (Flessa et al., 2000; Werth & Kuzyakov, 2010). The MRT of SOM can thereby be estimated in situ.

In the past 20 years, the C$_4$ plant Miscanthus has received increasing attention as a favored perennial bioenergy crop (Lewandowski et al., 2003; Ferrarini et al., 2017a) due to its benefits with regard to soil C and the greenhouse gas balance (Donini et al., 2009; Hillier et al., 2009). However, the effects on soil C stocks of land-use changes from cropland or grassland to Miscanthus remain unclear, and these effects depend heavily on soil texture, climate, plant productivity, and preexisting soil C levels (Poepplau et al., 2011). Conversion of cropland or grassland to bioenergy crops has long-term positive impacts on soil C sequestration and ecosystem services, including regulating (climate, water, and biodiversity), supporting (soil health), and provisioning services (biomass and energy yield) (Ferrarini et al., 2017b). Based on modeling, the average SOM accumulation rate in the top 30 cm after vegetation change from cropland to Miscanthus was estimated to be about 1 mg C ha$^{-1}$ yr$^{-1}$ (Anderson-Teixeira et al., 2009). Shortly after conversion from natural ecosystems (e.g., grassland or forest), however, initial soil C losses were detected (Anderson-Teixeira et al., 2009). To understand the SOM changes after planting Miscanthus, the Miscanthus-derived C can be distinguished, and the C dynamics can be assessed based on the $\delta^{13}$C value of SOM after the vegetation change from C$_3$ grassland.

**Miscanthus** is a perennial crop and, therefore, SOM is not disturbed by plowing during its cultivation. This provides a unique opportunity (in contrast to, e.g., maize cultivation) to estimate SOM turnover under undisturbed soil conditions. Nevertheless, most studies on Miscanthus have been conducted to explore short-term SOM changes and turnover within 10 years after vegetation change (Schneckenberger & Kuzyakov, 2007; Zimmermann et al., 2012). During the first several years after Miscanthus planting, however, SOM decomposition will be strongly affected by the development of new roots. For example, Miscanthus-derived C input increased in the topsoil during the planting period, which may strongly affect SOM decomposition and C sequestration (Poepplau & Don, 2014). Nearly all studies of SOM turnover based on C$_3$–C$_4$ vegetation changes have used only one sampling time and did not experimentally determine the stability of C turnover during the development of the new crop (maize or Miscanthus) (Flessa et al., 2000; Zimmermann et al., 2012; Poeplau & Don, 2014). To overcome this uncertainty, we estimated soil C changes and turnover with a repeated $^{13}$C natural abundance approach, 9 and 21 years after conversion from C$_3$ grassland to Miscanthus. Such a repeated $^{13}$C natural abundance approach is novel and extremely useful for the scope of the presented work, which enables (1) estimation of C stocks over chronosequences; (2) calculation of the C$_4$-C incorporation into SOM and the C$_3$-C decomposition rate with new crop plantation age; and (3) accurate assessment of the turnover of old C based on the decrease of C$_3$-C between two sampling times.

Agricultural practices and management of the perennial plant Miscanthus suggest higher C sequestration and contrasting patterns of SOM turnover compared with common annual crops (e.g., maize), especially in deep soil. There are several reasons: (1) Miscanthus is harvested aboveground every year after senescence, causing higher plant residue accumulation from pre- and direct-harvest losses compared with annual crops (Donini et al., 2009). (2) Miscanthus grows under no-tillage conditions, which lead to nonhomogeneous C distribution and input mainly into topsoil (0–30 cm). (3) Miscanthus has continuously growing horizontal underground stems (rhizomes). The rhizomes are concentrated at 0–20 cm soil depth and strongly affect C input and turnover (Christensen et al., 2016). (4) Miscanthus has a well-developed and deep-reaching root system (Neukirchen et al., 1999), which causes higher C input into the subsoil and may stimulate SOM turnover in deeper horizons (Fontaine et al., 2007). Limited information is available about the effects of increased new C input into subsoil, especially many years after a vegetation change. Maize monoculture contributed 10% and 2% of total SOM at 0–10 and 90–100 cm, respectively, after 10 years of cultivation (Flessa et al., 2000; Rasse et al., 2006). This amount, however, is less than half that of Miscanthus-derived C after 9 years (Schneckenberger & Kuzyakov, 2007). This indicates contrasting C inputs and turnover patterns under the perennial energy crop Miscanthus compared to annual crops. Thus, assessing SOM turnover in the topsoil alone may underestimate the soil C storage potential in deeper layers, especially for perennial plants with deep rooting systems (Baker et al., 2007). We therefore estimated the new C input and old C decomposition down to 100 cm to examine whether SOM accumulation and turnover change with depth.

