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Soil organic carbon allocation and dynamics under perennial energy crops and their feedbacks with soil microbial biomass and activity

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Abstract

The cultivation of perennial instead of annual energy crops has received growing interest. Previous studies identified numerous beneficial effects of perennial energy crop (PEC) cultivation for the agricultural landscape such as promotion of agrobiodiversity, reduced requirements for agrochemicals and fertilizers as well as a large potential for carbon accumulation in soil. However, the mere presence of soil organic matter (SOM) accumulation gives no indication about the persistence of the SOM for example after a recultivation of the stands. Therefore, this study focused on SOM pools of different density fractions and soil microbial parameters. Six different PECs were tested against a typical benchmark system as feedstock for anaerobic digestion. The study has shown that all PEC species increased soil microbial activity and provided an insight how they sequester carbon in soil. Moreover, significant modifications in basic soil properties caused by plant growth were observed. For example, the cultivation of giant knotweed has lowered the soil pH by more than 0.5 pH units compared to the benchmark system. After 5 years of PEC cultivation, total soil organic carbon stocks were increased between 1,500 ± 400 and 4,500 ± 1,500 kg C ha^{-1} for the upper 10 centimetres of soil. The distribution among different soil fractions showed species-specific patterns. Tall wheatgrass and Virginia mallow showed particular high accumulation rates in the mineral-associated SOM fraction which indicates long residence times of the SOM after a possible recultivation of the fields.

KEYWORDS

density fractionation, particulate organic matter fractions, perennials, silage maize, soil enzymes, soil microbiota

1 | INTRODUCTION

During the last two decades, the cultivation of silage maize has been expanded in whole Europe, resulting from its high viability to serve as feedstock for agricultural biogas plants (Weiland, 2010; Schmidt *et al.*, 2018). However, the enormous increase in agricultural land used for silage maize cultivation has stimulated the discussion about the sustainability of this cultivation system. Consequently, in recent years, the evaluation of land use systems focused on more holistic approaches aside direct economic factors (Adler *et al.*, 2007; Brandão *et al.*, 2011). The impacts of certain cropping systems on agrobiodiversity, on groundwater quality as well as on the net greenhouse gas (GHG) balance have been strongly evaluated (Immerzeel *et al.*, 2014).

Growing interest has been paid in replacing annual by perennial energy crops (PECs) like *Miscanthus* (*Miscanthus* × *giganteus*), cup plant (*Silphium perfoliatum* L.) or tall wheatgrass (*Agropyron elongatum*). Several PECs were tested to provide only slightly lower methane yields per area than silage maize coincidently with both significantly lower management efforts and expenses for fertilizers and plant protection measures (Lewandowski *et al.*, 2009). PECs may help to prevent environmental problems associated with intense silage maize cultivation such as soil erosion and compaction as well as dwindled agrobiodiversity (Mast *et al.*, 2012; Herrmann, 2013; Schorpp *et al.*, 2016). Moreover, investigations of the past years have demonstrated that these crops may have a large potential for carbon sequestration in soil (Chimento *et al.*, 2016; Emmerling *et al.*, 2017; Ruf *et al.*, 2018). The main reasons for this are the absence of tillage and the recirculation of large amounts of organic substances above as well as belowground in stands of PECs (Carvalho *et al.*, 2017; Haddaway *et al.*, 2017; Ruf *et al.*, 2017). The observed higher activity of soil organisms suggests the formation of complex humic compounds and increasing SOM contents over time (Don *et al.*, 2012; Emmerling, 2014; Hargreaves & Hofmockel, 2014). With regard to the climatic impact of energy crop cultivation, SOM accumulation can be considered as negative emissions improving the GHG balance of perennial compared to annual energy crops (Adler *et al.*, 2007; Brandão *et al.*, 2011; Don *et al.*, 2012; Felten *et al.*, 2013; Cadoux *et al.*, 2014).

Due to the expected life span of PECs stands, which is estimated to be between 10 and 20 years (KTBL, 2012; Gansberger *et al.*, 2015), the recultivation of such fields has certainly to be considered as a significant intervention in this equilibrium system. Soil tillage and herbicide application for termination of stands of PEC may have far-reaching consequences for the established SOM pool by a possible stimulation of the mineralization rates and thus on the GHG balance. In this process, one decisive factor is the stability of the SOM pool in soil. SOM compounds with a high degree of stabilization are physico-chemically protected against microbial decomposition whereas less humified substances will readily be available for mineralization (von Lützow *et al.*, 2007). Previous work by Li *et al.* (2018) showed that compared to annual crops, perennial crops can consistently increase soil organic carbon and nitrogen as well as the stable organic matter (i.e. mineral-associated organic matter fractions) in direct association with higher microbial biomass and better developed soil fractal aggregation. However, the aspect of SOM fractionation in pools with different stability in soils cultivated with annual and PECs is still little investigated.

Among several methods for extraction of different SOM fractions, the density fractionation has developed as state of the art. According to von Lützow *et al.* (2007), this method allows for a good correlation with functional pools and stabilization mechanisms of SOM. Thus, these approaches appear to be highly suitable for (a) estimation of the SOM sequestration potential and (b) predicting the legacy effect of PEC cropping systems.

With this study, we aimed to evaluate (a) the persistence of sequestered carbon and (b) determine the soil microbial activity in soil cultivated with different PECs. For this purpose, we combined methods for determining parameters of soil microbial activity with the assessment of SOM pools. However, by the fact that the distribution of SOC among particulate organic matter (POM) fractions heavily relies on inherent soil properties such as soil structure and texture, particularly the clay content, mineralogy and pH values (Golchin *et al.*, 1997; Crow *et al.*, 2007), we selected a rather small scale plot experiment setup in order to keep these key determinants equal. We hypothesized that PEC cultivation leads to species-specific patterns of SOM distribution among different pools reflecting the chemical composition of the plant litter and microbial activity. However, after 5 years, we expected only slightly increased SOM stocks under PEC cultivation in the recalcitrant SOM fractions.

