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RESEARCH ARTICLE

# ORGANIC CARBON STOCKS IN A SILTY TEXTURED SOIL FOLLOWING REINTEGRATION OF A 20 YEARS OLD *MISCANTHUS* × *GIGANTEUS* SITE INTO A CROP ROTATION

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**KEYWORDS**

biobased energy crops, C balance, humus accumulation

**HIGHLIGHTS**

- 0.98 $Mg \cdot ha^{-1} \cdot yr^{-1}$ $C_{org}$ accumulation under miscanthus over 26 years.
- $C_{org}$ accumulation under miscanthus continued even up to 26 years.
- Reintegration of a miscanthus site into a crop rotation induced decreasing C stocks at first after 6 years.

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**GRAPHICAL ABSTRACT**


**ABSTRACT**

*Miscanthus* × *giganteus* may play an important role in replacing fossil energy resources by bio-based alternatives. One further advantage of miscanthus production is the generally high soil organic carbon ($C_{org}$) enrichment in soils. Due to declining yields, miscanthus stocks are commonly reintegrated into crop rotation after approximately 20 years. Currently there is only few information, whether these high amounts of $C_{org}$ can be conserved while intensifying soil tillage and crop management after reintegration. Therefore, we monitored $C_{org}$ stocks in a control with more than 20 years of continuous miscanthus and in a treatment with reintegration of a 20-years old miscanthus stock into an organic crop rotation. Based on $\delta^{13}C$ soil values, we calculated an annual $C_{org}$ enrichment of 0.98 $Mg \cdot ha^{-1} \cdot yr^{-1}$ C under miscanthus. More than 95% of the miscanthus-C was determined in the upper 0.25 m of soil. Continuing miscanthus cultivation did not affect yields during the first five extension years and $C_{org}$ stocks increased further. Following reintegration, $C_{org}$ stocks remained constant during five years, which was mainly attributed to the humification and/or stabilization of high amounts of destroyed roots and rhizomes. A significant decrease in $C_{org}$ (−5.7 $Mg \cdot ha^{-1}$ C) compared to the continuing miscanthus cultivation was at first measured six years after reintegration into crop rotation, underlining the need of long-term investigations. Our data also show, that miscanthus production cycles can be extended in our region, and that sowing of the alfalfa grass mixture after rhizome/root destruction was efficient in preserving $C_{org}$ stocks for at least first five years after reintegration.



## 1 INTRODUCTION

*Miscanthus* × *giganteus* is a perennial member of the family Poaceae being a sterile triploid hybrid that was brought from Japan to Europe by Ollson in 1953[1] (referred to simply as miscanthus for the purposes of this paper). To date, this is the origin of most stocks of miscanthus used for agriculture in Europe[2]. Under favorable European climatic conditions, miscanthus can reach a height of 4 m with a correspondingly high biomass production[3]. When compared to other species of sweet grasses, the high biomass production of miscanthus is mainly an effect of its C4-metabolism. In contrast to C3-plants, a decisive advantage of the C4-carbon fixation at high temperatures is that plants are able to perform photosynthesis although stomata are closed[4]. Thereby C4-plants are able to generate an up to 10 times higher growth rate than C3-plants[5]. C4-plants show higher $^{13}C$-$CO_2$ uptake, increasing the $\delta^{13}C$ value of the plant and crop residues[6], which then can be used to determine the source of soil organic carbon ($C_{org}$)[7].

As a result of the high potential for biomass production, there is increasing interest in miscanthus production in terms of replacing fossil with biobased resources[8]. Miscanthus belongs to the low input crops, N fertilization is generally only practiced in the phase of crop establishment. It is mainly used as an energy crop for combustion or for biogas production. It needs two complete vegetation periods for establishment[9] and 3–5 years to full yield development[10]. During the vegetation period, highest yield in Germany was observed between end of September and beginning of October; between 30% and 50% of the above ground biomass can be lost until harvest for combustion purposes (in Germany usually in February/March) due to leaf fall and senescence [9,11] and dehydration over the winter months[12]. At temperate climate conditions, yields between 8 and 30 $Mg \cdot ha^{-1}$ DM can be expected for harvests in spring[2,11,13,14].

In times of a changing climate, C-cycling is a key determinant of atmospheric carbon dioxide, which is the most important greenhouse gas. Worldwide $CO_2$ accounted for 73.9% of greenhouse gas emissions in 2018[15]. Within the C-cycle, soils have a central role with about 2500 Pg C being bound in the soil matrix down to a depth of 2 m which is about twice the amount of C in the atmosphere[16]. As summarized by Söderstöm et al.[16], 12% of the soil C stock is present in cultivated soils which cover about 35% of the global land surface. Consequently, every measure that reduces C loss from agricultural soils potentially disburdens atmospheric $CO_2$ concentrations.

Beside its use as a biobased resource, and in comparison with other C4- and C3-energy crops, miscanthus shows further major advantage of positive humus balances[17]. According to Agostini et al.[18], the annual accumulation of organic C under miscanthus can vary largely between 0.7 and 2.2 Mg·ha$^{-1}$ C.

Miscanthus yield tends to decrease after about 20 years, therefore, reintegration into annual cropping is proposed after this production duration[19,20]. In a meta-analysis of 97 data sets, Guo and Grifford[21] reported a C loss of 59% when pasture soils were converted into arable land use systems. Despite the potentially different quality and the resulting different stability of C stored under miscanthus when compared to grassland, a high C loss can also be expected after reintegration of the miscanthus field into a crop rotation. Dufossé et al.[22] measured a release of 1.5 Mg·ha$^{-1}$ $CO_2$ in the first year following the removal of a 20 years old miscanthus stock. Determining the $^{12}C/^{13}C$ signature of $CO_2$ released after miscanthus removal, Drewer et al.[23] found that the $^{12}C/^{13}C$ signatures changed one year after miscanthus removal from signatures indicating miscanthus as main $CO_2$ source toward signatures from the succeeding C3 crops. They, therefore, claimed investigations covering a longer period than only one year after removal.

Although C losses can be expected after reintegration of a miscanthus field into crop rotation, high amounts of roots and rhizomes may serve as a potential source for humus reproduction offsetting C loss in the first year after reintegration[22].

The aims of our study were to estimate $C_{org}$ enrichment after 20 years of miscanthus cultivation, and to investigate the effect of reintegration on $C_{org}$ stocks. We therefore conducted a field experiment where we removed 20 years old miscanthus and reintegrated the field into an organic arable crop rotation. We monitored $C_{org}$ stocks over seven years. To assess the potential for $C_{org}$ reproduction in the reintegration treatment, we determined the amount of below-ground biomass (roots and rhizomes) and we also determined hot water extractable C as an indicator for easily available C as substrate for microbial C turn-over and subsequent C losses[24].

We hypothesized that (1) due to the age of the miscanthus, annual $C_{org}$ amounts in the control treatment with miscanthus do not increase anymore over this period, and (2) $C_{org}$ contents in the reintegrated arable system will significantly decrease.

