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Carbon sequestration and turnover in soil under the energy crop *Miscanthus*: repeated ^{13}C 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 C_3 grassland to the C_4 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 δ^{13}C signature of soil C. For the first time, we used a repeated ^{13}C natural abundance approach to measure C turnover in a loamy Gleyic Cambisol after 9 and 21 years of *Miscanthus* cultivation. This is the longest C_3–C_4 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 C_3-C increased strongly with depth.

Keywords: ^{13}C natural abundance, C_3–C_4 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 CO_2 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 ^{14}C radiocarbon dating, ^{14}C or ^{13}C labeling (e.g., bomb ^{14}C, δ^{13}C after free-air CO_2 enrichment (FACE), δ^{13}C after C_3–C_4 vegetation changes, tracer application), budget approaches, changes of SOM pools after land-use conversion, and

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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 (Dondini *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 (Poeplau *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 (Poeplau & 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 (Dondini *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 $\delta^{13}C$ analysis were collected in April 2003 and October 2015, corresponding to cultivation periods of 9 and 21 years, respectively. Grassland plots adjacent to the *Miscanthus* fields (about 20 m distant) were used as the C_3 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 $\delta^{13}C$ 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 C_3–C_4 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 C_3 (f_{C3}) and the C_4 (f_{C4}, *Miscanthus* derived) sources to total SOM were calculated according to Amelung *et al.* (2008):

$$f_{C_4} = (\delta^{13}C_t - \delta^{13}C_3)/(\delta^{13}C_4 - \delta^{13}C_3) \quad (1)$$

$$f_{C_3} = 1 - f_{C_4} \quad (2)$$

where $\delta^{13}C_t$ is the $\delta^{13}C$ value of the soil under *Miscanthus* and $\delta^{13}C_3$ is the $\delta^{13}C$ value of the corresponding layer in the reference soil (grassland). $\delta^{13}C_4$ was calculated based on the $\delta^{13}C$ value of *Miscanthus* (roots) and corrected for isotopic fractionation during humification by subtracting the differences between $\delta^{13}C_3$ of C_3 vegetation and $\delta^{13}C_3$ of SOM of the C_3 soil. This approach assumes equal isotopic fractionation during humification of C_3 plants and C_4 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 C_3-derived C in *Miscanthus* soil and the amount of C_3-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:

$$\mathrm{MRT} = 1/k = -t/\ln(1 - f_{C_4}) \quad (3)$$

where k stands for the turnover rate, t for the number of years after vegetation change, and f_{C4} for the proportional contribution of the C_4 (*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 C_3–C_4 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' C_3-C can be calculated according to Eqn (3) from the decrease of C_3-C in *Miscanthus* soil. Here, we use the term 'old' C for the C originating from preceding C_3 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 $\delta^{13}C$, 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, C_4-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 δ^{13}C

Miscanthus cultivation and the input of C_4-derived C strongly increased δ^{13}C values at all depths relative to the reference grassland ($P < 0.05$; Fig. 1). The δ^{13}C values increased with depth from −28.4 to −24.8‰ in the grassland soil, but decreased from −23 to −24‰ (9 years) and from −18 to −24‰ (21 years) under *Miscanthus*. The δ^{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 δ^{13}C values was found after 21 years, namely, a strong δ^{13}C increase down to 50 cm (between −18 and −23‰). 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).

The contribution of C_4-derived C after 9 years of *Miscanthus* was 27% in the A_h horizon. This amount of C_4-C was about 10 times higher than that in the B_wC_w horizon ($P < 0.05$). However, between 9 and 21 years after the vegetation change, the portion of C_4-C increased more than four times in the A_h horizon ($P < 0.05$), whereas no increase was recorded in the B_wC_w horizon (Fig. 2).

Mean residence time of C

The MRT of old C (>9 years), based on the changes in δ^{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_hB 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 B_{sw} and B_wC_w 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.

