Received: 7 January 2021 | Revised: 31 March 2021 | Accepted: 28 April 2021

DOI: 10.1111/gcbb.12861

GCB-BIOENERGY Bioproducts for a sustainable bioeconomy — Open Access

WILEY

ORIGINAL RESEARCH

Accumulation of C4-carbon from *Miscanthus* in organic-matter-rich soils

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Abstract

To evaluate the sustainability of biomass plantations, effects on soil organic carbon (SOC) need to be quantified. *Miscanthus* × *giganteus* is increasingly used as a bioenergy plant, and it has been hypothesized that, after conversion from cropland, *Miscanthus* cropping increases SOC storage, whereas conversion from grassland to *Miscanthus* provides, on average, no sequestration. All field studies hitherto were carried out on mineral soils with topsoil SOC contents of below 3.3%. Here, we analyze in the temperate zone of Switzerland five sites that have been cultivated with *Miscanthus* for 19–24 years and of which four sites are higher in topsoil SOC content (4.7%–16.2%) and storage (188–262 t SOC) than any previously studied *Miscanthus* plantation in Europe. We used the difference in carbon isotopic signature between C4 (*Miscanthus*) and neighboring plots with C3 vegetation (grassland) to quantify the accumulation of new SOC from *Miscanthus* down to 0.75 m. Annual C4-C accumulation rates were 1.66 (standard error ± 0.14) t C4-C ha^{-1} $year^{-1}$ (range: 1.26–2.01) in the upper 0.3 m of soil and 1.96 (±0.18) t C4-C ha^{-1} $year^{-1}$ (1.40–2.38) in 0–0.75 m. Average rates for 0–0.3 m were higher than those of mineral soils ($n = 37$) published previously (0.96 [±0.10] t C4-C ha^{-1} $year^{-1}$). However, high rates of C4-C accumulation were also reported previously for some mineral soils. Nevertheless, the one mineral soil in our study did not reveal a systematically different accumulation of *Miscanthus*-derived carbon compared with the four carbon-rich soils. We therefore conclude that soils rich in organic matter do not show a different C4-C accumulation pattern as compared with mineral soils. However, their C4-C accumulation rates are at the upper end of the data ensemble. Our results further underpin that conversion to *Miscanthus*, despite C4-C accumulation, provides no means to increase soil carbon stocks relative to grassland management.

KEYWORDS

bioenergy crop, biomass plantation, Cambisol, carbon sequestration, Gleysol, Histosol, *Miscanthus*, Switzerland, $\delta^{13}C$

1 | INTRODUCTION

Miscanthus × *giganteus* is a perennial grass currently cropped on approximately 25,000 ha in Europe and is hence the most widely used bioenergy crop after maize (Calderon et al., 2019). The plant can provide a harvestable aboveground productivity of more than 20 t dry matter (d.m.) per hectare even without additional fertilization (Zhuang et al., 2013) and is also suitable for being cropped on economically marginal land (Ouattara et al., 2021). Its dense root and rhizome network of between 8.5 and 15.1 t d.m. ha^{-1} deliver also high belowground inputs of up to 5 t d.m. ha^{-1} $year^{-1}$ (Christensen et al., 2016; Zatta et al., 2014).

As a plant with a C4 photosynthetic pathway, *Miscanthus* produces tissue carbon and, finally, soil organic matter (SOM) with a ^{13}C signature that differs from the one of SOM in soils with prevailing C3 vegetation. This characteristic has prompted extensive research on the amount of soil organic carbon (SOC) stemming from *Miscanthus* inputs in C3-dominated soils (Zang et al., 2018), based on the natural ^{13}C abundance method first described by Balesdent et al. (1987). Poeplau and Don (2014) showed, by summarizing *Miscanthus* studies existing at that time, that *Miscanthus*-derived C4-carbon (C4-C) accumulates steadily in soil with annual rates of 0.8 (standard error ± 0.2) t C4-C ha^{-1}. Rates increased with increasing mean annual temperature and increasing stand age. More recently, Zang et al. (2018) reviewed a larger number of studies, which indicated that after a change from cropland to *Miscanthus*, annual C4-C accumulation rates in topsoil horizons of 0–0.3 m depth were 1.0 (±0.1) t C4-C ha^{-1}, but only 0.7 (±0.1) t C4-C ha^{-1} when *Miscanthus* followed grassland. These authors also noted that C4-C accumulation does not equal a net soil carbon sink because it goes along with decomposition of old C3-carbon (C3-C) at the same time, and an increase in total SOC was observed only for conversion from cropland, as also reported earlier by Qin et al. (2016).