# 2 MATERIALS AND METHODS

## 2.1 Study site

The experimental site was located near Müllheim in southwest Germany (47°49′21′′ N; 7°36′24′′ E, 224 m a.s.l). Soil is a Luvisol[25] with a silty loam texture and a pH of 6.4 in $10^{-2}$ mol·L$^{-1}$ $CaCl_2$. Soil particle size distribution of the stone free soil of the plot experiment site, and the reference site used for determination of the $^{13}C$ background without C4 history (see Section 2.5.4) is given in Table 1.

Miscanthus was planted in the experimental field in 1995 and from 1990 to 1995 we are confident no C4-plants had been grown in this field. No reliable cultivation data are available prior to 1990, however, $\delta^{13}C$ measurements of the reference site (cultivated by the same farmer) indicate that no C4-plants had been cultivated in this field prior to 1990.

**Table 1 Soil particle size distribution of the experimental field and of the reference site**

| Site | Soil depth (m) | Clay (%) | Silt (%) | Sand (%) |
|---|---|---|---|---|
| Miscanthus experiment | 0.0–0.1 | 17.1 | 72.3 | 10.6 |
| | 0.1–0.2 | 17.3 | 72.4 | 10.3 |
| | 0.2–0.3 | 18.1 | 72.3 | 9.6 |
| | 0.3–0.4 | 20.0 | 72.5 | 7.5 |
| | 0.4–0.5 | 20.2 | 72.6 | 7.4 |
| | 0.5–0.6 | n.d. | n.d. | n.d. |
| Reference site | 0.0–0.3 | 14.6 | 76.2 | 9.2 |

Note: n.d., not determine.

## 2.2 Experimental design

In 2015 after 20 years of miscanthus production, we setup a randomized complete block design. The field was divided into four blocks with two subplots each (6 m × 16 m). In one of the subplots, the miscanthus was growing as before (miscanthus treatment), in the second subplot the miscanthus was removed and the plot was reintegrated into an organic arable farming system (reintegration treatment). The subplots were randomized in each block. To avoid shading of the reintegration plots by adjacent miscanthus plants, shadow stripes with the same crop as in the reintegration treatment where included (Fig. 1).

After the miscanthus harvest in 2015, the first samples were taken before a shallow cultivation in the reintegration treatment with a wing share cultivator on August 5, then with a cultivator (0.15 m) and a tiller (in order to destroy the rhizomes) on August 28. In September, an alfalfa-clover-grass mixture (*Medicago sativa*, *Trifolium pratense* and *Lolium perenne*) was sown. Sampling in 2016 was done before the alfalfa clover grass mixture was cut on July 13. After cutting, the plots were plowed and winter wheat (*Triticum aestivum*) sown. After winter wheat harvest soil was left bare during winter 2017/2018, and spring barley (*Hordeum vulgare*) was grown in 2018. In 2018/2019, winter rye (*Secale cereale*) was grown followed by spring barley in 2020. After spring barley, again an alfalfa-clover-grass mixture was sown. Management measures in the reintegration treatment are shown in detail in Table S1.

## 2.3 Weather conditions during the field experiment

The mean annual precipitation in the period before our investigation (1999–2014) was 721 mm·yr$^{-1}$ with an average air temperature of 11.3 °C and with 1782 sunshine hours per year (Fig. 2). Except for 2021, temperature and sunshine hours were higher between 2015 and 2019 when compared to the miscanthus cultivation years 1999–2014. In 2021, temperature was lowest and precipitation was highest within the whole period of investigation. In 2015, the highest annual temperature and the most sunshine hours were recorded, with very low precipitation at the same time. When compared to the period 1999–2014, the average annual temperature was 0.5 °C higher, precipitation decreased by 31.2 mm·yr$^{-1}$, and annual sunshine hours increased by 142.6 h·yr$^{-1}$ during our investigation (mean between 2015 and 2021).

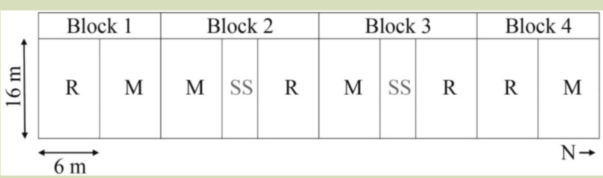


**Fig. 1** Experimental design of the plot experiment (R, plowed for reintegration; M, miscanthus; and SS, shadow strip).

## 2.4 Sample collection and preparation

Except for 2020, sampling took place annually in spring (April/May) after the harvest of the miscanthus. Since the date of harvest differed between the experimental years, soil sampling date varied between end of March and beginning May. Four soil cores were taken from each subplot with a steel cylinder with a Plexiglas inlay. The internal diameter of the 0.65 m long Plexiglas tube was 0.05 m. The inlay was held in the steel tube with a screwable lid and a screwable cutting edge. The steel cylinder was pressed 0.65 m deep into the soil using the tractor’s front hydraulics and implement bar. This corresponded to a filling height of the Plexiglas tube of 0.6 m. An eyelet was screwed into the lid, and the cylinder was removed with a chain attached to the tractor implement bar. Subsequently, the undisturbed soil column was removed and sealed in plastic bags. Thirty-two soil columns were collected each year. Of these, 16 were from miscanthus and 16 from reintegration plots. The cores were stored in the dark at 4 °C until further processing.

The plexiglas columns were grooved on opposite sides with a table saw. The soil core was not disturbed during this process but these grooves allowed the tube to be broken open with a hammer and chisel and for the upper half to be removed. The lower half with the soil column was then placed in a sawhorse, which had slots at 0.05 m intervals. The soil column was then divided into 12 sections with a knife, each 0.05 m heighth with a radius of 0.0275 m. Each section was transferred to an aluminum tray and the moist weight recorded. To determine the dry weight, about 25 g representative of the entire core was dried at 105 °C for 24 h. The remaining soil was stored air-dried and oven-dried immediately before further analysis.

For the determination of total C ($C_t$), $C_{org}$ as well as hot-water soluble C ($C_{hws}$), the samples had to be further crushed ⩽ 2 mm.

The $C_{hws}$, $^{13}C$ and carbonate analyses were performed on composite samples. These were prepared from the four

