







## RESEARCH ARTICLE

# Marginal land conversion to perennial energy crops with biomass removal enhances soil carbon sequestration

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## Abstract

Marginal land conversion to perennial energy crops can provide biomass feedstocks and climate change mitigation. However, the effect of perennial energy crop cultivation on soil organic carbon (SOC) sequestration and its underlying mechanism in marginal land still remains incomplete. Here, SOC turnover, stability, and its potential sequestration were evaluated based on 10 years of land use change from C<sub>3</sub> grass-dominated marginal land to C<sub>4</sub> energy crops *Miscanthus* and switchgrass cultivation. The naturally occurring <sup>13</sup>C signature down to 60 cm depth was used to determine the energy crops-derived C. Compared to reference marginal land, *Miscanthus* plantation increased the SOC stock at 0–60 cm depth by 17.8% and 64.3% in bulk and root zone, respectively. Similarly, the SOC stock under switchgrass was also 16.5% and 93.0% higher in bulk and root zone than in reference marginal land, respectively. The higher SOC stock in the root zone of switchgrass relative to *Miscanthus* was supported by the higher contribution of C<sub>4</sub>-derived C to SOC (44.5% vs. 32.4%). The mean residence time of old C was higher under switchgrass than *Miscanthus* in the bulk zone across 0–60 cm ( $p < 0.05$ ) but remained the same at 0–20 cm in the root zone. Specific SOC mineralization and temperature sensitivity were lower in soils under *Miscanthus* and switchgrass compared to reference marginal land. The partial least squares path model revealed that perennial energy crop cultivation enhances soil C stock via increased C<sub>4</sub>-derived C input and reduced mineralization. In conclusion, marginal land conversion to perennial energy crops is a win–win strategy for C sequestration to mitigate climate change and support the growing bioenergy sector with biomass supply.

## KEYWORDS

<sup>13</sup>C natural abundance, C<sub>3</sub>–C<sub>4</sub> vegetation change, marginal land, *Miscanthus*, soil C sequestration, switchgrass



## 1 | INTRODUCTION

Soil is the largest carbon (C) reservoir in the terrestrial biosphere containing three times as much C to depths of 1 m as the atmosphere (van Groenigen et al., 2011). Thus, even small proportions of soil C loss could induce large fluctuations in atmospheric CO<sub>2</sub> and trigger feedback on climate change (Bradford et al., 2016; Lal, 2004). Arable land conversion to perennial energy crops has been shown to increase soil C stocks on both regional and global scales (Chen, Lærke, & Jørgensen, 2022; Chen, Manevski, et al., 2022; Ledo et al., 2020). Thus, perennial energy crop cultivation can serve as a potential strategy for climate change mitigation. However, to avoid land conflict with food production, perennial energy crops must be cultivated on marginal land, which is unsuitable for food crop cultivation due to low fertility or high environmental stress. It is estimated that the marginal land area available for energy crop cultivation was 184.9 Mha accounting for 19.2% of the total land area in China (Zhang, Hastings, et al., 2020). Thus, marginal land conversion to perennial energy crops has the potential to provide biomass feedstocks for renewable energy and contribute to climate change mitigation. Yet, this strategy fails to consider the long-term effect on soil organic carbon (SOC) stocks under energy crop cultivation in marginal land, which may impede an adequate estimation of the sustainability of biomass plantations.

Plant-derived C input is a major source contributing to the C stocks under perennial energy crops (Rees et al., 2005). Nearly half of plant assimilated C is usually transferred to soil, either in the form of rhizodeposition (i.e., low molecular weight compounds) released from living roots and root litter input after harvest (Pausch & Kuzyakov, 2018). Given that the aboveground biomass of energy crops would be removed, it may cause an alteration in perennial energy crops-derived C input and consequently SOC stocks. For example, unchanged or even increased SOC stocks were found in various environments despite aboveground biomass removal due to the high belowground C input (Martani et al., 2021; Xu et al., 2021; Zhuang et al., 2013). As a plant with a C<sub>4</sub> photosynthetic pathway, energy crops produce tissue C and, finally, SOC with a <sup>13</sup>C signature that differs from the one of SOC in soils with prevailing C<sub>3</sub> vegetation. Thus, the C derived from the original (C<sub>3</sub>) and C<sub>4</sub> energy crops can be distinguished based on changes in the δ<sup>13</sup>C signature (Flessa et al., 2000; Zang et al., 2018). Though, most previous studies investigating the contribution of C<sub>4</sub>-derived C to SOC were conducted in grassland or agriculture ecosystems (Holder et al., 2019; Leifeld et al., 2021; Poeplau & Don, 2014; Zatta et al., 2014). So far, the accumulation of C<sub>4</sub>-derived C in marginal lands with low soil fertility and

high abiotic stress has not yet been studied. In addition, most studies available on the changes in soil C after perennial energy crops cultivation have not separated SOC into new and old pools, which may cause a vague estimation of the effects of land use on soil C dynamics as the old pools to have a much longer mean residence time (MRT) than labile pools (Novara et al., 2013; Zang et al., 2018).