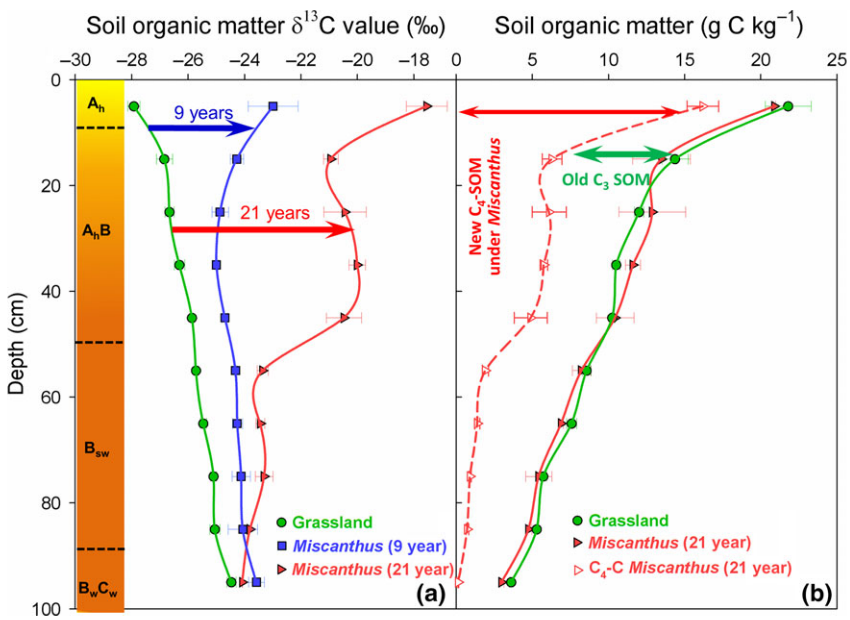

Fig. 1 Left: Soil organic matter δ^{13}C values down the soil profile after 9 and 21 years of *Miscanthus* cultivation (blue and red) and under the reference C_3 grassland (green). *P* values from the two-way ANOVA are as follows: treatments (grassland, 9 years, and 21 years *Miscanthus*), $P < 0.001$; depth, $P < 0.001$; treatment × depth, $P < 0.001$. Blue and red arrows: δ^{13}C value changes after 9 and 21 years of *Miscanthus* plantation, respectively. Right: Total (solid line) and *Miscanthus*-derived (dotted line) soil organic carbon (C_4-C) content after 21 years of *Miscanthus* cultivation (red) and under the reference grassland (green). *P* values from the two-way ANOVA are as follows: treatments (grassland and 21 years *Miscanthus*), *ns*; depth, $P < 0.001$; treatment × depth, *ns*. *ns* indicates no significant effect. Red and green double-headed arrows: the portion of new C_4-C and old C_3-C under *Miscanthus*, respectively. The error bars indicate standard error ($n = 3$). The yellow bar shows the horizons of the soil profile: A_h, A_hB, B_{sw} and B_wC_w.