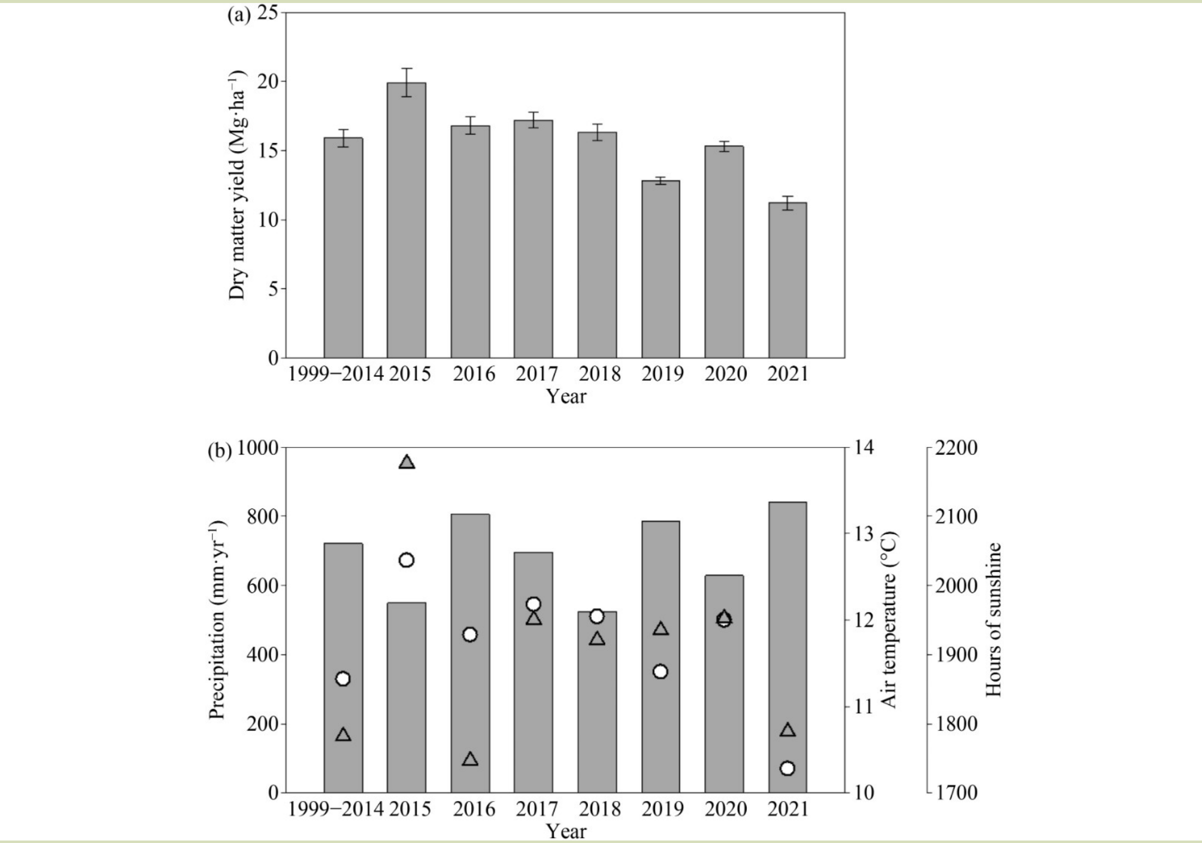


**Fig. 2** (a) Annual miscanthus yield (*n* = 4, mean ± standard error), and (b) average of annual temperature (○), average of annual precipitation (bars) and hours of sunshine per year (Δ) from 1999 to 2021, with 1999–2014 given as a combined mean being the period of miscanthus cultivation before the start of the experiment (between 1995 and 1998 yields were lower, since the crop was not fully established).

replicates within a plot of the same depth. Subsamples of 15 g from each depth and replicate were combined and homogenized.

## 2.5 Laboratory analysis

### 2.5.1 Determination of bulk density

Soil bulk density was determined gravimetrically on subsamples of each core that had been dried at 105 °C overnight.

### 2.5.2 Determination of carbonate C and total C

For the determination of carbonate C, 10–11 g of oven-dried soil was heated in a muffle furnace to 550 °C for 4 h. At this temperature, organic carbon is incinerated whereas inorganic carbonates remain stable[26]. The C concentration of the heated samples was measured using a CN analyzer (VarioMax, Elementar Analysensysteme, Hanau, Germany).

Total C ($C_t$) was measured with the same CN analyzer using 1 g of oven-dried soil. Organic C ($C_{org}$) concentration was then calculated by subtracting the inorganic carbon (carbonate, if detectable) from $C_t$.

Due to the soil pH of 6.4, we did not expect carbonate C to be present at our site. Nevertheless, we irregular found carbonate up to 0.11% in 2015 in few top soil samples (0.05 m) and attributed it to a liming measure in 2014. Therefore, carbonate determination was repeated in 2016. Here we used the first 0.05 m increments in the miscanthus plots and the upper five increments (corresponding to 0.25 m ploughing depth) in the reintegration treatment. In 2016, we could not detect carbonate in any of our samples and we consequently assumed no carbonate to be present in the subsequent samplings. However, mainly for 2015 we cannot exclude the possibility that small amounts of carbonate were present in some top soil samples. Carbonates can differ in their $\delta^{13}C$ value and this might have biased the $^{13}C$ results.

$C_{org}$ stocks were calculated by multiplying $C_{org}$ concentration with the corresponding bulk density of the soil cores. Tillage, as in our reintegration treatment, can lead to changes in bulk density and consequently to biased calculations of the $C_{org}$ stocks when related to fix soil depths. For the comparison of C stocks in soils with different bulk density, Ellert and Bettany[27] proposed the equivalent soil mass method which compares C stocks in a defined soil mass. We calculated the equivalent soil mass, but differences between the calculation of C amounts with fixed depths and the equivalent soil mass method were only minor. On average for the 0–0.6 m depth, only 0.015 m deeper soil profile of the reintegration treatment yielded in the same mass of soil as the miscanthus treatment. We, therefore, decided not to present the equivalent soil mass data in this paper.

#### 2.5.3 Exaction of hot-water soluble C ($C_{hws}$)

Analysis of $C_{hws}$ can be used to obtain an estimate of easily soluble and thus also easily available C in soil[24]. These are simple organic compounds as well as easily depolymerizable hydrocarbons[28]. $C_{hws}$ was determined for samples collected in 2015, 2016 and 2019. For this purpose, 20 g of soil was transferred to a 250-mL flask, mixed with 100 mL of deionized $H_2O$, connected to a reflux condenser and boiled gently for 1 h. The flasks were then stoppered and rapidly cooled in ice water. About 45 mL of the solution were transferred into 50 mL centrifuge tubes and centrifuged at 1800 g for 10 min with the addition of five drops of magnesium sulfate solution. About 20–30 mL of the supernatant was filtered through cellulose membrane filters. The filtrates were stored frozen until assayed using a total C analyzer for liquid samples (multi N/C 2100s, Analytik Jena, Germany).

#### 2.5.4 Analysis of isotopes

For isotope analysis, sieved soil samples were dried at 105 °C and plant samples (leaves, rhizomes and roots) at 60 °C. The $^{13}C$ abundances of the soil and plant samples were measured with an isotope ratio mass spectrometer (Delta Advantage, Thermo, Bremen, Germany) for samples collect in 2015 and 2019.

The isotope signatures were used to calculate the miscanthus-derived fraction of $C_{org}$[29]:

$$C_M = (\delta^{13}C_{\frac{M}{R}} - \delta^{13}C_F) \times \left(\delta^{13}C_{LR} - \delta^{13}C_F\right)^{-1} \times 100 \quad (1)$$

where, $C_M$ is the proportion of miscanthus-derived carbon (%), $\delta^{13}C_{\frac{M}{R}}$ is the $\delta^{13}C$ value in soil from the miscanthus or reintegration treatment (‰), $\delta^{13}C_F$ is the $\delta^{13}C$ value in soil from an adjacent field (reference site) without C4-plants history (‰), and $\delta^{13}C_{LR}$ is the $\delta^{13}C$ value in litter and rhizomes of miscanthus (‰) with $\delta^{13}C$ (‰) = $((^{13}C/^{12}C)_{Sample} \times (^{13}C/^{12}C)_{PDB\text{-}Standard}{}^{-1}) \times 1000$.