In this study, we estimated (1) total Miscanthus-derived C incorporated into SOM, depending on depth, 9 and 21 years after the land-use change from grassland; (2) the turnover of SOM depending on depth under Miscanthus; (3) changes in the Miscanthus-derived
C input into topsoil with time after a vegetation change, based on a literature review; and (4) the generalized changes of soil C stocks and turnover in topsoil over the decades following vegetation change, based on a literature review.

Materials and methods

Experimental set-up and soil sampling

The field was located at the experimental station of the University of Hohenheim, Baden-Württemberg, Germany (48°43’N, 9°13’E, 407 m above sea level), on a loamy Stagnic Cambisol (IUSS Working W.R.B. Group, 2014). Mean annual temperature was 10.4 °C, and average annual rainfall was 654 mm from 2000 to 2016. Soil texture was silty loam without any significant textural change in the soil profile. Miscanthus × giganteus (Greef et Deu.) was planted in May 1994 on a former grassland plot, and aboveground standing biomass has been harvested annually in February or March. Miscanthus yields at this site averaged 0.95 kg C m⁻² yr⁻¹ (Schneckenberger & Kuzyakov, 2007).

Soil and plant samples for SOM and δ¹³C were collected in April 2003 and October 2015, corresponding to cultivation periods of 9 and 21 years, respectively. Grassland plots (about 20 m distant) were used as the C₃ reference. In 2003, soil profiles both from grassland and Miscanthus fields were prepared to obtain volume samples. The distance between the replications was about 15 m. Please see detail in Schneckenberger & Kuzyakov (2007). In 2015, soil samples from both the grassland and Miscanthus sites were taken with an auger in 10 cm intervals to a depth of 100 cm. Three field replicates for grassland and Miscanthus were randomly selected; a distance of over 5 m between each replicate ensured independence of samples. Soil samples were taken from the middle of plant inter-row for Miscanthus in both 2003 and 2015.

Isotopic analysis

Soil samples were air-dried at room temperature and sieved (<2 mm). Afterward, all visible root and plant residues were removed, and the soil was ball-milled. Plant samples (shoots, roots, rhizomes) were dried at 60 °C and ball-milled. The δ¹³C of plant and soil was analyzed at the Center for Stable Isotope Research and Analysis (KOSI) at the University of Goettingen, with an Elemental Analyzer (Eurovector) coupled to an IRMS (Delta Plus XL IRMS, Thermo Finnigan MAT, Bremen, Germany).

Data collection from the literature

The synthesis was performed with published data (1990–2017) on SOM changes after conversion from cropland or grassland to Miscanthus using ISI Web of Science and Google Scholar. The criteria for selection of appropriate studies were as follows: (1) restriction to studies involving C₃-C₄ vegetation changes; (2) restriction to topsoil data (0-20 or 0-30 cm); and (3) focus solely on vegetation changes, with other factors excluded. In total, we extracted 41 observations from 12 studies.