2 | MATERIAL AND METHODS

2.1 | Field experiment

The study site was located northeast of Trier in Western Germany at an altitude of 170 m above sea level. The site is characterized by a long-term mean annual precipitation and temperature of 710 mm and 8.9°C (DLR, 2013). The soil at the experimental site developed from about 40 cm of loess overlaying Perminan fine sand and silt stones (Steingötter, 2005) and is free of soil skeleton. The soil at the study site has a silt loam (SiL) texture and can be classified as Haplic Luvisol (Epiloamic, Endoraptic) according to WRB 2014 (FAO, 2015).

The field experiment was established in spring 2011 and managed by the Agricultural Service Center, Rhineland-Palatinate (DLR RLP) with the primary objective to test six PECs with respect to their yield potential against a crop rotation including silage maize and winter wheat whole crop silage as benchmark system (in the following referred to as 'SM-WW-CR') (Table 1). Cultivated PEC species were tall wheatgrass (*A. elongatum*), reed canary grass (*Phalaris arundinacea*), Virginia mallow (*Sida hermaphrodita*), cup plant (*S. perfoliatum* L.), giant knotweed (*Fallopia* × *bohemica* cv. IGNISCUM) and a wild flower mixture composing of in total 24 annual (e.g. *Helianthus annus, Malva* spec.), biennial (e.g.

TABLE 1 Mean yields (years 2013–2015) of the different perennial species, excluding the phase of establishment (2011 and 2012), as well as of the crop rotation of maize and winter wheat whole crop silage based on data published in DLR (2016)

Species	Average yield (Mg d.m. ha^{-1} $year^{-1}$)
SM-WW-CR	13.96 (±7.71)
Tall wheatgrass	17.93 (±2.44)
Reed canary grass	17.68 (±0.84)
Cup plant	15.16 (±2.03)
Virginia mallow	4.09 (±1.08)
Giant knotweed	15.38 (±2.12)
Wild flower mixture	14.17 (±1.60)

Dipsacus sylvestris, Daucus carota) and perennial (e.g. *Inula helenium, Malva alcea, Medicago sativa*) species.

Each species was established on four plots with a size of 15 m^2 (three metres wide and five metres long) in spring 2011. The crops were managed in single (Maize, cup plant, Virginia mallow, wild flower mixture) or double (tall wheatgrass, reed canary grass, giant knotweed) cutting regime. Harvested biomass yields were removed from the experimental side. In the year of the establishment, the crops were not fertilized. From the second year on, all crops received 100 kg P ha^{-1} and 200 kg K ha^{-1} at start of vegetation in each year. The amount of N varied as follows: SM-WW-CR 200 kg N ha^{-1}, cup plant and Virginia mallow 160 kg N ha^{-1}, wild flower mixture 100 kg N ha^{-1}, each at the start of vegetation. Tall wheatgrass, reed canary grass and giant knotweed received 160 kg N ha^{-1} at vegetation start and additionally 100 kg N ha^{-1} after the first cut. Soil tillage in the SM-WW-CR benchmark system was done non-turning to a depth of about 10–12 cm using a disc cultivator.

2.2 | Soil sampling and pre-preparation

Composite soil samples were taken by six subsamples in each plot to a depth of 10 cm using an auger after 5 years of cultivation in April 2016. Samples were sieved to 2 mm and stored cool (4°C) until analysis. The soil was subdivided into three parts. The first share was dried at 105°C for 24 hr to determine the current water content of the soil. The second share was air-dried until constant weight was reached for subsequent density fractionation, total C measurement and pH measurement. The remaining amount of soil was moistened to reach a water content equivalent to 40%–60% of maximum water holding capacity for subsequent analysis of soil microbial parameters.

Moreover, in each plot, six undisturbed samples for bulk density determination representative for the soil depth of 0–10 cm were taken using sampling rings with 100 cm^3 volume.

2.3 | Soil analysis

2.3.1 | Determination of soil bulk density

Undisturbed soil samples were dried in a compartment drier at 105°C for 24 hr and weighted. Soil bulk density was calculated based on absolute dry soil mass and precisely known volume and mass of the single sampling rings.

2.3.2 | Determination of soil pH

The soil pH measurement was taken in a 10 mmol L^{-1} $CaCl_2$ solution using air-dried, sieved soil and a pH C^{-1}ond 340i glass electrode (WTW GmbH).

2.3.3 | Fractionation of SOM and carbon determination

Determination of organic matter pools was done using a stepwise, density-based approach acc. to Golchin *et al*. (1997) in order to obtain the following four fractions:

- Free particulate organic matter with a density lower than 1.6 g cm^{-3} (fPOM).
- Occluded particulate organic matter with a density lower than 1.6 g cm^{-3} (loPOM).
- Occluded particulate organic matter with a density lower than 2.0 g cm^{-3} (hoPOM).
- Mineral-associated organic matter with a density higher than 2.0 g cm^{-3} (MOM).

For the fractionation, 10 g of sieved (2 mm) and air-dried soil was weight in a 50 ml centrifuge tubes. In a first step, 30 ml of a sodium polytungstate solution (SPT, $Na_6(H_2W_{12}O_{40})$) (TC-Tungsten Compounds) with a density of 1.6 g cm^{-3} was added in order to obtain the fPOM fraction. The samples were then vortexed and centrifuged for 60 min at 2,800 *g*. The supernatants were vacuum-filtrated via 0.45 µm cellulose-acetate filter (Sartorius). The filter residue was then thoroughly rinsed with distilled water and transferred to evaporating dishes.

In a second step, each 10 glass beads and 30 ml of a 1.6 g cm^{-3} SPT solution were added to the remaining sediments in the centrifuge tubes, vortexed and overhead shaken for 16 hr at 15 rpm. Further processing for obtaining the loPOM was similar as in the first step.