For the determination of $^{13}C$ abundance in soil without C4-plant production for at least 25 years, we sampled soil from a comparable reference site under ecological management since 1990 that was only 70 m from our experimental site with a similar soil (Table 1). The $\delta^{13}C$ value of the top soil was −26.3 ‰, the mean $\delta^{13}C$ value of the rhizomes and roots sampled (see Section 2.6) was −12.8‰ and −12.5‰.

## 2.6 Rhizomes and root samples

Root samples were taken on October 11, 2017 from a part within the miscanthus field outside our experimental plots. Initially, three sampling positions were randomly selected. Miscanthus was removed at these positions and soil from 1 $m^2$ was taken up to a depth of 0.3 m and transferred to the laboratory in large bags.

The soil was first soaked in 0.5 $mol \cdot L^{-1}$ sodium chloride for 24 h to suspend soil attached at the roots and rhizomes. The roots and rhizomes were then rinsed over a 5-mm sieve and any remaining soil was removed by hand. The clean roots and rhizomes were spread on aluminum trays and dried at 60 °C for at least 4 days. The dry material was separated into roots, rhizomes and residual soil organic matter, with the latter including partially decomposed rhizomes[30]. The dry matter fractions was then weighed and subsamples were milled for C analysis.

## 2.7 Statistics

Single-year data were analyzed using the mixed model:

$$\bar{y}_{ijk} = \mu + b_k + t_i + \tau_j + (t\tau)_{ij} + (bt)_{ik} + e_{ijk} \quad (2)$$

where $\bar{y}_{ijk}$ is the mean value per horizon $i$ in block $k$ and treatment $j$ across the four column samples, $\mu$ is the intercept, $b_k$ is the fixed effect of block $k$, $t_i$ is the fixed effect of horizon $i$, $\tau_j$ is the fixed effect of treatment $j$, $(bt)_{ik}$ is the fixed effect of block $k$ within horizon $i$, $(t\tau)_{ij}$ is the fixed interaction effect of treatment $j$ and horizon $i$, and $e_{ijk}$ is the error of $\bar{y}_{ijk}$. A first order autoregressive variance-covariance structure with heterogeneous horizon-specific variances and an additional nugget variance was assumed. To reduce convergence problems, the same variance was assumed for horizons below 0.3 m.

Cumulative data across horizons, and thus data that was measured once per plot and year, were analyzed across years using the following model:

$$y_{jkl} = \mu + a_l + b_{kl} + \tau_j + (a\tau)_{jl} + e_{jkl} \quad (3)$$

where $y_{jkl}$ is the cumulative value across horizons at treatment $j$ in block $k$ and year $l$, $\mu$ is the intercept, $a_l$ and $(a\tau)_{jl}$ are the fixed effects of year $l$ and its interaction effect with treatment $j$, $\tau_j$ is the fixed effect of treatment $j$, and $e_{jkl}$ is the error of $y_{jkl}$ with a first order autoregressive variance-covariance structure with year specific variance. The variance-covariance structure was simplified to a first-order autoregressive variance-covariance structure with homogeneous variances or a compound symmetry structure if this decreases the AIC[31]. For both models, homogeneous variances (except the heterogeneity already fitted within the model) and normal distribution of residuals was checked graphically via residual plots. In case of deviations, a logarithmic transformation was used. In this case, means were back-transformed for presentation purpose only. These back-transformed means were denoted as medians. Standard errors were back-transformed using the delta method. Depending on the results of the global F tests, multiple comparisons were done using fishers LSD test. Results from multiple comparisons were shown via letter display[32]. Additionally, simple means of treatment-by-horizon combinations were calculated. In 2015, no reintegration treatment existed, thus the model was simplified by dropping all effects including the treatment.

# 3 RESULTS

## 3.1 Miscanthus yield

Highest miscanthus yield during the experiment was 19.9 Mg·ha$^{-1}$ DM in 2015 (Fig. 2). Over the following years, yields were largely similar to those between 1999 and 2014. The exceptions were 2019 and 2021, when yields were lower than expected. Average yield between 2015 and 2021 was 3 Mg·ha$^{-1}$ DM lower when compared to the period between 1999 and 2014. The lower mean yield was mainly the result of the low yield in 2021. In this year, yield was ~5 Mg·ha$^{-1}$ DM below the average value of the years 1999–2014.

## 3.2 Bulk density and $C_{org}$ concentration in the miscanthus and reintegration treatments

Figure 3 shows exemplarily the bulk density, $C_{org}$ concentrations and the corresponding $C_{org}$ amounts of the two treatments for 2019, the reminder data on bulk density and $C_{org}$ concentrations as well as $C_{org}$ amounts in the other years are shown in supplementary Figs. S1–S3. Except for the first depth (0–0.05 m), the miscanthus treatment had a higher bulk density than the reintegration treatment in the 0.05–0.3 m depth. The lowest bulk densities of all treatments were determined in the first depth (0–0.05 m) with 1.08 Mg·m$^{-3}$ for the miscanthus treatment. This was significantly lower when compared to the bulk density in the same depth in the reintegration treatment (1.25 Mg·m$^{-3}$) ($p = 0.0004$). The largest differences between the treatments occurred in the upper 0.25 m. With increasing depth, the bulk density of both treatments increased. In the reintegration treatment, plow compaction became apparent at about 0.25 m deep with an increase in bulk density from 1.47 to 1.56 Mg·m$^{-3}$. We did not find any significant differences between miscanthus and reintegration in bulk density below the plowed horizon, neither in 2019 (Fig. 3) nor in one of the remaining years (Fig. S1).

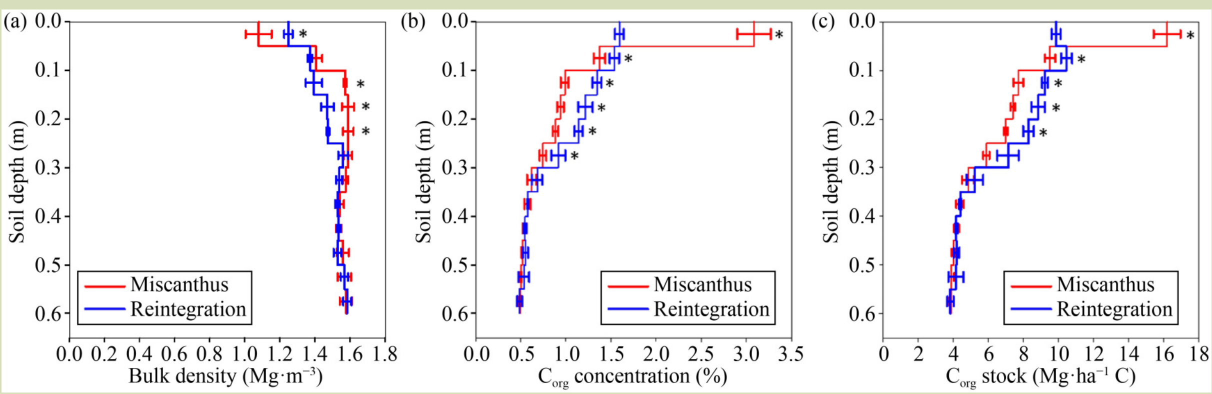


**Fig. 3** Bulk density (a), $C_{org}$ concentration (b), and mean $C_{org}$ stock (c) ($n = 4$, mean ± standard error) as a function of soil depth and treatment in 2019. Asterisks indicate statistically significant differences between the means of the treatments within a depth (LSD test, α = 0.05).