Calculations and statistics

The proportional contributions of the C₃ (f₃) and the C₄ (f₄) Miscanthus-derived sources to total SOM were calculated according to Amelung et al. (2008):

\[ f₃ = (δ^{13}C₁ - δ^{13}C₃)/(δ^{13}C₄ - δ^{13}C₃) \]  
\[ f₄ = 1 - f₃ \]

where δ¹³C₁ is the δ¹³C value of the soil under Miscanthus and δ¹³C₃ is the δ¹³C value of the corresponding layer in the reference soil (grassland). δ¹³C₄ was calculated based on the δ¹³C value of Miscanthus (roots) and corrected for isotopic fractionation during humification by subtracting the differences between δ¹³C₃ of C₃ vegetation and δ¹³C₃ of SOM of the C₃ soil. This approach assumes equal isotopic fractionation during humification of C₃ plants and C₄ plants (Schneckenberger & Kuzyakov, 2007).

In general, it is assumed that SOM decomposition follows first-order kinetics. Under steady-state conditions, the MRT of SOM was calculated using an exponential approach based on the difference between the amount of C₃-derived C in Miscanthus soil and the amount of C₄-derived C in grassland soil (Gregorich et al., 1995; Amelung et al., 2008). The MRT was calculated as the reciprocal of the turnover rate. Values were calculated according to the following equation:

\[ \text{MRT} = 1/k = -t/\ln(1 - f₄) \]

where k stands for the turnover rate, t for the number of years after vegetation change, and f₄ for the proportional contribution of the C₄ (Miscanthus-derived) source to the total C pool.

Equation 3 was always used for a single sampling time to estimate the C turnover, assuming that SOM was at steady state. The two sampling times (9 and 21 years) after the C₃–C₄ vegetation change in this study demonstrated that the steady-state assumption is not always valid. Nonetheless, for the time span between 9 and 21 years, the MRT of ‘old’ C₃-C can be calculated according to Eqn (3) from the decrease of C₃-C in Miscanthus soil. Here, we use the term ‘old’ C for the C originating from preceding C₃ vegetation, which is at least 9 years old.

Statistical analyses were carried out using Statistica (Version 7.0, StatSoft, Inc., USA). The values presented in the figures are means ± standard errors (SE). Significant difference between Miscanthus and grassland was tested by one-way analysis of variance (ANOVA) in combination with Tukey’s HSD (Honestly Significant Difference) test. Differences between Miscanthus and grassland as well as between soil depth on δ¹³C, SOC, and portion of Miscanthus-derived C in SOM were tested by two-way ANOVA, also three-way ANOVA was used when considering the sampling time (9 vs. 21 years). The Kruskal-Wallis ANOVA was used to compare the differences in total C stock changes, C₄-C changes, and mean residence time between previous land use (cropland or grassland) from literature review. All differences were considered significant at the P < 0.05 level.

Results

Soil organic C content and $\delta^{13}$C

Miscanthus cultivation and the input of C$_4$-derived C strongly increased $\delta^{13}$C values at all depths relative to the reference grassland ($P < 0.05$; Fig. 1). The $\delta^{13}$C values increased with depth from −28.4 to −24.8$\%_{oo}$ in the grassland soil, but decreased from −23 to −24$\%_{oo}$ (9 years) and from −18 to −24$\%_{oo}$ (21 years) under Miscanthus. The $\delta^{13}$C values increased strongly from 9 to 21 years after Miscanthus planting, especially in the top 50 cm of soil ($P < 0.05$). A specific pattern of $\delta^{13}$C values was found after 21 years, namely, a strong $\delta^{13}$C increase down to 50 cm ($P < 0.05$). This reflects strong root and rhizome development of Miscanthus in the upper 50 cm between 9 and 21 years.