In the third step, the hoPOM was obtained by adding 30 ml of a 2.0 g cm^{-3} SPT solution, vortexing and overhead shaking for 30 min at 15 rpm. Again, further processing of the supernatants was done similar as described for the fPOM.

In the fourth step, the remaining sediments were washed out in order to remove the SPT solution. Therefore, 30 ml of distilled water was added, the sample vortexed and centrifuged for 15 min at 4,000 rpm. The supernatants were discarded. This step was repeated three times. The sediments were finally transferred to evaporating dishes representing the MOM fraction.

The fractions in the evaporation dishes were dried at 45°C until constant weight and quantitatively scratched out, weight, grinded and analysed for their carbon contents using an Elemental Analyser EA3000 Series (HEKAtech). Carbon determination of the bulk soil was done using the same device. Each sample was prepared in duplicates.

2.3.4 | Determination of soil microbial parameters

Soil microbial parameters were determined after adjusting the soil to 40%–60% of its maximum water holding capacity and a conditioning phase of 8 days in which the samples were protected against water loss, simultaneously allowing gas exchange.

2.4 | Soil microbial biomass carbon

Microbial biomass carbon (MBC) was determined by the chloroform fumigation extraction method according to Vance *et al.* (1987). Extraction was performed using a 0.01 M $CaCl_2$ solution acc. to Joergensen (1996). Measurement of the total organic C in the extracts was done with a TOC-TN Analyzer (Shimadzu TOC-V + TNN). MBC contents were calculated using a kEC coefficient of 0.45 as stated by Joergensen (1996).

2.5 | Basal respiration

Soil respiration measurements were conducted according to Heinemeyer, Insam, Kaiser, & Walenzik, (1989). Therefore, 30 g dry equivalent moist soil samples were weighted in tubes that were flushed with 200 ml min^{-1} of CO_2-free, humid air for two days. The formed CO_2 was measured after the soil passage using an infrared gas analyzer (ADC 225 MK3, The Analytical Development).

2.6 | Enzyme activity

Enzyme activity of cellulose 1,4 β-cellobiosidase and 1,4 β-glucosidase was determined using a fluorimetric microplate enzyme assay according to Marx *et al.* (2001). MUB-ß-D-cellobioside (EC 3.2.1.91) and MUB-ß-D-glucopyranoside (EC 3.2.1.3) served as substrates for assessing the activity of cellobiohydrolase and β-glucosidase, respectively.

For the measurements, homogenous soil suspensions were prepared from 0.5 g dry matter equivalent field moist soil samples and 50 ml of autoclaved water. After vigorously stirring for 2 min, 50 µl aliquots were transferred to black 96-well microtiter plates (Biozyme Scientific). Subsequently, 50 µl of MES buffer (100 mM, pH: 6.1) and 100 µl of the respective substrate solution were added (chemicals purchased from Sigma Aldrich Chemicals). Plates were incubated at 30°C under absence of light. Fluorescence measurements were taken in 30 min intervals over a period of two hours using a Victor3 MultiLabel Reader (Perkin Elmer) with an excitation wavelength of 355 nm and emission wavelength of 460 nm.

2.7 | Data evaluation and statistical analysis

Carbon stocks in different soil fractions (fPOM, loPOM, hoPOM, MOM) under the seven crops investigated were calculated as follows: Firstly, the relative share of a certain soil fraction on the total soil and the respective carbon concentration of the specific fraction were multiplied. Secondly, the mean bulk density of all replicates of a single crop was used to estimate the soil mass in the depth interval of 0–10 cm. In the last step, the carbon contents were multiplied with the calculated soil mass. Changes in total SOC and in the different soil fractions after 5 years of PEC cultivation were calculated by subtracting the SOC stock of a certain fraction from the SOC stock of the same fraction in the benchmark system.

Due to the low number of field replicates ($n = 4$) against seven crop species established, multiple comparisons could statistically not be conducted with adequate discriminatory power. Therefore, the perennial species were individually tested against the crop rotation of maize silage and winter wheat whole crop silage as benchmark system.

In this regards, the data of the different crops were firstly tested for normality using a Shapiro–Wilk test (Shapiro & Wilk, 1965) and secondly for homoscadacity (Levene test) (Levene, 1960). Testing of the single crops cultivated against the benchmark system was done using two-sample *t* tests and Wilcoxon signed rank test, depending on fulfilment of the test conditions. The level of probability was chosen at $\alpha = 0.05$, unless otherwise specified. Statistical analysis was conducted using RStudio Version 3.3.2 (R Core Team, 2016).

3 | RESULTS

Significant modifications in soil parameters by different energy crops could be observed after 5 years of cultivation. The