The $C_{org}$ concentration decreased with depth in both treatments (Fig. 3). In the upper 0.05 m, the $C_{org}$ concentrations of both treatments were highest. With 3.09% $C_{org}$ the miscanthus treatment had a significantly higher $C_{org}$ concentration ($p < 0.0001$) than the reintegration treatment (1.59%). Significant differences in $C_{org}$ concentration were found in 0.05–0.3 m with no deviation between the two treatments below.

Carbon distribution in the profile was affected by soil tillage (Fig. 3 and Fig. S3). After the shallow tillage to kill roots and rhizomes (~0.1 m) in summer 2015, the $C_{org}$ distribution in the reintegration treatment differed from the miscanthus treatment in the upper 0.1 m in 2016. Plowing of the alfalfa-clover-grass mixture after our 2016 sampling resulted in even incorporation to 0.3 m in 2017. In the following years, the redistribution to depth remained similar.

## 3.3 Total C stocks and C distribution

After 20 years of miscanthus production, the initial $C_{org}$ stock (0–0.6 m) at the beginning of our measurements was 75.4 $Mg \cdot ha^{-1}$ C (Table 2). About 66% of the $C_{org}$ was located in the upper 0.3 m.

From 2015 to 2021 (except 2020), mean C stocks down to 0.6 m increased by 4.8 $Mg \cdot ha^{-1}$ C in the miscanthus treatment (Table 2). A decrease of 0.9 $Mg \cdot ha^{-1}$ C was observed in the reintegration treatment during this period with these differences being significant in the uppermost 0.3 m. Except for 2019, the miscanthus treatment had higher mean C stocks than the reintegration treatment. These differences were statistically significant in 2018 and 2021 (Table 2).

For the temporal dynamics of the $C_{org}$ stocks in the 0–0.3 m depth in miscanthus, we found a significant increase from 50.2 $Mg \cdot ha^{-1}$ C in 2016 to between 53.3 and 54.4 $Mg \cdot ha^{-1}$ C in the following years (Table 2).

The lowest $C_{org}$ stock (0–0.3 m) in the reintegration treatment was 48.6 $Mg \cdot ha^{-1}$ C in 2021. In the first 3 years after miscanthus removal (2016, 2017 and 2018) $C_{org}$ stocks remained unaffected. The $C_{org}$ stocks increased in 2019, the year which had the highest mean $C_{org}$ stocks (54.0 $Mg \cdot ha^{-1}$ C) within the reintegration treatment.

## 3.4 Proportions of miscanthus-derived C to total C

The $\delta^{13}C$ values in the miscanthus treatment in 2015 and 2019 varied between −14.7‰ and −24.2‰ (Fig. 4). Generally, in both years, the highest $\delta^{13}C$ values and thus the greatest impact of miscanthus cultivation on soil $C_{org}$ was found in the upper 0–0.25 m. The highest $\delta^{13}C$ value of −14.7‰ was determined in the upper 0.05 m under miscanthus in 2019. The values dropped to about −19.0‰ in 0.05–0.1 m and further decreased to about −22.5‰ in 0.1–0.2 m. Below 0.25 m, the $\delta^{13}C$ values shifted toward the isotopic signature of $C_{org}$ from C3-plants.

Reintegration resulted in relative even portions of miscanthus-C within the plow horizon (0–0.25 m) in 2019 (Fig. 4). The $\delta^{13}C$ values there ranged between −19.4‰ and −20.9‰. Similar to the miscanthus treatment, values below 0.25 m deep were lower (ranging between −22.3‰ and −24.6‰) indicating higher portions of non-miscanthus-C.

The highest accumulation of C4 carbon was observed in the 0–0.05 m depth (Fig. 5). Miscanthus contributed 80% (2015) and 84% (2019) to the total $C_{org}$ stocks of this depth, corresponding to 10.2 and 13.6 $Mg \cdot ha^{-1}$ C. At a depth of 0.05–0.1 m, the C4-C fraction accounted for 50% in 2015 (4.6 $Mg \cdot ha^{-1}$ C) and 51% in 2019 (4.9 $Mg \cdot ha^{-1}$ C) of the total C in this depth. In the reintegration treatment, the C4-C proportions were distributed evenly in the upper 0.25 m. The

**Table 2** $C_{org}$ stocks ($n$ = 4, mean ± standard error) of miscanthus and reintegration treatment

| Treatment | Depth (m) | $C_{org}$ ($Mg \cdot ha^{-1}$ C) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 2015* | 2016** | 2017*** | 2018 | 2019 | 2021 |
| Miscanthus | 0–0.6 | $75.4^{ns}$ (1.0) | $75.4^{ns}$ (4.5) | $79.3^{ns}$ (2.2) | $80.4^{ns}$ (3.1) | $79.2^{ns}$ (1.2) | $80.2^{ns}$ (1.1) |
| Reintegration | 0–0.6 | $75.4^{ns}$ (1.0) | $75.9^{ns}$ (4.5) | $75.1^{ns}$ (2.2) | $75.7^{ns}$ (3.1) | $80.1^{ns}$ (1.2) | $74.5^{ns}$ (1.1) |
| Miscanthus | 0–0.3 | $50.2^{A,b}$ (0.8) | $50.2^{A,a,b}$ (3.4) | $53.7^{A,a,b}$ (2.2) | $54.4^{A,a,b}$ (3.1) | $53.8^{A,a,b}$ (0.6) | $53.3^{A,a}$ (0.5) |
| Reintegration | 0–0.3 | $50.2^{A,a,b}$ (0.8) | $49.7^{A,a,b}$ (3.4) | $48.7^{A,a,b}$ (2.2) | $51.6^{A,a,b}$ (3.1) | $54.0^{A,a}$ (0.6) | $48.6^{B,b}$ (0.5) |

Note: Mean values with the same capital letter within a year or with the same lowercase letter within the time course of a treatment are not significantly different (LSD test, α = 0.05). ns, not significant. *Sampling before tillage in the reintegration treatment; **first sampling after shallow tillage in the reintegration treatment; ***first sampling after plowing in the reintegration treatment.