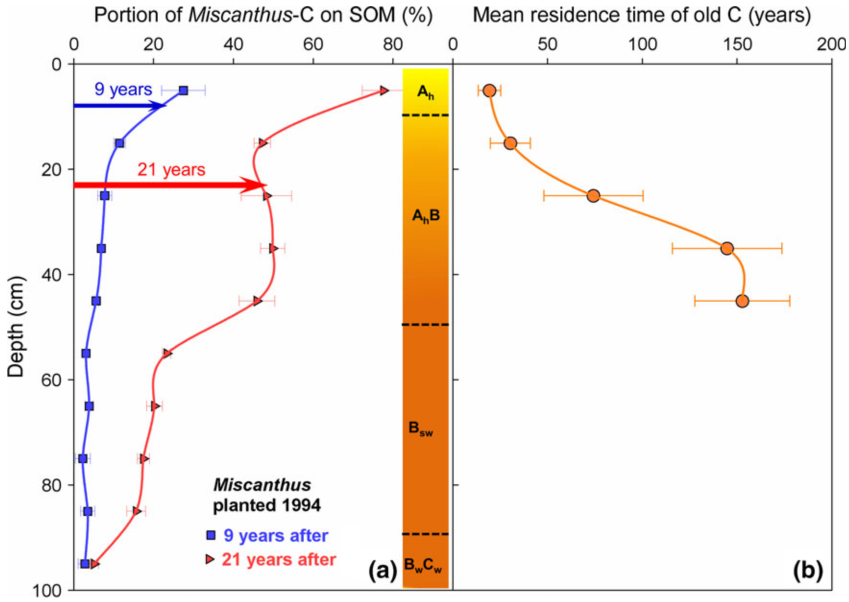

Fig. 2 Left: Portion (±SE, *n* = 3) of *Miscanthus*-derived C in SOM at 0–100 cm depth after 9 and 21 years of *Miscanthus* cultivation. *P* values from the two-way ANOVA are as follows: treatments (9 and 21 years), $P < 0.001$; depth, $P < 0.001$; treatment × depth, $P < 0.001$. The data after 9 years of *Miscanthus* were recalculated from Schneckenberger & Kuzyakov (2007). Right: Mean residence time of old C under *Miscanthus* cultivation. Here, we use the term 'old' C for the C originating from C_3 vegetation (>9 years prior). With no significant decrease of C_3-C below 50 cm depth from 9 to 21 years, we did not calculate the MRT at these depths. Blue and red arrows on the left: the portion of C_4-C after 9 and 21 years of *Miscanthus* cultivation, respectively. Yellow bar: soil profile horizons A_h, A_hB, B_{sw} and B_wC_w.

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 C_4-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 C_4-SOM with depth from the A_h, to A_hB, B_{sw}, and B_wC_w horizons correlated with C_4-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 C_4-C incorporated into SOM is located in the A_h horizon after 21 years under *Miscanthus* (Fig. 1). This is consistent with the 42.6% of C_4-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 C_4-C (35% of the total C_4-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 B_{sw} horizon of our loamy Gleyic Cambisol was restricted because of the oxygen limitation. Accordingly, C_4-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.

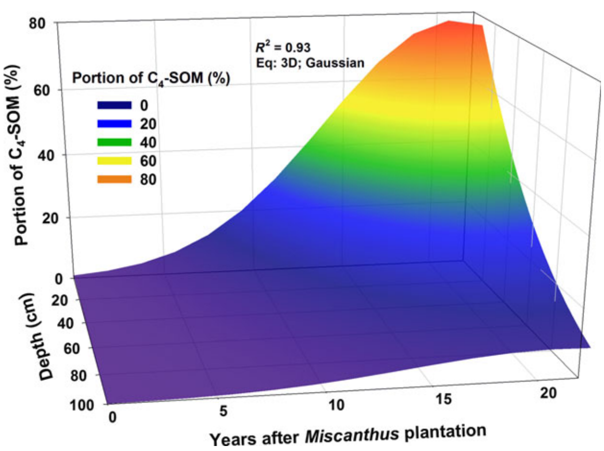

Fig. 3 The contribution of *Miscanthus* (C_4) derived C within 100 cm soil depth over 21 years. The figure was modeled based on field data from 9 and 21 years of *Miscanthus* cultivation. Dark blue, light blue, green, yellow, and red represent the increased proportions of C_4-SOM within 100 cm soil depth under *Miscanthus*.

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 ha^{-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 (Gurgel *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