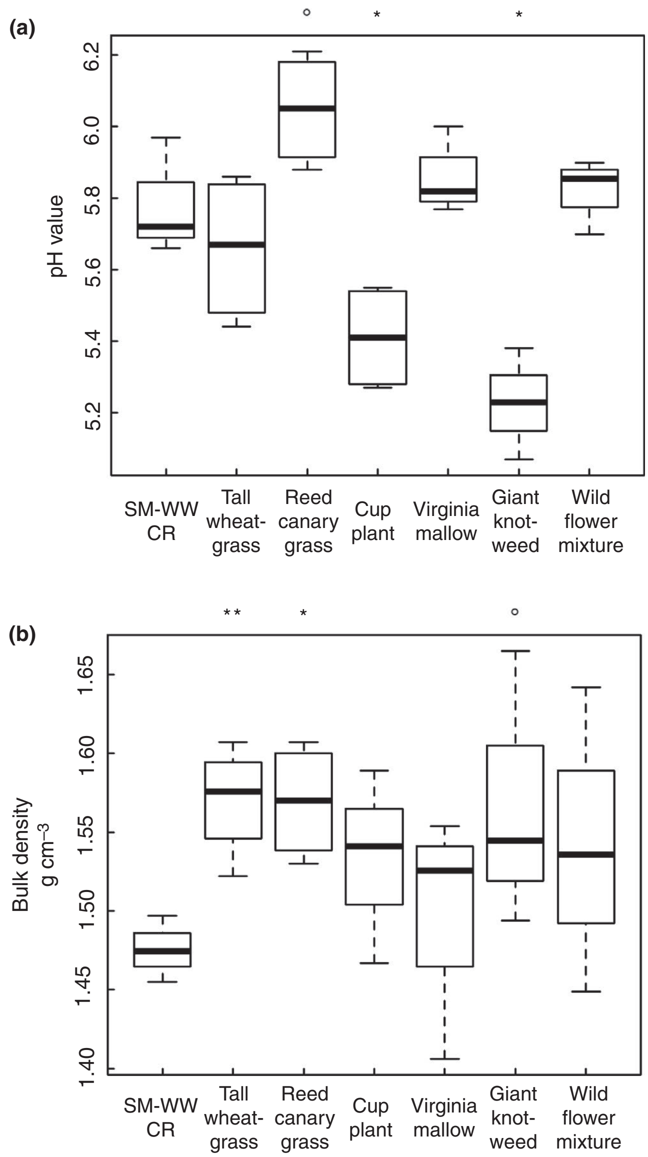

FIGURE 1 Mean values ($n = 4$, ± standard deviation) of pH value and soil bulk density under different crops. Significant differences between the benchmark system 'SM-WW-CR' (silage maize – winter wheat whole crop silage) and a certain crop are indicated as follows: °$p < .10$, *$p < .05$, **$p < .01$

pH values showed a range from 5.23 ± 0.13 (giant knotweed) to 6.05 ± 0.16 (reed canary grass). The soils cultivated with these both species as well as with cup plant (5.78 ± 0.16) were significantly different from the soil pH of the benchmark system (5.41 ± 0.15), whereas only small differences were observed to tall wheatgrass and Virginia mallow (Figure 1).

Increased bulk densities with typical values of 1.55 g cm^{-3} were observed in all perennial systems which were characterized by absence of tillage for 5 years compared to the benchmark system (1.48 ± 0.02 g cm^{-3}) which was regularly tilled before seedbed preparation. However, these apparent differences were not significant in all cases due to relatively large standard deviations in all the perennial cultivation systems.

For soil microbial biomass, higher but also significantly lower values were observed for the plots cultivated with PECs as compared to the benchmark system (Table 2). Highest values were determined under reed canary grass (144.8 ± 4.5 µg C g^{-1} d.m.) and tall Wheatgrass. The lowest value was observed for giant knotweed (49.8 ± 5.2 µg C g^{-1} d.m.). The benchmark system took an intermediate position with 117.6 ± 11.2 µg C g^{-1} d.m.

For soil respiration, lowest values were determined for giant knotweed (0.18 ± 0.02 µg CO_2-C g^{-1} d.m. hr^{-1}) and highest for reed canary grass (0.35 ± 0.04 µg CO_2-C g^{-1} d.m. hr^{-1}). The metabolic quotient clearly responded to low MBC and high soil respiration observed for giant knotweed and cup plant which were significantly higher ($p < .01$) than the benchmark system and all other PECs (Table 2).

Enzyme activities in the perennial systems were usually higher than in the annual benchmark system (Figure 2). Significant differences in the glucosidase activity compared to the benchmark system (49.7 ± 14.9 nmol g^{-1} d.m. hr^{-1}) were observed for reed canary grass (81.7 ± 17.2 nmol g^{-1} d.m. hr^{-1}), Virginia mallow (72.7 ± 9.8 nmol g^{-1} d.m. hr^{-1}) and the wild flower mixture (69.1 ± 16.6 nmol g^{-1} d.m. hr^{-1}). Cellobiosidase activity was generally lower compared to glucosidase; however, significantly higher cellulase activities in the plots cultivated with reed canary grass (54.4 ± 12.0 nmol g^{-1} d.m. hr^{-1}) and the wild flower mixture (65.0 ± 3.1 nmol g^{-1} d.m. hr^{-1}) compared to the benchmark system (36.8 ± 4.8 nmol g^{-1} d.m. hr^{-1}) were observed.

For all crops, the distribution of the different soil fractions followed a similar pattern. The fPOM fraction always accounted for less than 1% and the loPOM fraction usually made up less than 0.5% of the total soil (Table 3). The hoPOM fraction typically amounted to 1.0% to 1.5% of total soil. Resulting from that, the predominant fraction was represented by the MOM fraction which accounted for 97%–98% of the total soil. Remarkably, reed canary grass showed, compared to all other crops, a different pattern with significantly higher shares of loPOM and hoPOM fractions. Similarly, the share of hoPOM was also significantly higher for giant Knotweed, whereas the loPOM fraction was almost not existent.

Data showed a clear grouping of SOC contents (Table 4). Compared to the annual benchmark system with a SOC content of 10.77 (±0.40) g kg^{-1}, the SOC contents were significantly increased after 5 years of perennial crop cultivation, except for cup plant. Usually, perennial crop cultivation has led to an increase of about 1.0 to 2.0 g kg^{-1} SOC compared to the benchmark system in the upper 10 cm of soil. Highest value was observed under tall wheatgrass cultivation with 13.01 (±0.93) g kg^{-1}.