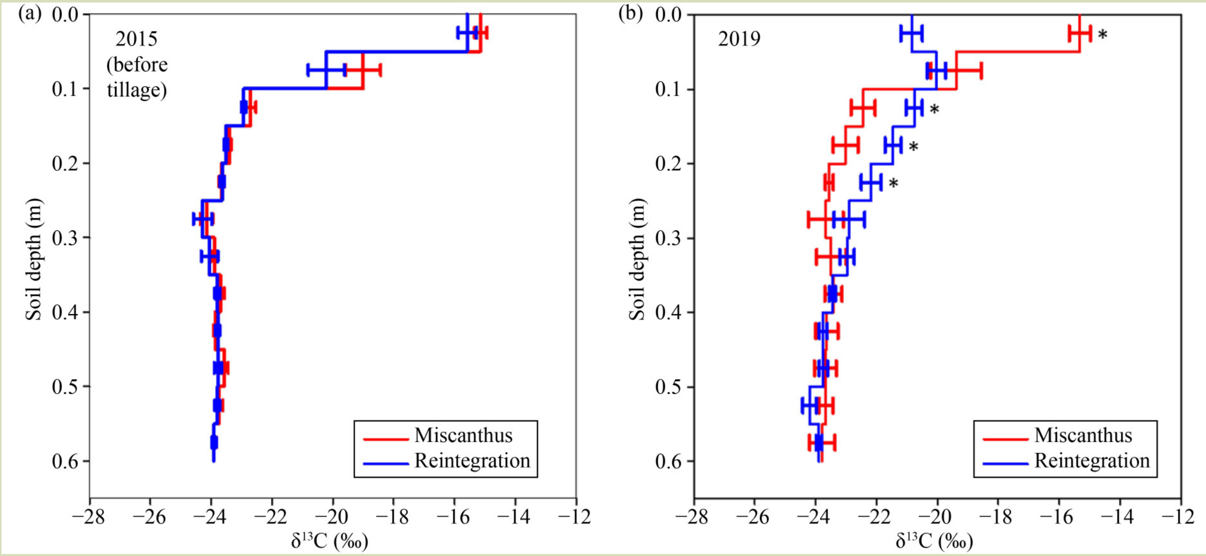


**Fig. 4** $\delta^{13}C$ values ($n$ = 4, mean ± standard error) of miscanthus and reintegration treatment as a function of soil depth in 2015 (a) and 2019 (b). Asterisks indicate statistically significant differences between the means of the treatments within a depth (LSD test, α = 0.05).

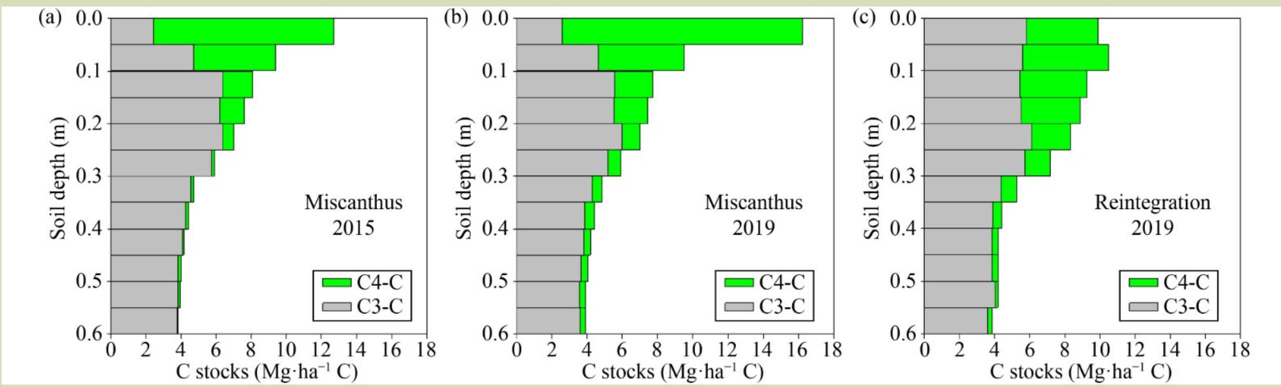


**Fig. 5** Mean C4- and C3-C stocks ($n$ = 4) of the miscanthus treatment in 2015 (a), of the miscanthus treatment in 2019 (b), and of the reintegration treatment in 2019 (c).

C3 stocks were proportionally larger, also in comparison with the initial values determined in 2015. In 2019, C3-C stocks were 5.5 Mg·ha$^{-1}$ C higher in the reintegration treatment than in the miscanthus treatment (0–0.6 m). Main differences were found in the 0–0.05 m depth (3.2 Mg·ha$^{-1}$ C, more C3-C).

Over the 0.6 m depth, we determined 19.6 Mg·ha$^{-1}$ C4-C in the miscanthus treatment in 2015 (Fig. 5). More than half of this $C_{org}$ (10.2 Mg·ha$^{-1}$ C corresponding to 52.7%) was found in the upper 0.05 m. About a quarter of the C4-C was located in soil 0.05–0.10 m deep (23.9%). Over 95% of the total C4-C in both treatments was determined in the 0–0.25 m depth.

For the prior 20 years of production of miscanthus, the 19.6 Mg·ha$^{-1}$ C4-C corresponded to an annual increase of the $C_{org}$ pool of 0.98 Mg·ha$^{-1}$·yr$^{-1}$ C.

## 3.5 Effect of the treatments on hot-water soluble C ($C_{hws}$)

$C_{hws}$ accounted for between 3% and 6% of the total C ($C_{hws}$) in the top soil (0–0.3 m) and for between 1% and 2.5% in deeper soil (data not shown). Similar to the $C_{org}$ content, $C_{hws}$ was the highest in the 3 years of determination in soil depths with the highest $C_{org}$ stocks (compare Fig. 3 and Fig. 6). In 2016 and

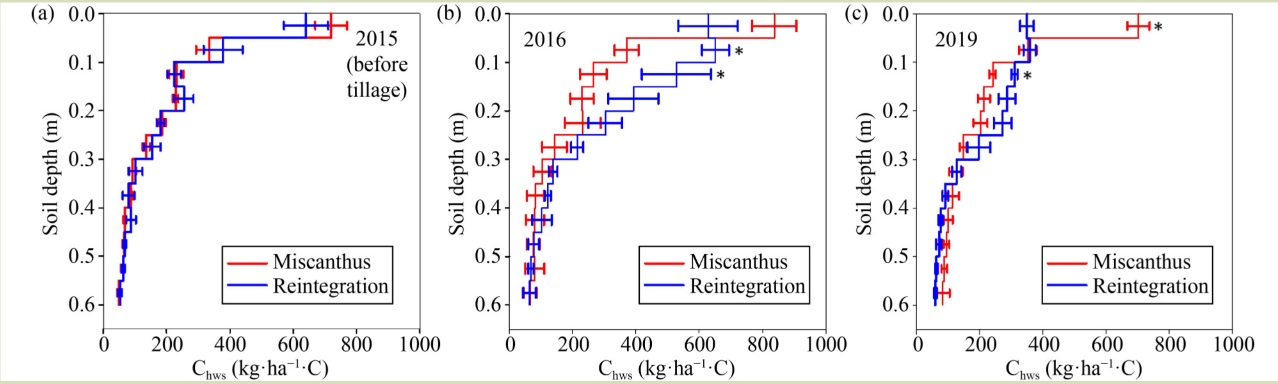


**Fig. 6** $C_{hws}$ stocks ($n$ = 4, mean ± standard error) as a function of soil depth and treatment in 2015 (a), 2016 (b) and 2019 (c). Asterisks indicate statistically significant differences between the means of the treatments within a depth (LSD test, α = 0.05).