All of the studies reviewed by Zang et al. (2018), who compiled the most comprehensive overview to date, were carried out on mineral soils with mean SOC concentrations of 1.80% (±0.10%) in the upper 0.3 m. Mineral soils provide various means of SOC stabilization via formation of aggregates and interaction of SOM with soil minerals (Von Lützow et al., 2006). Consequently, *Miscanthus*-derived carbon does not only accumulate as aboveground and belowground plant litter but becomes part of stable aggregates and of silt and clay size fractions already a few years after establishment of the crop (Poeplau & Don, 2014; Rehbein et al., 2015). So far, accumulation of *Miscanthus*-derived carbon has not been studied in soils with high SOC content, for example, organic soils that formed in peatlands that degrade after conversion to agriculture. Organic soils have naturally high SOC contents and are increasingly used for cropping worldwide (Leifeld & Menichetti, 2018). They can be highly fertile because the peat, after drainage, degrades and releases organically bound nutrients (Wang et al., 2016), which may foster plant productivity also without fertilizer addition. However, owing to their already high SOC content, their potential for accumulating new carbon may be smaller than that of mineral soils due to less available mineral binding sites. It is yet unknown whether soils with high SOM content differ in the accumulation of new C4-C as compared with mineral soils. Here, we evaluate the accumulation of *Miscanthus*-derived carbon at five sites with different SOC content, which are all situated in the same region and exposed to the same climate. At all sites, C3-grassland was grown adjacent to *Miscanthus* that had been introduced 19–24 years before sampling. Furthermore, we analyze whether SOC stocks differ between *Miscanthus* and grassland and compare C4-C accumulation rates with the ones reported in previous studies.

2 | MATERIALS AND METHODS

The five study sites are situated in the southwestern part of Switzerland in the region "Grosses Moos" with widespread occurrence of organic soils that developed after the last glaciation. Four of the sites are located in a former peatland area that has been drained since AD 1864, and a mineral soil has developed on moraine deposits at the fifth site. The mean annual temperature in the region Grosses Moos is 10.0°C, and the mean annual precipitation is 1145 mm (Bader et al., 2018). Details on the sites and their soils are provided in Table 1. One site was classified as organic soil, whereas at the three other sites on former peatland the decomposition of peat was so much progressed that these soils were classified as humic Gleysols. However, these sites contained visible peat remains in their subsoil >0.3 m and had higher SOC contents than the mineral soil (Cambisol, site Uettlingen). We will collectively refer to those four soils with high SOC contents (Histosol, Gleysols) as "organic-matter-rich soils" in this study. All sites have been used for agriculture for at least 150 years.

Each site consisted of a *Miscanthus* field situated adjacent to a neighboring field with C3 grassland serving as reference for our evaluation of the $\delta^{13}C$ signature and SOC storage. Grasslands were established at the same time as *Miscanthus* at three of the five sites, whereas on two reference fields, grassland replaced cropland in 2008 and 2009 (sites Gals and Le Landeron, respectively). The mineral soil sites (both *Miscanthus* and grassland) were fertilized with horse manure once a year, whereas no fertilizer was applied to any of the other *Miscanthus* or grassland sites. Yields of *Miscanthus*, harvested always in early spring, were regularly estimated by the farmers. These estimates were between 12.8 and 14.5 t d.m. ha^{-1}.

TABLE 1 Site overview

Site	Coordinates N, E	Soil type[a]	Soil pH (*Miscanthus*/Reference)	Year of sampling	Age *Miscanthus* (years)	Land use before *Miscanthus*	Crop on reference field
Le Landeron	47.0449, 7.0438	murshic limnic Histosol	7.4/7.4	2014	19	CL	GL (CL until 2009)
Galmiz	46.9500, 7.1429	eutric humic Gleysol	7.2/7.0	2016	22	GL	GL
Gals	47.0179, 7.0413	eutric humic Gleysol	6.9/7.1	2016	24	GL	GL (CL until 2008)
Bellechasse	47.0181, 7.1287	eutric humic Gleysol	7.0/7.1	2016	22	CL	GL
Uettlingen	46.9726, 7.3837	eutric Cambisol	5.6/5.8	2016	22	CL	GL

Note: Soil pH for 0–0.3 m soil depth.

Abbreviations: CL, cropland; GL, grassland.

[a]Following IUSS (2014).

We took four soil cores, 5 cm in diameter, down to 0.75 m on every *Miscanthus* and reference plot. On each field, sampling points had a distance of 25 m to each other and were in approximately 10 m distance to the field margin. As such, each *Miscanthus* core was neighbored by a grassland core approximately 20 m away. The excavated soil cores were cooled, brought to the laboratory, and cut into segments as indicated in Table 2. From each sample, coarse living plant material from roots and rhizomes was carefully removed on a 2 mm mesh and eventual stone content determined before the sample was weighed, dried at 105°C, and weighed again. Fresh sieved soil was measured for pH in 0.01 M $CaCl_2$.

Soil samples were ground and homogenized in a vibrating ball mill (MM 400, Retsch, Germany) and pre-treated with HCl fumigation in a desiccator for 24 h to remove carbonates. Stable carbon isotopes and carbon content were measured with a mass spectrometer combined with an SL elemental analyzer (Integra2, Sercon) following standard processing techniques. Stable carbon isotope ratios are reported as $\delta^{13}C$ (‰) relative to the V-PDB standard. The instrumental standard deviation is 0.1% for $\delta^{13}C$.