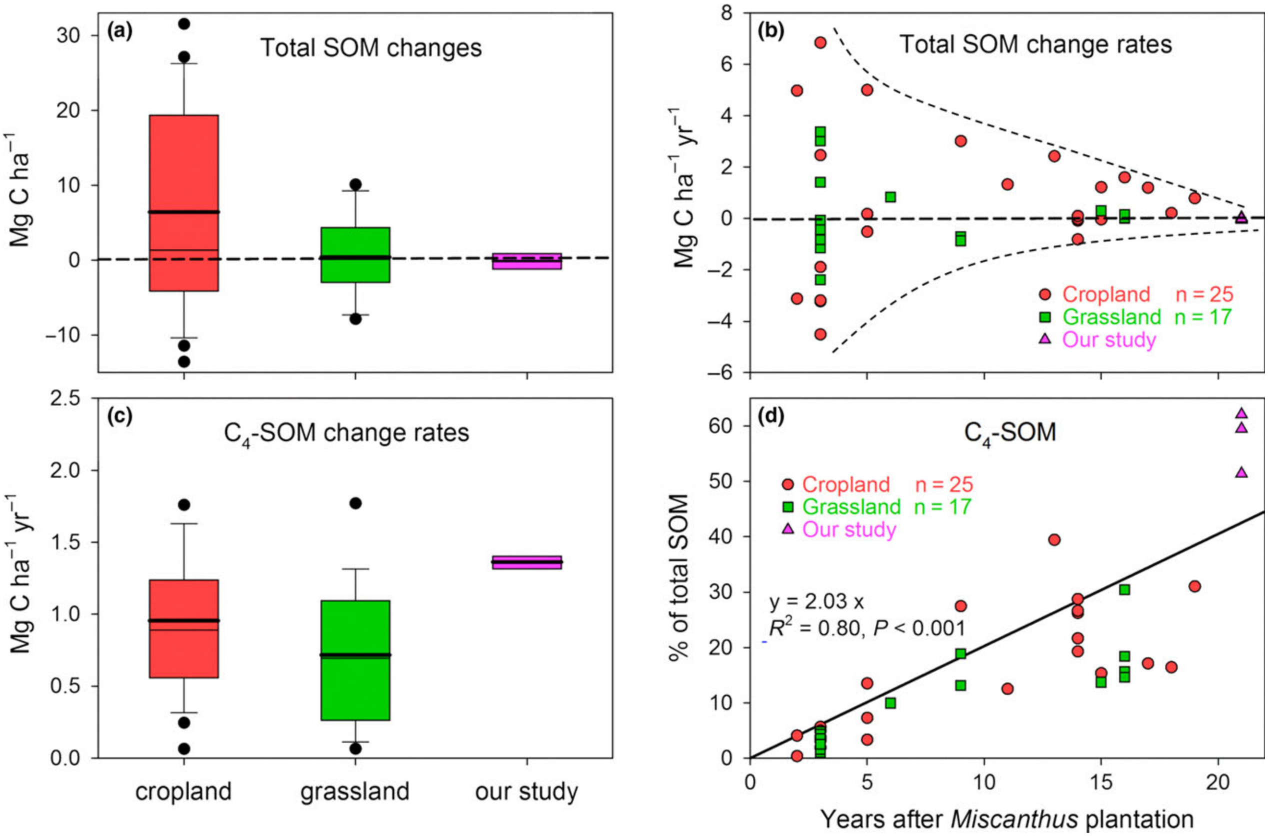

Fig. 4 Results of the literature review on total soil organic C and C_4-C changes in topsoil after conversion of former C_3 grassland (green) or C_3 cropland (red) to *Miscanthus*. Topsoil means 0–20 or 0–30 cm soil depths. The bars in Fig. 5a show the total SOM changes for conversion from cropland (red) or grassland (green) to *Miscanthus* based on 41 observations, as well as in our study (pink). Black line in Fig. 5b: trend of total SOM rates of change with *Miscanthus* cultivation time. Bars in Fig. 5c: C_4-SOM rates of change after conversion from cropland (red) or grassland (green) to *Miscanthus* based on the literature, as well as our study (pink). Black line in Fig. 5d: trend of C_4-C changes with *Miscanthus* cultivation time. In Fig. 5a,c: the black points are outliers beyond the 10th and 90th percentiles; the boxes are 25th and 75th percentiles; central thin horizontal line represents median, and central bold horizontal line represents mean. No significant effect of previous land use type on total SOM changes was observed based on the Kruskal–Wallis ANOVA ($P > 0.05$). This figure is based on the literature review (Hansen *et al.*, 2004; Clifton-brown *et al.*, 2007; Schneckenberger & Kuzyakov, 2007; Dondini *et al.*, 2009; Felten & Emmerling, 2012; Zimmermann *et al.*, 2012; Cattaneo *et al.*, 2014; Poeplau & Don, 2014; Zatta *et al.*, 2014; Richter *et al.*, 2015; Christensen *et al.*, 2016; Ferchaud *et al.*, 2016). For details of data selection, see text.

explained by the restricted root growth in the Bsw horizon of loamy Stagnic Cambisol because of the oxygen limitation. These variations in C_4-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 ^{13}C 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