2019, the reintegration treatment had lower $C_{hws}$ contents in the 0–0.05 m depth than the miscanthus treatment ($p$ = 0.0008). The decrease in the reintegration treatment from 2015 to 2019 of almost 56% in the upper 0.05 m was particularly notable. In contrast, at the depth of 0.05–0.3 m the reintegration treatment had higher $C_{hws}$ content than the miscanthus in both years. This was also statistically confirmed in 2016 for the 0.05–0.15 m depth ($p \leqslant 0.0001$, both depths) and in 2019 for the 0.1–0.15 m depth ($p$ = 0.0255). In contrast, in the reintegration treatment there was an increase from 2293 to 3293 kg·ha$^{-1}$ $C_{hws}$ between 2015 and 2016.

### 3.6 Rhizomes and roots

In October 2017, a mean dry mass of 10.6 Mg·ha$^{-1}$ (ranging between 8.8 and 13.6 Mg·ha$^{-1}$) was determined for miscanthus roots at a depth of 0–0.3 m. The corresponding dry mass of the rhizomes was 15.2 Mg·ha$^{-1}$ (ranging between 13.6 and 17.4 Mg·ha$^{-1}$) (data not shown).

Roots had lower $C_t$- and higher $N_t$-concentrations than the rhizomes (Table 3). Simultaneously the spatial distribution (standard error) in the roots was particularly high. In total, 3.83±0.08 Mg·ha$^{-1}$ C were found in the roots, and 6.63±0.07 Mg·ha$^{-1}$ C in the rhizomes. Due to the strongly different C amounts and the almost identical N amounts, the C/N ratio between roots and rhizomes varied significantly.

## 4 DISCUSSION

### 4.1 Yield development during time of measurement

In contrast to the decreases in yield of miscanthus after 15–20 years described in literature[19,20,22], these initially remained constant in our study. One reason for the constant yields could be the different weather conditions during our measurement period, compared to the years 1999–2014. During measurements, on average there were higher temperatures and more sunshine hours, with only slightly less precipitation. The fact that higher temperatures and water availability have a beneficial effect on the yield of miscanthus was also reported by Gauder et al.[33]. However, Lesur et al.[34] mentioned, that to date, there are no clearly identified monocausal factors explaining yield stability of miscanthus. They reported from miscanthus experiments that gave no yield decrease after more than 20 years whereas yields in other fields had a strong decreased during this time.

The lower yields in 2019 at our site can be explained by the

**Table 3** Total C ($C_t$) concentrations, C/N ratio, and C amounts in roots and rhizomes (0–0.3 m depth) on Nov. 10, 2017 ($n$ = 3, mean ± standard error)

| Plant parts | $C_t$ (%) | C/N | C (Mg·ha$^{-1}$) |
|---|---|---|---|
| Roots | 37.0±3.1 | 50.1±16.5 | 3.83±0.9 |
| Rhizomes | 43.6±0.5 | 94.8±6.2 | 6.63±0.6 |

very low rainfall during the growing season in 2018. As noted by Lewandowski et al.[9], water availability can often be a limiting factor for the growth of miscanthus. A first indication of decreasing yield could be the low yield in 2021, 26 years after planting, with only 11.2 Mg·ha$^{-1}$ DM. In the previous year, weather conditions were quite favorable, and no environmental influence was observed that might have had a negative impact on yield.

With 15.2 Mg·ha$^{-1}$ DM, the rhizome dry matter was identical to the dry matter determined by Amougou et al.[19] at a site in northern France (15.5 Mg·ha$^{-1}$ DM). With a dry matter of 10.6 Mg·ha$^{-1}$ DM, roots were almost one-third less than the dry matter of rhizomes. Although root dry matter was lower than rhizome dry matter, amounts measured in our study were high. In Neukirchen et al.[35], root dry matter of 4.4 Mg·ha$^{-1}$ DM was found over a sampling depth of 0.3 m and Amougou et al.[19] determined only 1.8 Mg·ha$^{-1}$ DM. Reasons for the considerable differences in dry matter could be different site conditions and the substantially younger miscanthus stocks of 2–3 years[19] and 3–5 years[35]. Miscanthus and thus a distinct root system, are not fully developed until 3–5 years[10] and site conditions can have a major effect on rooting capacity[36].

## 4.2 Development of $C_{org}$ stocks during extended miscanthus production

Since decreasing yields were expected at the beginning of the study, lower miscanthus-C input to the soil was assumed. However, given the constant miscanthus yields observed, further constant resupply of $C_{org}$ from preharvest and harvest losses, as well as from dead roots and rhizomes was observed[37]. With regard to the development of the total $C_{org}$ stocks over the measurement period, an increase was determined from 2017 onwards. This was also statistically confirmed over a depth of 0.3 m in comparison to 2015 and 2021. In 2021, a total increase of 4.1 Mg·ha$^{-1}$ $C_{org}$ was observed compared to 2015. Of this C, 82% were found in the upper 0.1 m. Considering the observed total amount of about 26 Mg·ha$^{-1}$ DM of roots and rhizomes, it is likely that the dead parts of the rhizomes in particular contributed to the $C_{org}$ supply in addition to the litter layer. This is consistent with the observations of Neukirchen et al.[35], who reported that the rhizomes were located mainly in the upper 0.15 m of soil. Felten & Emmerling[38] also assumed that dead and regenerating root and rhizomes contributed a large degree to $C_{org}$ supply.

In line with the initial hypothesis that $C_{org}$ stocks would not increase further, it was expected that the $C_{org}$ stocks of miscanthus treatment would remain at a stable level due a steady-state of C4-C accumulation and C4-C depletion. However, the partially significant increases in C already indicated a continuous accumulation of C4-C stocks. This was confirmed by an increase of C4-C stocks in 2019. The increase contradicts the assumption of a steady-state. The accumulation of C was obviously higher than C-depletion. One reason for the C4-C accumulation could be that some of the miscanthus-C accumulated in stable fractions of the soil. Poeplau & Don[39] noted that in addition to the accumulation of C4-C in the easily available fraction, an amount of C4-C was associated with silt and clay particles through organic-mineral interactions. Thus, this portion of C4-C is stabilized and protected against mineralization processes[40]. Due to the high proportions of silt and clay in the soil of the experimental site, the storage capacity for stabilized C may not have been reached suggesting that stabilized portion may also be responsible for the accumulation of C. With increasing sampling depth, C4-C stocks decreased, consistent with the natural distribution of humus[38,39]. The main accumulation occurred in the upper 0.1 m of soil, this was also reported by Poeplau & Don[39] and by Schneckenberger et al.[41]. In our study, an increase of 3.6 Mg·ha$^{-1}$ C was observed within the upper 0.1 m of soil over a 4-year period. As summarized by Agostini et al.[18] in their meta-analysis, average annual $C_{org}$ accumulation in soils under miscanthus cultivation is about 1.2 Mg·ha$^{-1}$·yr$^{-1}$ C. Beuch et al.[37,42] reported a range between 2 and 4.5 Mg·ha$^{-1}$·yr$^{-1}$ C. Since C stocks increased by about 1 Mg·ha$^{-1}$·yr$^{-1}$ C until 2019, it can be assumed that the miscanthus stock was not in yield depression during that time. In contrast to our hypothesis, neither miscanthus yield nor C accumulation decreased after 15–20 years, and we have to reject this hypothesis, at least for the first 24 years of miscanthus production at our study site.