We calculated the contribution of *Miscanthus*-derived carbon based on the method of Balesdent et al. (1987). The fraction of C4-C (fC4SOC) using that approach is given as:

$$\text{fC4SOC} = \frac{(\delta^{13}\text{Csoil}_{\text{misc}} - \delta^{13}\text{Csoil}_{\text{ref}})}{(\delta^{13}\text{Cmisc} - \delta^{13}\text{Csoil}_{\text{ref}})},$$

where $\delta^{13}\text{Csoil}_{\text{misc}}$ is the $\delta^{13}C$ signature of the soil carbon from *Miscanthus* sites, $\delta^{13}\text{Csoil}_{\text{ref}}$ is the $\delta^{13}C$ signature of soil carbon from the grassland plot, serving as reference with no C4-C accumulation, and δ^{13}Cmisc is the average $\delta^{13}C$ signature of *Miscanthus* tissue, using the value of −12.4‰ measured for *Miscanthus* stalks and rhizomes by Bader et al. (2017) for site Le Landeron. For all sites, the average isotopic signature of samples from the four grassland replicates of the same depth as the corresponding section from the *Miscanthus* site was used as reference to account for variations in $\delta^{13}C$ with depth that might occur in degrading organic soil (Krüger et al., 2015).

The volumetric sampling of the peat enabled us to determine the soil bulk density. The SOC stocks and the share of C4-C were calculated by carbon content and bulk density for depth increments of each single core. All results are presented on a volumetric basis (0–0.3 and 0–0.75 m) as means ± 1 SE. We analyzed the possible effect of site on C4-C accumulation rates using univariate ANOVA and a post hoc least significant difference test. Soil organic carbon stocks for *Miscanthus* and their respective reference were tested per site for difference by a *t* test. This was done for stocks in 0–0.3 and 0–0.75 m. The C4-C accumulation rates and carbon stocks and concentrations of soils from this study, in 0–0.3 m, were also compared with results from 12 previously published studies encompassing 37 sites in total. Pearson's correlation coefficients were calculated for the correlation between C4-C accumulation rates and two variables, namely, the mean annual temperature and stand age.

For comparing stocks, differences in soil density are often accounted for by using the equivalent soil mass approach (Ellert & Bettany, 1995). This is achieved by subtracting part of the mass of subsoil from those cores with the highest overall mass before calculating SOC stocks to get the same soil masses across replicates and sites. For mineral soils, results are little sensitive to cutting off subsoil layers owing to the relatively small and steadily declining SOC concentration with soil depth. However, four of our studied sites were different from typical mineral soils in that (i) their SOC concentrations were higher in deeper than in shallower layers and overall did not always show the same or any clear trend

TABLE 2 Mean soil organic carbon (SOC) concentrations, soil bulk densities (bd), and $\delta^{13}C$ values of five *Miscanthus* (Misc.) and reference grassland (Ref.) sites

Site	Land use	Sampling depth (m)	SOC (mg g^{-1})	bd (g cm^{-3})	$\delta^{13}C$ (‰)
Le Landeron	Misc.	0–0.1	160.7 (6.4)	0.4 (<0.1)	−24.5 (0.5)
		0.1–0.2	164.9 (6.1)	0.5 (<0.1)	−23.9 (0.4)
		0.2–0.3	150.8 (18.6)	0.5 (0.1)	−25.2 (0.8)
		0.3–0.5	204.2 (21.9)	0.4 (<0.1)	−26.5 (0.2)
		0.5–0.75	378.7 (18.0)	0.2 (<0.1)	−27.1 (0.1)
	Ref.	0–0.1	166.8 (6.4)	0.5 (<0.1)	−26.2 (0.1)
		0.1–0.2	167.9 (6.1)	0.6 (<0.1)	−26.1 (<0.1)
		0.2–0.3	164.8 (5.4)	0.5 (<0.1)	−26.1 (<0.1)
		0.3–0.5	260.9 (15.0)	0.3 (<0.1)	−27.1 (0.2)
		0.5–0.75	281.6 (48.9)	0.2 (0.1)	−27.7 (0.2)
Galmiz	Misc.	0–0.125	77.2 (13.7)	1.1 (0.1)	−24.0 (0.4)
		0.125–0.25	69.3 (13.8)	1.3 (0.1)	−25.9 (0.1)
		0.25–0.50	105.9 (28.1)	1.0 (0.2)	−26.9 (0.1)
		0.5–0.75	34.1 (29.0)	1.7 (0.3)	−26.1 (0.3)
	Ref.	0–0.125	102.2 (13.8)	1.0 (<0.1)	−27.8 (<0.1)
		0.125–0.25	83.2 (11.8)	1.1 (0.1)	−27.3 (0.1)
		0.25–0.50	60.4 (21.3)	1.5 (0.3)	−27.1 (<0.1)
		0.5–0.75	32.0 (28.6)	1.7 (0.3)	−26.4 (0.3)
Gals	Misc.	0–0.125	61.7 (17.4)	1.0 (0.3)	−20.8 (0.2)
		0.125–0.25	41.2 (3.2)	1.7 (0.1)	−22.9 (0.4)
		0.25–0.50	55.7 (9.8)	1.5 (0.1)	−26.2 (0.5)
		0.5–0.75	43.5 (17.1)	1.6 (0.1)	−26.9 (0.3)
	Ref.	0–0.125	47.2 (9.6)	1.4 (<0.1)	−26.9 (0.2)
		0.125–0.25	39.9 (7.2)	1.6 (0.1)	−25.5 (0.4)
		0.25–0.50	50.9 (6.3)	1.6 (0.1)	−27.0 (0.3)
		0.5–0.75	41.6 (12.4)	1.7 (0.1)	−27.2 (0.1)
Bellechasse	Misc.	0–0.125	48.0 (2.2)	1.3 (0.1)	−23.5 (0.3)
		0.125–0.25	43.8 (2.4)	1.3 (0.2)	−24.7 (0.5)
		0.25–0.50	68.0 (17.9)	1.3 (0.2)	−26.3 (0.2)
		0.5–0.75	51.3 (26.1)	1.5 (0.2)	−26.1 (0.3)
	Ref.	0–0.125	58.4 (3.6)	1.2 (0.1)	−27.5 (0.2)
		0.125–0.25	48.3 (2.2)	1.3 (0.1)	−26.9 (0.1)
		0.25–0.50	42.1 (8.0)	1.4 (0.2)	−27.0 (0.1)
		0.5–0.75	18.4 (9.5)	1.6 (0.3)	−26.6 (0.2)
Uettlingen	Misc.	0–0.125	25.1 (2.8)	1.3 (0.1)	−20.0 (0.2)
		0.125–0.25	11.6 (0.3)	2.1 (0.1)	−24.2 (0.3)
		0.25–0.50	4.6 (0.3)	2.1 (0.1)	−25.1 (0.1)
		0.5–0.75	1.5 (0.2)	2.1 (0.1)	−23.9 (0.3)
	Ref.	0–0.125	21.6 (1.2)	1.6 (0.1)	−28.5 (0.2)
		0.125–0.25	10.3 (0.4)	1.9 (0.1)	−27.1 (<0.1)
		0.25–0.50	4.5 (0.8)	2.1 (0.1)	−26.5 (0.3)
		0.5–0.75	1.5 (0.2)	2.2 (0.1)	−25.8 (0.3)