TABLE 2 Mean values ($n = 4$, ± standard deviation) of soil microbial parameters under different crops. Total SOC and SOC values of the soil density fractions are presented together in Table 4

Species	MBC (µg C g^{-1} d.m.)	MBC:SOC (%)	Soil respiration (µg CO_2-C g^{-1} d.m. hr^{-1})	Metabolic quotient (µg CO_2-C mg^{-1} MBC g^{-1} d.m. hr^{-1})
SM-WW-CR	117.6 (±11.2)	1.04 (±0.08)	0.24 (±0.03)	2.01 (±0.03)
Tall wheatgrass	129.6 (±8.6)	0.98 (±0.11)	0.32 (±0.07)	2.49 (±0.52)
Reed canary grass	144.8 (±4.5)**	1.27 (±0.19) †	0.35 (±0.04)*	2.30 (±0.18)
Cup plant	76.2 (±5.5)**	0.65 (±0.07)***	0.28 (±0.06)	3.25 (±0.12)**
Virginia mallow	132.5 (±7.9)†	1.04 (±0.01)	0.28 (±0.02)†	2.14 (±0.22)
Giant knotweed	49.8 (±5.2)***	0.38 (±0.04)***	0.18 (±0.02)*	3.28 (±0.06)**
Wild flower mixture	132.0 (±7.4) †	1.06 (±0.04)	0.34 (±0.04)*	2.48 (±0.30) †

Significant differences between the benchmark system 'SM-WW-CR' (silage maize – winter wheat whole crop silage) and a certain crop are indicated as follows:
†$p < .10$,
*$p < .05$,
**$p < .01$,
***$p < .001$.

Carbon concentrations in different soil fractions showed a species-specific distribution (Table 4). However, the carbon concentrations in the soil cultivated with PECs were usually significantly higher in the lighter fractions (fPOM, loPOM). In the heavy occluded fraction (hoPOM), significant differences between perennial crops and the benchmark system could only be observed for the cup plant and giant knotweed. However, the carbon concentrations in the hoPOM fraction were still on a high level with about 170–210 g kg^{-1}. In the fraction considered to represent the mineral-associated soil organic matter (MOM), the carbon concentrations were by more than on order of magnitude lower than for all other fractions.

Compared to the benchmark system, tall wheatgrass, Virginia mallow and wild flower mixture showed significantly higher carbon concentration in the MOM fraction whereas the carbon contents of the other crops did not differ from the reference.

Resulting from both, the higher SOC contents and the elevated bulk density under perennial crop cultivation, the soil organic carbon stocks showed a more distinct differentiation and were significantly higher in soils cultivated with PECs compared to the benchmark system. Generally, the soil organic carbon stocks were dominated (typically 70%) from the carbon sequestered in the MOM fraction resulting from the fact that this fraction represented about 97 to 98% of the soil mass (Table 3), although the carbon concentrations in this fraction were quite low (Table 4). Significantly increased SOC stocks in the MOM were calculated for four of the six perennial species. Compared to the benchmark system, increases in total SOC content in the upper 10 cm of the soil ranged between 1524.1 ± 378.1 kg ha^{-1} (cup plant) and 4,544.2 ± 1,454.4 kg ha^{-1} (tall wheatgrass) after 5 years of PEC cultivation (Table 5). Similarly, significantly higher carbon stocks in the hoPOM fraction could be observed ranging from 2,840 ± 150 (benchmark system) kg ha^{-1} up to 4,604 ± 321 (giant knotweed) kg ha^{-1} (Figure 3). With respect to the total SOC stock, the loPOM fraction was of minor importance due to the much smaller mass share (Table 3) compared to the other fractions. For this fraction, slight reductions in SOC amounts compared to the benchmark ranging between −29.4 ± 21.6 kg ha^{-1} (Virginia mallow) and −184.3 ± 31.7 kg ha^{-1} (giant knotweed) were observed for all forb species investigated (Table 5). In relative figures, the carbon stock related to the fPOM fraction showed the greatest range. Whereas an amount of only 765.27 ± 141.50 kg ha^{-1} was calculated for the benchmark system, more than twice of this amount was identified for giant knotweed (1,610.17 ± 451.30 kg ha^{-1}) (Figure 3). For the other perennial crops, significantly higher values could at least be proven at p-values < 0.10. SOC accumulation in the fPOM fraction was highest for giant knotweed (844.9 ± 451.3 kg ha^{-1}) and lowest for cup plant (301.6 ± 129.6 kg ha^{-1}).

4 | DISCUSSION

4.1 | Impact of PEC cultivation on soil bulk density and soil pH

Perennial cultivation systems are characterized by a long-term absence of each kind of soil tillage. As a result, changes in basic soil properties can be observed in the course of time. In this study, after 5 years of PEC cultivation, the bulk

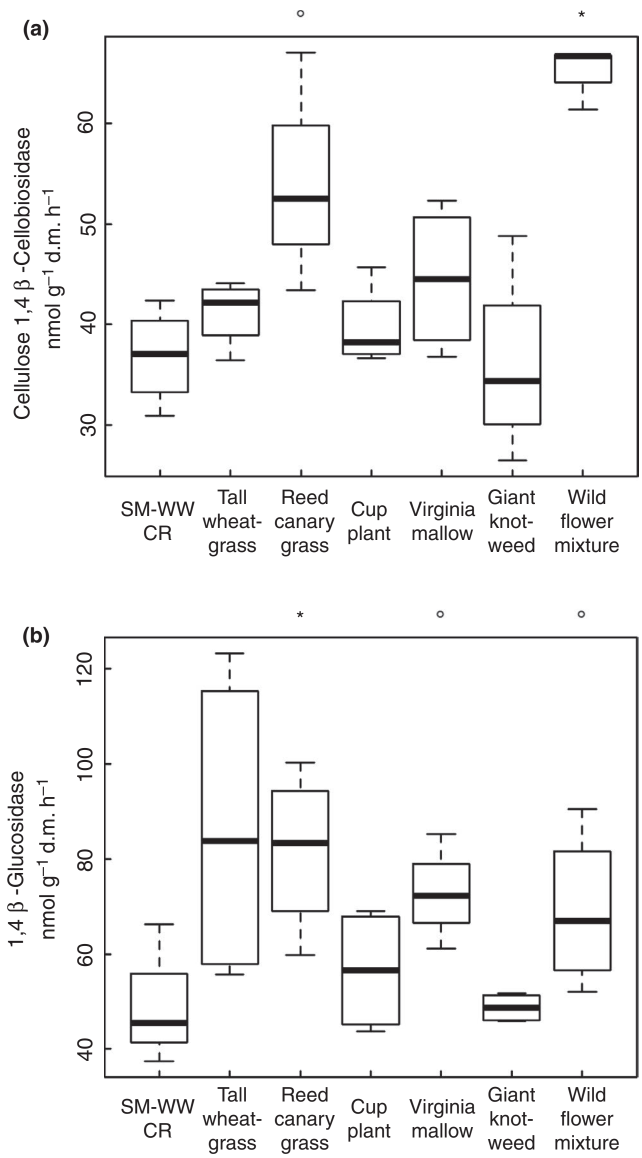

FIGURE 2 Mean values ($n = 4$, ± standard deviation) of cellulose 1,4 β-cellobiosidase and 1,4 β-glucosidase enzyme activity soil under different crops. Significant differences between the benchmark system 'SM-WW-CR' (silage maize – winter wheat whole crop silage) and a certain crop are indicated as follows: °$p < .10$, *$p < .05$