Amounts of hot-water extractable C under miscanthus did not change over time. However, it can be assumed, that some of the miscanthus-derived inputs have been degraded despite the higher C4-C accumulation. According to Poeplau and Don[39], a large portion of the miscanthus-derived C accumulates in an easily available C fraction (particulate organic matter). Also in our study, a large portion of the easily available C was miscanthus-derived C. This was indicated by the accumulation of about 80% of $C_{hws}$ stocks in the upper 0.3 m. In the miscanthus treatment there was no C3 plant input during the past 26 years, and consequently C3 contribution to $C_{hws}$ stocks between 2015 and 2019 seemed to be negligible. Thus, only minor degradation processes occurred in the C3 pool, as indicated by the same share of C3-C in the miscanthus samples of 2015 and 2019. According to Lorenz et al.[43], this was to be expected because the mean residence time and stability of $C_{org}$

increases with depth which was confirmed by increased C3-C portions with depth.

### 4.3 Development of $C_{org}$ stocks during reintegration

Guo & Gifford[21] showed in their meta-analysis that conversion of pasture to cropland results in average $C_{org}$ losses of 59%. Carbon stocks of permanent grassland are similar to the stocks under miscanthus[44]. Therefore, we also assumed high C losses after reintegration due to tillage practices and other arable land use. Comparing miscanthus with the reintegration treatment, we found only a significant decrease in C stocks of 4.7 Mg·ha$^{-1}$ C in the reintegration treatment in 2021 in the 0–0.3 m depth. When compared to the initial C stocks in 2015, total C loss over the measurement period was only 1.6 Mg·ha$^{-1}$ C. This discrepancy could be explained by temporary stable C stocks between 2016 and 2019.

One reason for stable $C_{org}$ stocks in 2016 and 2017 could be the decomposition of the remaining miscanthus-derived roots and rhizomes, but also of the litter that remained on the field after harvest in 2015. We did not determine litter C, but Agostini et al.[18] reported amounts of about 2 Mg·ha$^{-1}$·yr$^{-1}$ C. Due to the two cultivation measures in August 2015 and plowing in September 2016, rhizomes, roots and litter were incorporated and contributed to humus reproduction. This was also assumed by Dufossé et al.[22], who reported a trend of increase in C stocks one year after reintegration of a 20-year-old miscanthus stock. The C amounts of the below-ground biomass (roots and rhizomes) in our case were 10.46 Mg·ha$^{-1}$ C at a sampling depth of 0–0.3 m. Dufossé et al.[22] reported a similar C input of 9.1 Mg·ha$^{-1}$ C in below ground biomass at a sampling depth of 0–0.2 m. The assumption that humus reproduction and thus building up of $C_{org}$ stocks were fueled through the humification of roots, rhizomes and litter after soil tillage in the context of reintegration was also supported by an increase in hot-water extractable C amounts in 2016 (3.29 Mg·ha$^{-1}$ $C_{hws}$). As noted by Franko[45], $C_{hws}$ is an indicator for easily available C from young organic matter.

The still high $C_{org}$ amounts in 2018 may also be the result of high root biomass incorporation from the alfalfa clover grass mixture. As demonstrated by Li et al.[46], alfalfa promoted higher soil organic C accumulation than alfalfa-grass mixtures, and thus the high portion of 25% alfalfa in the mixture might have also contributed to this phenomenon.

The amount of C3-C in the 0–0.1 m depth increased from 38% in 2015 to 56% in 2019, indicating that the increase in $C_{org}$ stocks was also a result from young (C3-C) organic matter from crops and residues emerging after miscanthus cultivation (alfalfa-clover-grass, wheat, barley and rye).

One possible explanation for the higher C stocks in 2019 and the significantly lower stocks in 2021 could also be the weather conditions in 2018. The low precipitation combined with high temperature could have reduced the degradation of C in 2019[47]. In particular, higher rainfall in the subsequent 2 years (2019 and 2020) may have amplified the decrease in C stocks until 2021. The expected decrease in C stocks after reintegration was significant first in 2021, suggesting that when miscanthus is reintegrated into an organic arable crop rotation, $C_{org}$ losses occur later or to a lesser extent when compared to conversion of pasture to arable land.

Carbon stocks in the upper 0.1 m of soil decreased by 6.9 Mg·ha$^{-1}$ C until 2017, but in deeper soil, this C stocks increase simultaneously by 5.8 Mg·ha$^{-1}$ C, indicating a redistribution of C through soil tillage. A similar redistribution of C after conversion of pasture to arable land use was also reported by Don et al.[48]. In the current study, a soil redistribution effect was still evident over the entire measurement period. Lower $C_{org}$ stocks were found in the first 0.05 m in the restoration reintegration treatment compared to the miscanthus treatment. However, significantly higher stocks were found in the 0.1–0.25 m depth of the reintegration treatment. In 0.3–0.6 m depth, there were no more differences. Corresponding to Lorenz & Lal[43], this could be expected, because the mean residence time of slowly-degrading plant components increases with soil depth. Thus, the $C_{org}$ stocks of subsoil are more stable and changes, if any, emerge only on the long-term.

## 5 CONCLUSIONS

After 20 years of miscanthus production, yield remained constant for at least four more years, indicating that miscanthus does not necessarily decline in yield after 20 years and that longer crop cycles are possible in our study region.

The cultivation of miscanthus shows a very high potential for the accumulation of soil organic C with about 1 Mg·ha$^{-1}$·yr$^{-1}$ C. Stable C stocks in the first years after destruction of roots and rhizomes show, that sowing of alfalfa clover grass mixture was an efficient strategy to reintegrate the former miscanthus field into arable farming system.

The main problem for C accumulation and potential C losses after reintegration seems to be the shallow storage of the young miscanthus C with over 70% in the uppermost 0–0.1 m depth. This means that even systems with shallow tillage affect about 70% of the miscanthus C during soil management measures thus stimulating the turnover, degradation and potential loss of organic C. A single deep ploughing of the highly C enriched top soil, below the common tillage depth after miscanthus removal should be tested as a measure to preserve this C in the soil system in future studies.

Cultivation of miscanthus revealed a high potential for the enrichment of soil organic C with approximately 1 $Mg\cdot ha^{-1}\cdot yr^{-1}$ C. Stable $C_{org}$ stocks in the first years after destruction of roots and rhizomes showed, that sowing of alfalfa clover grass mixture was an efficient strategy to re-integrate the former miscanthus field into arable farming system. However, the reduction in $C_{org}$ stocks in the 6th year after reintegration clearly indicates, that a reliable assessment of the effect of reintegration on $C_{org}$ stocks after miscanthus removal measures needs more long-term investigations.

### Supplementary materials

The online version of this article at https://doi.org/10.15302/J-FASE-2023485 contains supplementary materials (Table S1; Figs. S1–S3).


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### Compliance with ethics guidelines

Lisa Essich, Reiner Ruser, Jens Hartung, Anne Hanemann, Meike Gassner, Liam Oberdorfer, Jörn Breuer, Jürgen Recknagel, Helmut Nußbaumer, and Torsten Müller declare that they have no conflicts of interest or financial conflicts to disclose. This article does not contain any studies with human or animal subjects performed by any of the authors.

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