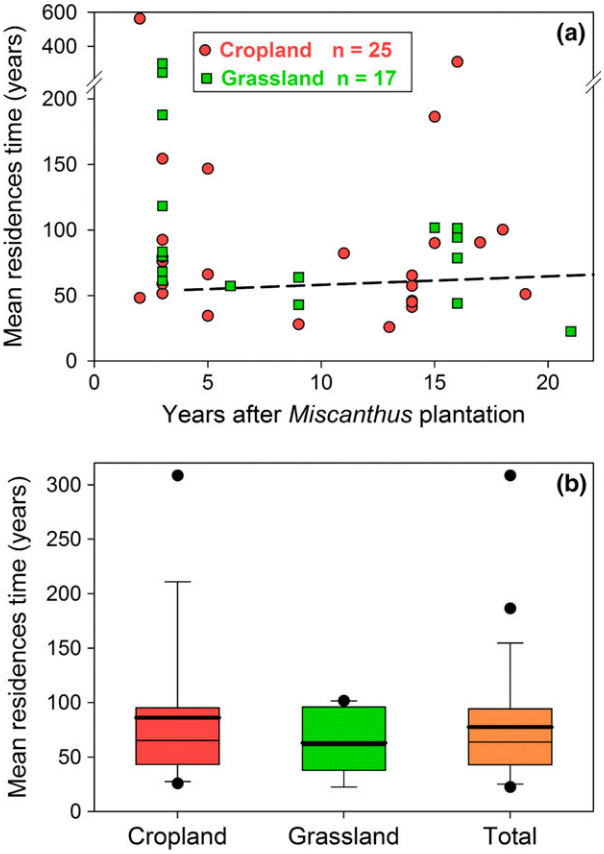

Fig. 5 Changes in mean residence time of soil organic matter in topsoil (0–20 or 0–30 cm) after vegetation change from cropland (red) or grassland (green) to *Miscanthus* (>5 years) according to the literature review (41 observations). Black line: trend of MRT of SOM with *Miscanthus* cultivation time (>5 years). No significant effect of previous land use type on MRT of SOM was observed based on the Kruskal–Wallis ANOVA ($P > 0.05$). This figure is based on our study and the literature review (Hansen *et al*., 2004; Clifton-brown *et al*., 2007; Schneckenberger & Kuzyakov, 2007; Dondini *et al*., 2009; Felten & Emmerling, 2012; Zimmermann *et al*., 2012; Cattaneo *et al*., 2014; Poeplau & Don, 2014; Zatta *et al*., 2014; Richter *et al*., 2015; Christensen *et al*., 2016; Ferchaud *et al*., 2016). For details of data selection, see text.

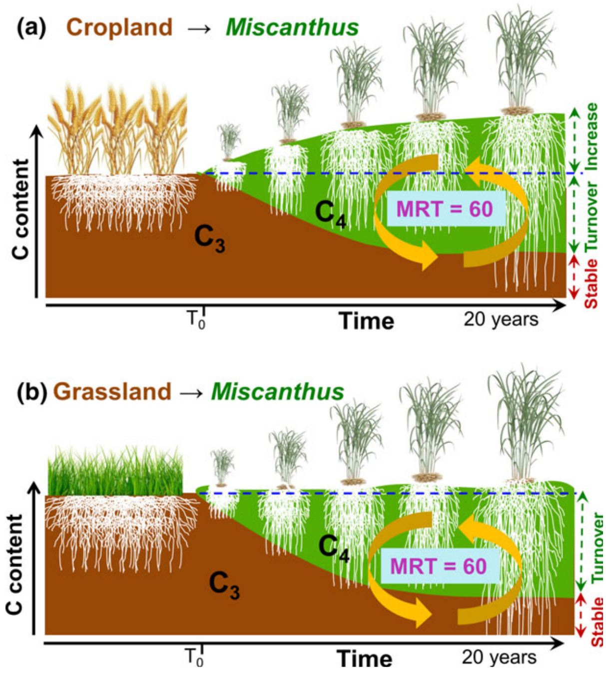

Fig. 6 Schematic representation of total soil organic C and C_4-C changes in topsoil after conversion of former C_3 cropland (a) or C_3 grassland (b) to *Miscanthus*. The *green color* of soil organic C represents the contribution of *Miscanthus*-derived C_4-C, and the *brown color* represents the C derived from C_3 cropland or grassland. The *yellow circle* shows the mean resident time of soil organic matter under *Miscanthus*, which was around 60 years. T_0 represents the starting point of land-use change to *Miscanthus*.

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 C_3-C_4 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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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 $\delta^{13}C$ 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 A_h, A_hB_w, B_{sw} and B_wC_w horizons.