Note: Values in parentheses are 1 SE ($n = 4$).

with depth and (ii) bulk densities in at least some of the cores declined with depth along with the increasing SOC content. Furthermore, these soils continue to subside. Under these conditions, a correction toward the same soil mass by cutting off deeper layers of higher density cores was not considered meaningful.

We compared our results with those of previously published studies on C4-C accumulation with *Miscanthus*. Only publications from Europe were considered to ensure similar climatic conditions. We further restricted the comparison to publications in which C4-C accumulation rates (t C4-C ha^{-1} year^{-1}) for the upper 0.3 m of soil were reported. In total, 12 studies encompassing 37 datasets could be included in the analysis. To obtain values for 0–0.3 m from those of our sites where the original segments were 0–0.25 and 0.25–0.50 m (see Table 2), we added one-fifth of the SOC and C4-C stock of the segment 0.25–0.50 m to the segment 0–0.25 m to obtain an estimate for the layer 0–0.3 m. This simple approach was based on the reasoning that, in our studied soils, SOC does both increase and decrease with soil depth, that is, the directional change within the segment 0.25–0.50 m was considered unknown.

3 | RESULTS

The four sites with organic-matter-rich soils developed on weakly alkaline peat deposits with a pH of around 7, whereas the mineral soil at site Uettlingen was weakly acidic (Table 1). Under both land uses (i.e., *Miscanthus* and grassland), SOC concentrations were the highest in the Histosol (site Le Landeron) and the lowest in the Cambisol (site Uettlingen), with Gleysols (i.e., former Histosols after prolonged drainage) situated in between (Table 2). Soil organic carbon stocks in 0–0.3 m soil depth followed the same order and reached between 188 and 266 t ha^{-1} in Gleysols and the Histosol, but only 71–75 t ha^{-1} in the Cambisol (Table 3). Topsoil carbon storage was significantly different between land uses only in the Histosol ($p < 0.04$), whereas neither for the other topsoils nor for any of the other depth segments significant differences in carbon stocks were observed. Cumulated over the

TABLE 3 Mean soil organic carbon (SOC) storage under *Miscanthus* (Misc.) and grassland (Ref.), fraction of C4-carbon (fC4), amount of C4-carbon (C4-C), and annual C4-C accumulation rates for soil depths of 0–0.3 and 0–0.75 m

Site	SOC (t ha^{-1})	fC4 (-)	C4-C (t ha^{-1})	Rate (t C4-C ha^{-1} year^{-1})
Le Landeron				
Misc.$_{0–0.3}$	204.3 (13.3)*	0.187 (0.028)	38.1 (5.4)	2.01 (0.28)
Misc.$_{0–0.75}$	578.5 (46.5)	0.093 (0.013)	45.2 (4.9)	2.38 (0.26)a
Ref.$_{0–0.3}$	265.5 (18.3)			
Ref.$_{0–0.75}$	571.3 (24.5)			
Galmiz				
Misc.$_{0–0.3}$	261.1 (25.1)	0.139 (0.010)	36.1 (3.7)	1.64 (0.17)
Misc.$_{0–0.75}$	530.5 (31.0)	0.075 (0.009)	38.7 (4.4)	1.76 (0.20)ab
Ref.$_{0–0.3}$	265.5 (22.4)			
Ref.$_{0–0.75}$	447.2 (40.8)			
Gals				
Misc.$_{0–0.3}$	187.5 (6.7)	0.244 (0.017)	45.8 (3.8)	1.91 (0.16)
Misc.$_{0–0.75}$	546.6 (39.3)	0.108 (0.008)	54.6 (5.5)	2.28 (0.23)a
Ref.$_{0–0.3}$	200.4 (26.1)			
Ref.$_{0–0.75}$	504.6 (42.3)			
Bellechasse				
Misc.$_{0–0.3}$	191.8 (13.8)	0.173 (0.021)	33.2 (4.5)	1.51 (0.21)
Misc.$_{0–0.75}$	553.8 (172.6)	0.098 (0.023)	44.0 (5.1)	2.00 (0.23)ab
Ref.$_{0–0.3}$	192.8 (3.5)			
Ref.$_{0–0.75}$	387.7 (32.5)			
Uettlingen				
Misc.$_{0–0.3}$	75.3 (5.5)	0.366 (0.011)	27.7 (2.7)	1.26 (0.12)
Misc.$_{0–0.75}$	102.4 (5.5)	0.298 (0.017)	30.7 (2.6)	1.40 (0.12)b
Ref.$_{0–0.3}$	70.9 (2.6)			
Ref.$_{0–0.75}$	100.2 (5.3)			