density in the soils was significantly increased by about 10% compared to the annual system.

Simultaneously, a distinct differentiation in the soil pH values under the different crops was observed. The initial soil pH of 6.4 decreased in the course of the experiment for all cultivated species. In 2016, the pH values ranged between 5.2 ± 0.1 (giant knotweed) and 6.1 ± 0.2 (reed canary grass). Thus, with regard to the results of Emmerling *et al.* (2017) for the same site, the species-specific trend in soil pH modification has continued. Moreover, the results of this field trial confirm results of a mesocosm experiment that among PECs, the forb species stronger decreased the soil pH than grasses (Ruf *et al.*, 2019).

Although not investigated in this study, it might be concluded that the changes in the soil pH predominantly result from root exudates which are specific in composition and quantity depending on the plant species and the soil. In addition, plants interact via root exudation with the soil microbiome, whereby supporting or suppressing effects have been documented (Walker *et al.*, 2003; Gregory, 2006). As a general rule, Brimecombe *et al.* (2007) stated that perennial species allocate more assimilates via root exudates to the soil than annual ones.

4.2 | Impact of PEC cultivation on soil microbial activity

Compared to the annual benchmark system, MBC and microbial activity in the soils cultivated with PECs were changed in both directions. Highest and lowest soil MBC were observed in reed canary grass and giant knotweed, compared to the benchmark system. Similar observations were made for the basal respiration and also the activities of cellulose 1,4 β-cellobiosidase and 1,4 β-glucosidase, which can be regarded as a tracer for the ability of soils for SOM stabilization (Bakshi & Varma, 2011). From the information available to date, no study has dealt with effects on enzyme activities in soils cultivated with dicotyledonous PECs. Nonetheless, Hargreaves & Hofmockel (2014) compared the effects of an annual (*Zea mays*) and a perennial (*Panicum virgatum* L.) energy crop, both treated in no-till cultivation, on soil microbial parameters. They observed higher MBC, lignocellulose-degrading enzyme activities and soil respiration in the plots cultivated with the perennial species particularly in summer. Similar results were observed by Culman *et al.* (2010) and DuPont *et al.* (2010) for MBC in perennial grasslands compared to cropland.

These results are in line with the results of this study for both of the investigated grass species (tall wheatgrass and reed canary grass), Virginia mallow and the wild flower mixture. For cup plant and giant knotweed, this only applies to a certain extent. For the latter, the MBCs were significantly lower and the activities of both enzymes in the range of the annual benchmark system. However, the phytobiologically altered soil pH impedes the interpretation of soil microbial parameters due to its direct effect on soil enzyme activity (Sinsabaugh *et al.*, 2008).

4.3 | Effects of PEC cultivation on distribution of soil fractions, their SOC contents and SOC pools

This study revealed only slight differences in the mass distribution of the soil among the four analysed soil fractions

TABLE 3 Relative shares of the different soil fractions under different crops on a mass basis

Species	fPOM fraction (%)	loPOM fraction (%)	hoPOM fraction (%)	MOM fraction (%)
SM-WW-CR	0.59 (±0.02)	0.27 (±0.09)	1.14 (±0.14)	98.01 (±0.23)
Tall Wheatgrass	0.76 (±0.18)	0.27 (±0.05)	1.18 (±0.25)	97.79 (±0.34)
Reed canary grass	0.72 (±0.15)	0.58 (±0.12)**	1.54 (±0.09)**	97.17 (±0.20)**
Cup plant	0.65 (±0.08)	0.24 (±0.04)	1.12 (±0.13)	97.86 (±0.24)
Virginia mallow	0.73 (±0.08) †	0.22 (±0.01)	1.21 (±0.19)	97.97 (±0.15)
Giant knotweed	0.83 (±0.21)	0.08 (±0.04)*	1.41 (±0.13)*	97.69 (±0.28)
Wild flower mixture	0.68 (±0.08)	0.22 (±0.03)	1.15 (±0.12)	97.17 (±0.20)

Significant differences between the benchmark system 'SM-WW-CR' (silage maize – winter wheat whole crop silage) and a certain crop are indicated as follows:
†$p < .10$,
*$p < .05$,
**$p < .01$,
***$p < .001$

(fPOM, loPOM, hoPOM, MOM). In contrast to that, the carbon concentrations in the different fractions were consistently increased due to PEC cultivation which can likely be traced back to larger amounts of plant-derived organic material that has not been exported with the harvested biomass but remained onsite. In general, pre-harvest losses, direct yield losses and harvest residuals are worth to be mentioned (Ruf *et al.*, 2017). Moreover, some PECs are characterized by a post-harvest regrowth which freezes down during winter (Schittenhelm *et al.*, 2016). Amounts of aboveground litter production strongly depend on the cultivated species but also on site and weather conditions. According to own observations, pre-harvest losses primarily occur during the vegetation period as a result of leaf shedding caused by reduced light intensity in dense stands (Schittenhelm *et al.*, 2016) and internal nutrient allocation to young shoots and root. Although comprehensive data are missing for the PECs investigated in this study, it can be concluded that forb species did not produce as much litter as grasses. Hedĕnec *et al.* (2014) observed that bare soil is visible between plants of *S. perfoliatum*. However, in perennial cropping systems, the carbon inputs via belowground pathways, like root exudation and root biomass turnover, were significantly higher compared to annual cropping systems (Carvalho *et al.*, 2017; Kantola *et al.*, 2017)—findings that may explain the higher carbon contents under PECs observed in this study.