The total SOM stock down to 1 m under Miscanthus was similar to that under reference grassland in both sampling years (2003 and 2015, $P < 0.05$; Fig. S1). However, SOM significantly increased by 30–80% from 9 to 21 years under Miscanthus at 0–10 and 30–60 cm depths ($P < 0.05$; Fig. S1). Down the soil profile, the SOM contents declined gradually from the top 10 to 90–100 cm depth (Fig. 1). After 21 years under Miscanthus, 61 mg C ha$^{-1}$ in the upper 1 m was C$_4$-derived. The contribution of Miscanthus-derived C to total soil C within 100 cm depth increased strongly from 9 years (7.5%) to 21 years (45%) after conversion to Miscanthus ($P < 0.05$; Fig. 2).

Mean residence time of C

The MRT of old C (>9 years), based on the changes in $\delta^{13}$C values after 9 and 21 years of Miscanthus cultivation, gradually increased from topsoil down to 50 cm depth: from 19 to 30 and to 152 years within the A$_h$, A$_h$B horizons (Fig. 2). Below 50 cm depth, the absence of a significant decrease in C$_3$-C between 9 and 21 years indicates the stability of old C pools in Bsw and BwCw horizons. Considering the whole soil profile down to 1 m, the MRT of old C$_3$-C was around 60 years from 9 to 21 years of Miscanthus cultivation.

![Fig. 1](https://example.com/fig1.png)
Discussion

The C stock is mainly determined by the balance between new C input and incorporation into SOM (here: derived from Miscanthus) and the decomposition of old C (here: derived from grassland and from Miscanthus). This has been related to the duration of land-use change and to soil depth (Schneckenberger & Kuzyakov, 2007; Felten & Emmerling, 2012). The increased SOM stock reflects the increase in new C input after conversion to Miscanthus and the concomitant increase of the C4-C fraction in the soil. However, a similar increase in C stock was also observed under grassland. Miscanthus is a good proxy for grassland because it is perennial, the roots extend much deeper than those of agricultural crops, it is not plowed (no soil disturbance), and it is not or only minimally fertilized (Lewandowski et al., 2003; Ferrarini et al., 2017a). The increasing C stocks therefore may indicate that the land was used as arable land decades before grassland was established.

The decreasing trend of C4-SOM with depth from the Ah to A h and B and Bsw, and BwCw horizons correlated with C4-C input; it reflected the natural distribution of SOM and decreased root and rhizodeposition input with depth (Neukirchen et al., 1999; Fontaine et al., 2007). In our study, about 77% of C4-C incorporated into SOM is located in the Ah horizon after 21 years under Miscanthus (Fig. 1). This is consistent with the 42.6% of C4-C incorporated at 0–15 cm depth after 14 years (Dondini et al., 2009). New C incorporation in the plow layer reached 15% of total C after 10 years and only 29% after 17 years of maize cultivation (Balesdent et al., 1990; Rasse et al., 2006). The aboveground plant residues that accumulated on the soil surface under perennial Miscanthus are incorporated into the soil partly by earthworms (Beuch et al., 2000). In that study, the preharvest losses accounted for 16–34% of the total aboveground biomass and additional losses during harvest amounted to 6–23% (Beuch et al., 2000). Elsewhere, the absence of soil tillage resulted in lower SOM decomposition rates and slower C transport into deeper horizons (Clifton-brown et al., 2007). In our study, therefore, the C4-C (35% of the total C4-C down to 1 m depth) mainly accumulated at 0–10 cm depth after 21 years, which is three times more than after 9 years (Fig. 1). Although Miscanthus roots can penetrate down to 3 m depth, the main root mass of Miscanthus is concentrated within the upper 60 cm (Monti & Zatta, 2009; Christensen et al., 2016). Root growth in the Bw horizon of our loamy Gleyic Cambisol was restricted because of the oxygen limitation. Accordingly, C4-C decreased markedly below 50 cm compared to the topsoil (Fig. 1). Nonetheless, up to 33% of total Miscanthus-derived C
accumulated below 50 cm after 21 years. The input of C_{4}-C may reflect fine root turnover, particle-mediated translocation, and leaching of Miscanthus-derived organic matter derived from the upper soil (Hansen et al., 2004). Based on the contribution of Miscanthus-derived C to SOM at different depths 9 and 21 years after land-use change, we simulated the changes in C_{4}-C proportions with depth and time as a 3D figure (Fig. 3). The proportion of C_{4}-C in SOM reached about 80% in topsoil 20 years after the C_{3}-C_{4} vegetation change. The incorporation of C_{4}-C in the topsoil was 16 times higher than in the subsoil.