Note: Values in parentheses are 1 SE ($n = 4$). The asterisk in column "SOC" indicates the only significant difference ($p < 0.04$) per site between SOC stocks of *Miscanthus* and reference fields. Small letters in the last column indicate significant differences (post hoc least significant difference test) between sites for 0–0.75 m soil depth.

upper 0.75 m of soil, SOC stocks of between 100 (Uettlingen grassland) and 579 t ha^{-1} (Le Landeron *Miscanthus*) were measured. At none of the sites, SOC stocks in 0–0.75 m depth differed between grassland and *Miscanthus*.

Between 19 and 24 years of *Miscanthus* cropping shifted the δ^{13}C isotopic signature at all sites toward less negative values (Table 2), from on average over all sites and layers −26.8‰ to −24.8‰. Isotopic shifts were more pronounced in topsoils, and isotopic signatures under *Miscanthus* approximated those of reference soils below 0.5 m at all sites except the mineral soil at site Uettlingen, where a C4-C imprint was visible also below that depth. During 19–24 years, between 28 (Uettlingen) and 46 (Gals) t C4-C ha^{-1} were accumulating in 0–0.3 m soil depth (31–55 t C4-C ha^{-1} in 0–0.75 m). This range corresponds to an annual accumulation of between 1.26 and 2.01 t C4-C ha^{-1} (1.40–2.38 t C4-C ha^{-1} in 0–0.75 m; Table 3). The C4-C accumulation rate was the smallest in the mineral soil, and ANOVA revealed that "site" was a significant factor (Tables 3 and 4) when analyzing the whole core (0–0.75 m), whereas it was not significant for 0–0.3 m. However, C4-C accumulation was not less in the mineral soil as compared with all others, but it differed significantly from two of the four organic-matter-rich soils. Owing to the large difference in total carbon stocks, the contribution of new carbon from *Miscanthus* accounted for less than 10% of total carbon in the four organic-matter-rich soils and for around 30% in the mineral soil at site Uettlingen (Figure 1). On average, 85.4% (±3.1%) of the total C4-C accumulated in 0–0.3 m soil depth. This pattern was not different among sites.

A comparison with previously reported C4-C accumulation rates under *Miscanthus* is provided in Figure 2. The figure includes only data from 0 to 0.3 m because different studies encompassed different soil depths, but all provided results for at least the upper 0.3 m. Mean soil carbon stocks in previous studies were 66.1 (±3.3) t carbon ha^{-1}, whereas the mean for the current dataset is 184.0 (±30.2) t carbon ha^{-1}. The corresponding mean carbon concentrations were 1.82% (±0.10%; previous studies) and 6.91% (±2.50%; this study), and the mean C4-C accumulation rates were 0.96 (±0.10) t C4-C ha^{-1} year^{-1} (previous studies) and 1.66 (±0.14) t C4-C ha^{-1} year^{-1} (this study). Figure 2 also implies that over a time scale of at least 24 years (longest experiment, site Gals, this study), the accumulation of C4-C from *Miscanthus* seems to continue. We tested annual C4-C accumulation rates against mean annual temperature and stand age. For mean annual temperature, we found no significant effect ($r = +0.27$; $p = 0.09$), whereas C4-C accumulation rates increased significantly with increasing stand age by 0.031 (±0.014) t C4-C ha^{-1} year^{-1} ($r = +0.33$; $p = 0.03$).

The distribution of all C4-C accumulation rates at study sites from previous work and from this study ($n = 42$) reveals a mean value of 1.04 t C4-C ha^{-1} year^{-1} with a median and mode of 0.89 and 0.60 t C4-C ha^{-1} year^{-1}, respectively, and 5% and 95% percentiles of 0.31 and 2.35 t C4-C ha^{-1} year^{-1}, respectively, for *Miscanthus* cropping in Europe (Figure 3).