TABLE 4 Carbon concentrations in the different soil density fractions under different crops.

Species	Total SOC (g kg^{-1})	C in fPOM fraction (g kg^{-1})	C in loPOM fraction (g kg^{-1})	C in hoPOM fraction (g kg^{-1})	C in MOM fraction (g kg^{-1})
SM-WW-CR	10.77 (±0.40)	88.75 (±15.27)	88.55 (±10.93)	171.62 (±16.50)	7.64 (±0.23)
Tall wheatgrass	13.01 (±0.93)*	110.05 (±9.13) †	135.70 (±31.04) †	184.49 (±0.72)	8.83 (±0.43)**
Reed canary grass	11.75 (±0.58)*	123.33 (±10.31)*	93.12 (±4.65)	170.87 (±8.52)	7.73 (±0.57)
Cup plant	11.35 (±0.25)	106.96 (±9.91) †	83.13 (±9.47)	212.78 (±21.45)*	7.76 (±0.36)
Virginia mallow	12.40 (±0.12)**	98.25 (±18.92)	108.03 (±11.28)*	176.99 (±4.74)	8.87 (±0.37)**
Giant knotweed	12.42 (±1.04)*	125.04 (±18.89)*	161.64 (±56.19) †	206.53 (±1.15)*	7.87 (±0.42)
Wild flower mixture	12.29 (±0.46)***	122.85 (±16.86)*	95.98 (±21.02)	186.49 (±3.10)	8.92 (±0.76)**

Significant differences between the benchmark system 'SM-WW-CR' (silage maize – winter wheat whole crop silage) and a certain crop are indicated as follows:
†$p < .10$,
*$p < .05$,
**$p < .01$,
***$p < .001$

TABLE 5 Mean values ($n = 4$, ± standard deviation) of soil organic carbon changes observed for the perennial energy crops, against SM-WW-CR as reference, in different soil density fractions and total soil organic carbon stock after 5 years of cultivation.

Species	Total SOC (kg C ha^{-1})	C in fPOM fraction (kg C ha^{-1})	C in loPOM fraction (kg C ha^{-1})	C in hoPOM fraction (kg C ha^{-1})	C in MOM fraction (kg C ha^{-1})
Tall wheatgrass	4,544.2 (±1,454.4)**	563.8 (±415.4) †	217.7 (±169.3) †	620.0 (±759.9)	2,510.0 (±638.7)**
Reed canary grass	2,553.9 (±917.0)**	610.5 (±235.9)**	379.3 (±191.9)**	1,287.6 (±324.1)**	744.7 (±847.3)
Cup plant	1524.1 (±378.1)**	301.6 (±129.6) †	−55.5 (±37.2)	781.5 (±393.6)	628.0 (±535.3)**
Virginia mallow	2,745.6 (±174.5)**	304.1 (±209.7)	−29.4 (±21.6)	365.8 (±431.0)	2004.7 (±572.1)**
Giant knotweed	3,511.4 (±1,620.6)*	844.9 (±451.3)*	−184.3 (±31.7)*	1763.8 (±321.1)*	973.5 (±627.8) †
Wild flower mixture	3,048.0 (±714.1)***	407.5 (±204.8)**	−42.6 (±27.4)	437.0 (±290.9)*	2,414.1 (±1,137.8)*

Significant differences between the SM-WW-CR benchmark system (silage maize – winter wheat whole crop silage) and a certain PEC species and soil fraction are indicated as follows:

†$p < .10$,

*$p < .05$,

**$p < .01$,

***$p < .001$.

Besides the amounts of organic substances that are available as potential sources for increased carbon contents in soil, their quality determines the pathway of decomposition (Castellano *et al.*, 2015). Based on the results of Schrama *et al.* (2016) and Emmerling *et al.* (2017), litter of PECs is typically characterized by high carbon to nitrogen ratios, as well as high shares of cellulose and lignin. These physico-chemical characteristics make the organic residues more recalcitrant to microbial decomposition leading to long residence times in soil (von Lützow *et al.*, 2007; Prescott, 2010). However, the differences among the PEC species should not be neglected. According to Emmerling *et al.* (2017), the lignin contents of