As the SOM increased from 9 to 21 years for both Miscanthus and grassland at 0–10 and 30–60 cm depth, the soil was not at steady state, probably due to ongoing grassland establishment. Therefore, the MRT of SOM cannot be calculated based on the difference in the amount of C_{4}-derived C in Miscanthus soil (Gregorich et al., 1995; Amelung et al., 2008). However, based on the C_{3}-C decrease in Miscanthus soil between 9 and 21 years, the MRT of old C_{3}-C (>9 years) was estimated at 19 years (Fig. 2). A similar MRT of SOM in the top 10 cm (16.8 years) was calculated based on δ^{13}C value changes after 12 years of Miscanthus cover at the same site (Blagodatskaya et al., 2011). To our knowledge, the present study is the first to estimate the turnover of old C based on direct application of a repeated ^{13}C natural abundance approach. This proved that C in the upper 50 cm was still active even after more than 9 years, whereas old C below 50 cm was relatively stable. The average MRT of old C_{3}-C down to 1 m was around 60 years, which is similar to the approximately 50 years assessed in literature reviews (Amelung et al., 2008; Schmidt et al., 2011). The results for ‘old’ C are remarkably similar to those for C_{4} (i.e., ‘new’) C in C_{3}-C_{4} vegetation change studies. This indicates that the processes determining the fate of soil C are similar irrespective of C age: High turnover in the topsoil leads to relatively short MRT for both the ‘old’ and the ‘new’ C (Flessa et al., 2000). The results from our repeated ^{13}C natural abundance approach call for caution when assessing SOM turnover based on single sampling.

To compare the MRT of old C_{3}-C under Miscanthus, we analyzed the effects of land-use change (to Miscanthus cultivation) on new C sequestration and old C decomposition from 12 studies with 41 observations (Figs 4 and 5). The average total SOM changes in cropland and grassland converted to Miscanthus were 6.4 and 0.4 mg C ha^{-1} yr^{-1}, respectively (Figs 4a and 6). As SOM under grassland is higher than under cropland, the conversion to Miscanthus from grassland generally resulted in modest C losses at establishment stages because of the disturbance of native or restored ecosystems (Anderson-Teixeira et al., 2009). The C losses will remain until the new C input reaches a level that restores the initial losses, over a period of decades (Gur- gel et al., 2007; Schneckenberger & Kuzyakov, 2007). In contrast, Miscanthus growing on former cropland (C-depleted) sequesters C and thus increases the soil C stock (Fig. 6). These results indicate that the potential for C sequestration under Miscanthus largely depends on the previous land use (Dondini et al., 2009). The variation of total SOM rates of change in the first 5 years after planting Miscanthus was very high, ranging from -4 to 7 mg C ha^{-1} yr^{-1} (Fig. 4b). A similar finding was reached elsewhere for the first 2–3 years after Miscanthus planting: -6.9 to 7.7 mg C ha^{-1} yr^{-1} (Zimmerman et al., 2011). The variation of annual SOM change decreased with time and was negligible after 15 years (Fig. 4b). Miscanthus establishment in the first few years is strongly affected by soil properties and environmental conditions (Lewandowski et al., 2003). This causes changing patterns of C partitioning within the plant and soil, and influences the SOM content after land-use conversion (Anderson-Teixeira et al., 2009). Thus, the precision of overall SOM change estimates increases with the duration of Miscanthus growth.