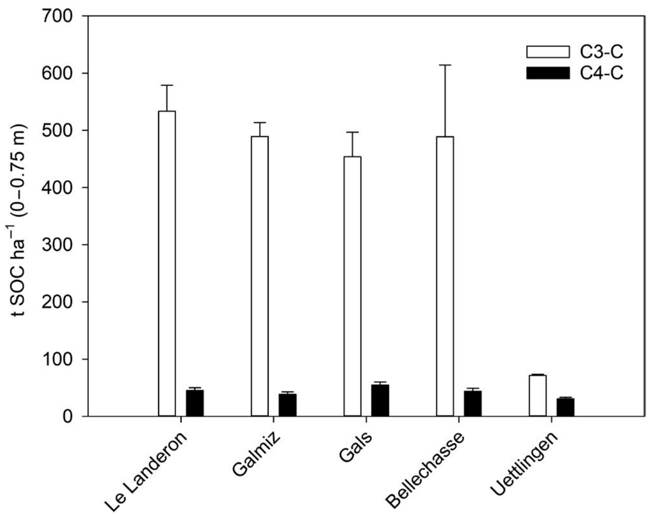

FIGURE 1 Mean share of old C3-carbon (C3-C) and young, *Miscanthus*-derived C4-carbon (C4-C) in the overall soil organic carbon (SOC) storage in 0–0.75 m soil depth at the five studied sites after 19–24 years of *Miscanthus* cropping. Error bars are 1 SE ($n = 4$)

TABLE 4 ANOVA results for C4-carbon accumulation rates for soil depths of 0–0.3 and 0–0.75 m

Source	Degrees of freedom	Sum of squares	Mean square	*F*	*p*
0–0.3 m					
Between sites	4	1.4686	0.3671	2.4130	0.0948
Within sites	15	2.2823	0.1522		
Total	19	3.7509			
0–0.75 m					
Between sites	4	2.5421	0.6355	3.5439	0.0316
Within sites	15	2.6900	0.1793		
Total	19	5.2321			

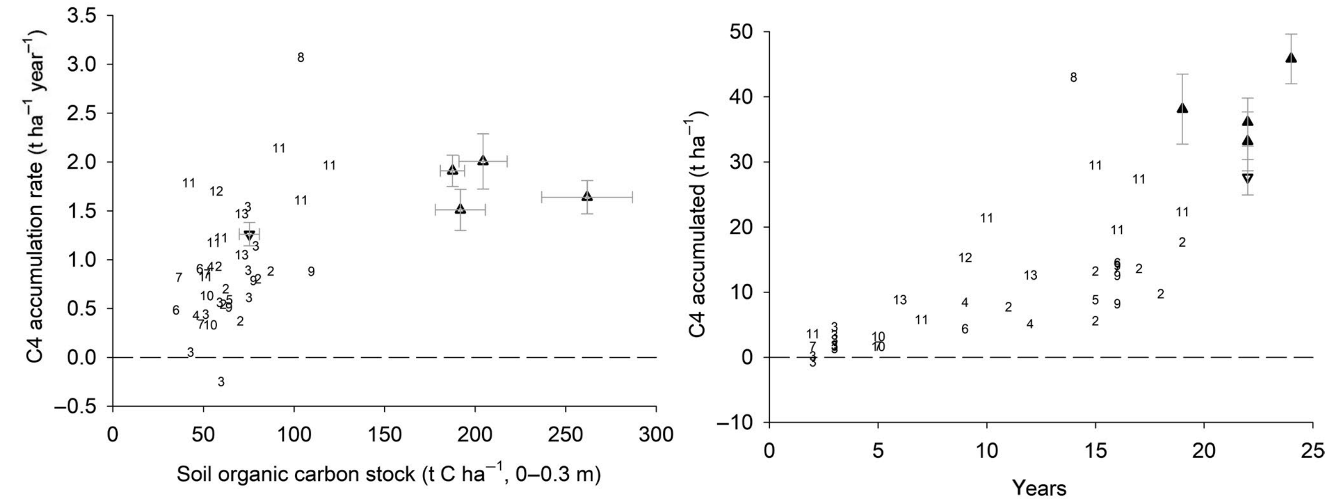

FIGURE 2 Accumulation rates of *Miscanthus*-derived C4-carbon (C4-C) at 42 study sites in Europe for the soil layer 0–0.3 m plotted against topsoil carbon storage (left panel) and accumulation for the same study sites plotted against the experimental duration (right panel). Triangles: this study; reversed triangle shows the mineral soil at site Uettlingen. Numbers refer to studies 2 (Poeplau & Don, 2014), 3 (Zimmermann et al., 2012), 4 (Schneckenberger & Kuzyakov, 2007), 5 (Clifton-Brown et al., 2007), 6 (Hansen et al., 2004), 7 (Felten & Emmerling, 2012), 8 (Dondini et al., 2009), 9 (Christensen et al., 2016), 10 (Ferchaud et al., 2016), 11 (Rehbein et al., 2015), 12 (Cattaneo et al., 2014), and 13 (Holder et al., 2019)