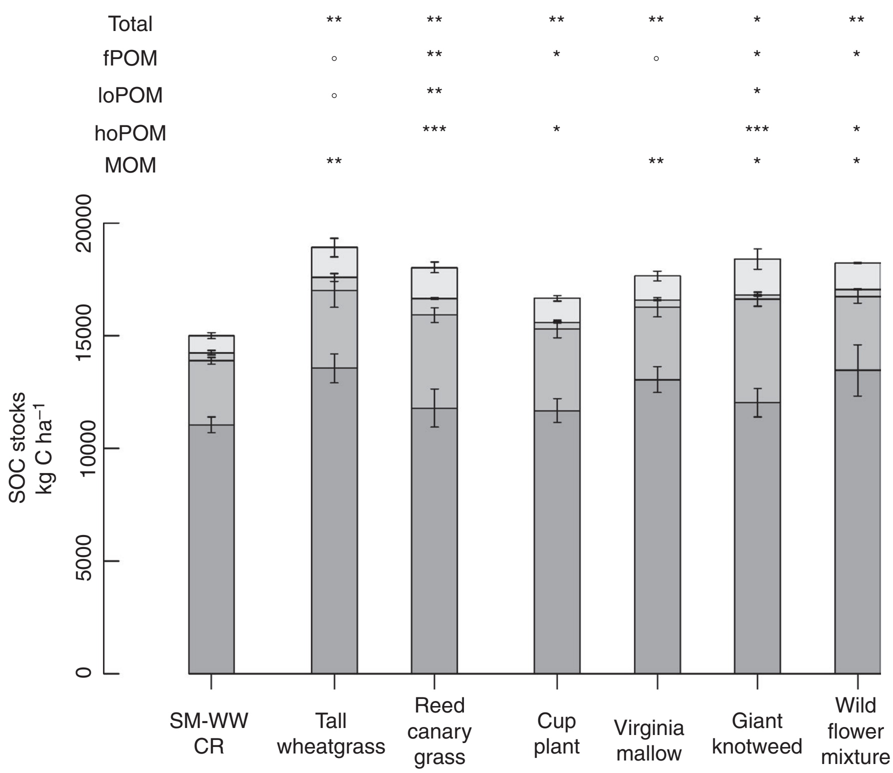

FIGURE 3 Mean values ($n = 4$, ± standard deviation) of soil organic carbon stocks in different soil fractions and total carbon stock. Significant differences between the SM-WW-CR benchmark system (silage maize – winter wheat whole crop silage) and a certain PEC species and soil fraction are indicated as follows: °$p < .10$, *$p < .05$, **$p < .01$, ***$p < .001$

PECs were usually two to three times higher than that of silage maize (3.4% of d.w.), whereas the lignin content of giant knotweed even exceeded 18% of d.w. Cellulose contents varied in a wide range between 19.1% of d.w. (silage maize) and 41.8% of d.w. (tall wheatgrass). In the present study, the elevated activities of cellulose 1,4 β-cellobiosidase and 1,4 β-glucosidase, both catalysing the hydrolysis of oligo- and polysaccharides (Bakshi & Varma, 2011) underpin the lignocellulosic character of the crop residues. It should furthermore be considered that in the tilled soil of the benchmark system, the aeration and accessibility of organic residuals are higher presumably leading to accelerated mineralization rates.

Total SOM contents and, by taking into account the established bulk densities, the total SOC stock under PEC cultivation were significantly increased regardless the specific species. In the topmost horizon, about 1,500–4,600 kg ha^{-1} of carbon was additionally stored. The magnitude of the presented results is in line with several studies that also observed significantly increased total SOC stocks after land-use change from annual to PEC cultivation or more extensive land use such as reduced tillage (DeGryze *et al.*, 2004; Don *et al.*, 2012; Ferchaud *et al.*, 2016). However, to conclusively account for SOC storage changes by PECs, deeper measurements are necessary. They should preferably be conducted after a longer cultivation period of PECs when changes in soil parameters are to be expected for deeper layers.

As shown in Table 3 and Figure 3, particularly the fPOM fraction was enriched in carbon under giant knotweed which is producing litter with an unfavourable composition for degradation. This coincided with low MBC and basal respiration. From assessment of SOM quality in forest soils, it is well known that the fPOM fraction becomes the more dominant, the less favourable the litter and the lower the pH values are (Koch, 2015). Thus, the carbon stock in the fPOM fraction (1,610 ± 451 kg C ha^{-1}) was twice that of the benchmark system (765 ± 142 kg C ha^{-1}).

In contrast to that, more easily degradable litter, for example from reed canary grass or Virginia mallow, seems to be faster incorporated and physically protected in the heavier soil fractions (hoPOM, MOM). Thus, these two species significantly increased the carbon stocks in the hoPOM and MOM compared to the benchmark system by nearly 1,300 kg C ha^{-1} (reed canary grass, hoPOM) and slightly more than 2,000 kg C ha^{-1} (Virginia mallow, MOM) after 5 years of cultivation in the topmost ten centimetres of soil. Similar observations were made by DeGryze *et al.* (2004) and Kantola *et al.* (2017). However, due to the non-harmonized sample pretreatment and density fractionation methods, direct comparisons to other studies are difficult. Although it was not expected after only 5 years of PEC cultivation, significant increases in the carbon content and SOC stock in the MOM fraction were determined in the framework of this study. In line with this result, also DeGryze *et al.* (2004) detected distinct rises in the mineral-associated organic matter pool in the upper seven centimetres 10 years after a land-use change from agricultural land to afforested and successional systems. They calculated carbon accumulation rates in the MOM of 415 kg C ha^{-1} $year^{-1}$ in the topmost seven centimetres for the successional system. The accumulation rates observed in this study for tall wheatgrass and Virginia mallow were quite similar with 502 and 401 kg C ha^{-1} $year^{-1}$, respectively.

5 | CONCLUSION

The results of this study demonstrated that PECs may modify soil properties in a species-specific manner. Nonetheless, compared to the annual benchmark system in this study, all perennial systems were characterized by higher soil microbial activity and formation of SOC. Enhanced SOC contents were observed for all soil fractions investigated (fPOM, loPOM, hoPOM, MOM) substantiating the assumption of higher input of organic substances from both, above and belowground sources to the soils cultivated with PECs. However, the SOC accumulation in the different fractions was species-specific likely resulting from different litter qualities. The significantly higher SOC contents particularly in the heavier fractions (hoPOM, MOM) further indicate that the dynamic equilibrium between C-input, assimilation and humification has not yet been reached after 5 years of PEC cultivation in the topmost ten centimetres of soil. There was reasonably indicated that grass species outclass forbs with regard to promote soil microbial activity and long-term carbon sequestration potential. According to the elevated activity of 1,4 β-glucosidase activity, there are credible reasons to assume that PEC cultivation systems have a great potential to store significant amounts of stabilized SOM. It is hypothesized that after a possible recultivation of PEC fields, these SOM pools will not readily be mineralized and thus sustaining the soils' carbon storage and soil quality. Nonetheless, long-term research and soil monitoring of PEC cultivation, particularly of deeper soil layer, is crucial in order to assess the absolute potential of these cultivation systems for carbon sequestration. In this context, changes in SOC and distribution among different fractions in the subsoil are supposed to be the key in durable carbon sequestration.

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