The SOM derived from Miscanthus increased with time in the topsoil (Fig. 5d): 2% of total SOM was replaced by C_{4}-C each year. In the reviewed literature, C_{4}-SOM sequestration in the topsoil was 1.0 ± 0.1 mg C ha^{-1} yr^{-1} from cropland and 0.7 ± 0.1 mg C ha^{-1} yr^{-1} from grassland (Fig. 4c). However, the C_{4}-C accumulation in our study was 1.8 mg C ha^{-1} yr^{-1}, nearly two times higher than the average results from the literature review. The higher accumulation of Miscanthus-derived C in top 30 cm is...
explained by the restricted root growth in the Bsw horizon of loamy Stagnic Cambisol because of the oxygen limitation. These variations in C₄-C sequestration rates are mainly caused by the soil texture and climate at different experimental sites (Schneckenberger & Kuzyakov, 2007; Poeplau & Don, 2014).

The variation of MRT of SOM in the first 5 years after Miscanthus planting was very high (50–300 years; Fig. 5). Shortly after land-use changes, the variation in ¹³C abundance is always very high and results in variable MRT estimations. On the other hand, the MRT calculation was based on the important assumption that the total SOM was under steady state. The high variation of total SOM turnover rates in the first 5 years (Fig. 4b) indicated strong SOM disturbance caused by Miscanthus planting. We therefore excluded the MRT results from the initial 5 years and found a very stable level of SOM turnover thereafter (Fig. 5). Remarkably, the MRT of SOM was constant and was independent of vegetation change period. The MRT of SOM was around 60 years, after land-use change from both cropland and grassland to Miscanthus, which is comparable to values of around 50 years found elsewhere (Amelung et al., 2008; Schmidt et al., 2011). The MRT of the ‘old’ C determined in our study (41 years at 0–30 cm) is also similar to these literature results for SOM turnover, which shows the remarkably similar turnover of ‘new’ and ‘old’ C. A long-term incubation experiment proved that C losses during recycling of microbial biomass are much lower than C losses during initial metabolism of available substrates (Basler et al., 2015a,b). In the context of our results, this indicates that after initial assimilation

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of added substrate, the processes determining the fate of soil C are similar irrespective of C age. This corroborates the idea that the actual turnover of soil C is much faster than the turnover based on C3-C4 vegetation changes because C dynamics are dominated by recycling processes rather than C stabilization in soil (Gleixner et al., 2002; Basler et al., 2015a,b).

Our literature review shows that the average total SOM changes from conversion of cropland and grassland to Miscanthus were 6.4 and 0.4 mg C ha\(^{-1}\), respectively. The Miscanthus-derived C replaced 2% of the existing SOM in topsoil per year. The MRT of SOM at 0–30 cm depth was relatively stable (~60 years) independent of the duration of Miscanthus cultivation. Globally, growing Miscanthus on C-poor soils (e.g., degraded cropland) will provide immediate SOM sequestration.

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References

Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

**Figure. S1** Soil organic carbon content under *Miscanthus* cultivation (red) and the reference grassland (green) at 2003 (dotted line) and 2015 (solid line). *P* values from the three-way ANOVA are as follows: land-use type (grassland vs. *Miscanthus*), *ns*; years, *P* < 0.001; depth, *P* < 0.001; land-use type × years, *ns*; land-use type × depth, *ns*; years × depth, *P* < 0.001; land-use type × years × depth, *ns*. *ns* indicate no significant effect. The error bars indicate standard error (n = 3).

**Figure. S2** Left: Regressions between soil organic carbon and total nitrogen after 21 years of *Miscanthus* cultivation (red) and under reference grassland (green). Right: Regressions between the logarithm of C content and soil δ¹³C after 9 and 21 years of *Miscanthus* cultivation (blue and red), as well as under the reference grassland (green). Brown lines at top: location of points within the A₅w, A₅h, B₅w, and B₅hC₅w horizons.