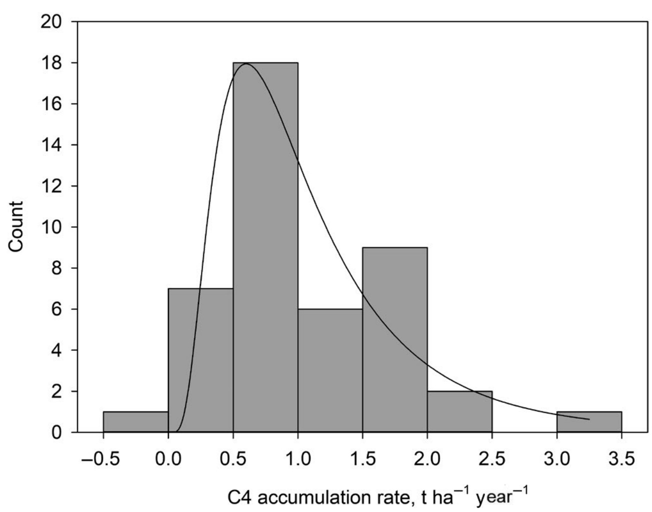

FIGURE 3 Distribution of annual C4-carbon (C4-C) accumulation rates under *Miscanthus* cropping in Europe for the soil layer 0–0.3 m based on results from 42 study sites. The curve fit follows [f = if(x<=0; 0;a*exp(−0.5*(ln(x/x0)/b)^2)/x)] with values for a, x0, and b being 13.335, 0.920, and 0.654, respectively

4 | DISCUSSION

4.1 | Methodological considerations

In accordance with other studies that addressed the effect of *Miscanthus* cropping on soil carbon, we made use of the change in carbon isotopic signature after a switch from C3 to C4 vegetation by comparing it with the signature on adjacent C3 plots. Most studies on *Miscanthus* use such a chronosequence approach (or paired plot design) where the assumed δ^{13}C reference signature is taken from neighboring C3 plots but not from the *Miscanthus* field at the beginning of the experiment. Because carbon is subject to isotopic discrimination upon transformation and transport in soils, δ^{13}C varies spatially also under permanent C3 vegetation for mineral soils (e.g., Ferchaud et al., 2016) and for organic soils (Krüger et al., 2015). Hence, any estimate on C4-C accumulation based on a paired plot design is associated with an error owing to variability in δ^{13}C between the reference site and the treatment at the beginning of the conversion. We tried to minimize this error by placing the sampling pairs as closely as possible to each other. This assumption is supported by the measured carbon stocks, which, apart from site Gals in 0–0.3 m soil depth, were not significantly different in any of the layers.

In this study, experimental constraints led to only four cores being taken per site. For our study of C4 accumulation rates, this was sufficient, however, for wider studies of C stock change other authors suggest a greater number of cores is preferable (e.g., Conant & Paustian, 2002; Schrumpf et al., 2011).

4.2 | Accumulation of C4-carbon at the studied sites

The accumulation of C4-C in this study represents a duration of between 19 and 24 years and is, according to our knowledge, for four of the five sites longer than any previously published records, which were at maximum 21 years (Zang et al., 2018). Poeplau and Don (2014) found that the age of the *Miscanthus* stand and the mean annual temperature explained some of the variability in C4-C accumulation rates.

With our larger dataset, including all available studies, we could not confirm such an effect of mean annual temperature, most probably because other site and management factors that influence SOC dynamics were too different across the 42 sites analyzed in Figure 2. Our compilation of 42 study sites supports the notion of a general and steady increase in C4-C storage over time. This increase in the C4-C stock has been suggested to be linear (Poeplau & Don, 2014; Rehbein et al., 2015). However, we found a significant positive effect of stand age on the annual accumulation rate of C4-C. With increasing stand age, the belowground root and rhizome network continue to develop, providing higher inputs when plants are getting better established (Richards et al., 2017). This development might drive an increase in C4-C accumulation rates over time. The observed increase in C4-C stocks and accumulation rates across studies is in apparent contradiction to evidence from SOC modeling and long-term experiments that both suggest that annual accumulation rates decline over time when SOC or C4-C approaches its new equilibrium (Ludwig et al., 2003; Poulton et al., 2018; Robertson & Nash, 2013). We argue that during 24 years of *Miscanthus* establishment accumulation rates remain high and that even longer observational studies are required to finally identify the complete accumulation kinetics. As discussed above, it should also be considered that the dataset does not represent a time series but a chronosequence and true time series from single sites are needed to further support this interpretation.

4.3 | Comparison with other *Miscanthus* studies from Europe

Mean rates of C4-C accumulation in our dataset, which included four soils with high organic matter content, greatly exceeded that of the mean and median of 37 studied European sites on mineral soils taken for comparison. However, similarly high rates were found for sites on mineral soils in Germany (Rehbein et al., 2015) and Italy (Cattaneo et al., 2014) and even a higher one for a *Miscanthus* plot on mineral soil in Ireland (Dondini et al., 2009). Of the five sites in this study, the one mineral soil had the smallest C4-C accumulation but was significantly different from only two out of four organic-matter-rich soils in its C4-C accumulation rate. Therefore, we cannot identify a systematic positive effect of high SOM contents on the accumulation of C4-C, and our data indicate that, although C4-C accumulation rates at our sites were among the highest ever measured, their high SOM content is not driving it. Because management of both, *Miscanthus* and grassland sites, was similar among our five sites, and climatic conditions were the same, we conclude that the high C4-C accumulation might be related to the favorable climate of the Swiss Central Plateau with sufficient rainfall also in summer and, for the soils rich in organic matter, a high nutrient delivery from the decomposition of the native SOM as well as access to the groundwater for the roots. Together, the comparison across all sites and the skewed distribution of rates in Figure 3 suggest that a reliable estimate for average accumulation rates is ideally based on the median value of 0.89 t C4-C ha^{-1} $year^{-1}$ (0–0.3 m), rather than on mathematical means.

How do these 0.89 t C4-C compare to typical carbon inputs under *Miscanthus*? Approximately 85% of the C4-C accumulation occurred in the topsoil (0–0.3 m), independently of SOC content and stand age. A higher accumulation in topsoils was found in all *Miscanthus* studies cited here and is related to the depth distribution of aboveground and particularly belowground organic matter inputs. Aboveground input from harvest residues was found to be 4.2–7.2 t d.m. ha^{-1} (1.9–3.2 t C4-C ha^{-1}) in two experiments in Denmark (Hansen et al., 2004), and estimated total input from mature *Miscanthus* stands (Wales, UK) to the topsoil varied between 3.5 and 4.4 t C4-C ha^{-1} (Zatta et al., 2014). The ratio of the latter values to the annual median increment of 0.89 t C4-C ha^{-1} calculated above gives a retention coefficient of 0.20–0.25. This range is close to the estimates of 0.26–0.29 by (Hansen et al., 2004), who reported for 0–1 m soil depth.

4.4 | Effects of *Miscanthus* cropping on soil organic carbon storage

The high C4-C accumulation we measured did not result in different soil carbon storage as compared with the grassland reference fields at any site, calculated for the total sampling depth of 0–0.75 m. Whereas SOC stocks of the two crops were similar at four of our five sites (Table 3), site Bellechasse revealed a higher but still non-significant difference for grassland compared to *Miscanthus*. The large variability in its SOC stock under *Miscanthus* could be attributed to one core replicate with relatively high SOC concentration below 0.25 m. When that core was excluded from the comparison between *Miscanthus* and grassland, SOC stocks between grassland and *Miscanthus* were still not different ($p = 0.37$) at Bellechasse. We therefore consider our result as robust. The average within-site variability of SOC stocks of our 10 fields (mean coefficient of variation 17.4% for all fields and 14.1% excluding Bellechasse *Miscanthus*) is at the upper end of that reported for six sites in Europe by Poeplau and Don (2014; mean coefficients of variation 9.7% and 17.0% for topsoil and subsoil, respectively), but this may be expected given the complex nature of degraded organic soils which leads to higher spatial variability.

We found no evidence for *Miscanthus* cropping to induce a net soil carbon sink at our sites relative to grassland. This finding supports the observation by Zang et al. (2018) that, on average, a conversion to *Miscanthus* does result in a net SOC increase only after conversion from cropland, not from grassland. Importantly, in their synthesis some croplands did not sequester carbon when converted to *Miscanthus*. In our study, three of the Miscanthus sites were converted from cropland 19 and 24 years ago. Management intensity and residue return to soil may be high also in croplands, particularly in Switzerland where leys and cover crops are widespread (Keel et al., 2019), which might explain why net SOC increases were not found. Only for the topsoil of the degrading Histosol at Le Landeron, a significant stock difference was measured (Table 3), but with higher SOC storage under grassland than under *Miscanthus*. This result can possibly be ascribed to the higher topsoil bulk density as induced by more frequent field traffic, and thus soil compaction, in this managed grassland on peat.

For mineral soils considered to be close to a dynamic equilibrium in their SOC balance, a non-change in SOC storage indicates that the loss of "old" C3-C occurs at a similar rate as the accumulation of "young" C4-C. Our finding that cropping of *Miscanthus* also does not change overall SOC storage in organic-matter-rich soils can be explained by two mechanisms. First, in drained Histosols and Gleysols with their strong disequilibrium toward long-lasting carbon losses, C4-C accumulation may be of the same rate as the accumulation of C3-C from the permanent grassland on the reference fields. Second, it might also be possible that the accumulation of new carbon does not occur at the same rate under C3 and C4 vegetation, respectively, and that the decomposition rate of peat differs between grassland and *Miscanthus*. Such dynamics might maintain a similar carbon stock but with different underlying dynamics. Indeed, the radiocarbon signature of soil samples taken from the site Le Landeron revealed significantly older ages under grassland (depth: 0.15–0.25 m) than under *Miscanthus*, suggesting that the dilution of the old peat by newly introduced aboveground and belowground residues might be stronger under *Miscanthus* (Bader et al., 2017).

5 | CONCLUSIONS

Do organic-matter-rich soils under *Miscanthus* behave differently in terms of C4-C dynamics as compared with mineral soils? By selecting five long-term paired plots from the same region but different soils, we could exclude possible confounding factors such as climate or management. Our analysis revealed that accumulation of C4-C at site Uettlingen with mineral soil was significantly different from only two of our four sites rich in organic matter, despite a difference in SOC stock by a factor of five. This finding suggests that the net dynamics of C4-C from *Miscanthus* are little influenced by SOM content and that other, hitherto unknown, site factors contribute to the variation in C4-C accumulation.

ACKNOWLEDGEMENTS

The authors thank Andrea Engler, Isabel Haas, and Cédric Bader for taking soil samples and analyzing them in the laboratory. We thank the five farmers for providing management information and access to their sites.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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How to cite this article: Leifeld, J., Alewell, C., & Paul, S. M. (2021). Accumulation of C4-carbon from *Miscanthus* in organic-matter-rich soils. *GCB Bioenergy*. 2021;13:1319–1328. https://doi.org/10.1111/gcbb.12861