In addition to C input, the C loss via mineralization is also a vital factor affecting SOC stocks (Mary et al., 2020; Zhou, Wen, et al., 2021). Typically, perennial energy crop cultivation reduces soil C mineralization by enhancing physical protection derived from aggregation due to no-till systems, a large number of root exudates, and a longer growth period (Austin et al., 2017; Sartori et al., 2006; Tiemann & Grandy, 2015). Therefore, perennial energy crop cultivation has the potential to enhance soil C sequestration by reducing soil C mineralization. Collectively, how marginal land conversion to perennial crop cultivation affects soil C stocks and its controlling mechanisms remains elusive.

We established a 10 years field study with vegetation change from C<sub>3</sub> grass-dominated marginal land to C<sub>4</sub> energy crop cultivation. *Miscanthus* and switchgrass were selected as leading energy crops suitable for marginal land cultivation. *Miscanthus* has a coarse and broad root system, while switchgrass has a fine and deep root system (Winkler et al., 2020; Xue et al., 2015; Zheng et al., 2019). We aimed to (1) evaluate soil C sequestration from the conversion of marginal land to *Miscanthus* and switchgrass; (2) quantify the plant-derived C input and turnover, as well as soil C mineralization; and (3) identify the controlling factors for soil C sequestration under energy crops in marginal land.

## 2 | MATERIALS AND METHODS

### 2.1 | Experimental setup

The field was located at the Hunan Agricultural University experimental station, Liuyang, Hunan province, China (27°51'N, 113°10'E, 11.4 m.a.s.l.). The average temperature and rainfall were 17.4°C and 1529 mm, respectively. The soil (collected in April 2011 at a depth of 0–20 cm before the field trial establishment) is loam soil with a pH of 5.12 and contained 5.19 g kg<sup>-1</sup> soil organic matter, 30.63 mg kg<sup>-1</sup> available nitrogen, 2.69 mg kg<sup>-1</sup> available phosphorus, and 90.77 mg kg<sup>-1</sup> available potassium. This land can be classified as marginal land since it is unsuitable for food crop cultivation due to low soil fertility and severe acidification (Fu et al., 2022).

Before the establishment of the experiment, the site was an abandoned land dominated by a mixture of C<sub>3</sub>

weeds for more than 20 years. The field experiment was conducted as a randomized block design with three treatments. Each treatment contained three replicates, with a 37.5 m<sup>2</sup> (5 m × 7.5 m) plot size. The three treatments included two energy crops: *Miscanthus* (*Triarrhena lutarioriparia* L., hybrid Xiangzamang NO.1) and switchgrass (*Panicum virgatum* L., lowland ecotype, Alamo), as well as a C<sub>3</sub> reference grassland. The reference grassland was dominated by a C<sub>3</sub> weed mixture (*Cyperus rotundus* L. and *Setaria viridis* L.) without human disturbance. The energy crops were planted with a row space of 1 m and a plant space of 1 m. Aboveground biomass was harvested annually from late November to early December for bioenergy production. No additional management practices (e.g., fertilization, irrigation, weeding, and pest control) were used.

## 2.2 | Plant and soil sampling

Soil and plant samples were collected in January 2021, corresponding to a cultivation period of 10 years. For energy crop plots, soil cores were taken in two different positions to account for the different inter-row spacing for each species due to the tussock forming condition of *Miscanthus* and switchgrass (Martani et al., 2021). For the energy crop plot, five energy crop plants in the “S” pattern were randomly selected and harvested for further isotopic analysis. Soil samples from each plot were obtained after harvesting biomass using a hand-operated soil core (diameter of 4 cm) down to a depth of 60 cm according to the following steps: (1) 10 cm from the center of the plant four soil cores in four locations were collected and mixed to get a root zone (R) composite soil sample (Figure 1); (2) 10 cm from the edge of the plant (i.e., between the plant rows) four soil cores were collected and mixed to get a bulk zone (B) composite sample (Figure 1); and (3) the 20 cores (5 plants × 4 positions) from the root zone and the bulk zone

was mixed to get a final composite soil sample from each plot. For C<sub>3</sub> reference plots, 10 randomized soil cores were pooled to form a mixed soil sample in each plot. Soil cores were divided into four depth intervals (0–10 cm, 10–20 cm, 20–40 cm, and 40–60 cm). For each of these depths, soil samples were divided into two sub-samples, with one stored at room temperature to measure soil C and isotopic signature, and another stored at 4°C to measure enzyme activity within 1 week. Additional undisturbed soil samples were taken to determine the bulk density and soil aggregate separation. The dry sieving method was used to separate soil aggregate (Yan et al., 2022).

## 2.3 | Isotopic analysis

Soil samples were air-dried at room temperature and sieved (<2 mm) where all visible root and plant residues were removed, and the soil was milled. Plant samples (roots and rhizomes) were dried at 60°C and ball-milled. The organic C and δ<sup>13</sup>C signature of the plant and SOC and total nitrogen were measured using an ANCA-IRMS (PDZE Europa Limited).

## 2.4 | Soil enzyme activities

Three hydrolytic enzymes related to soil C cycling (β-glucosidase, BG, β-cellobiohydrolase, CEL, and β-xylosidase, XYL) were measured using fluorogenic labeled substrates (Ma et al., 2022; Zhang, Kuzyakov, et al., 2020; Zhou, Gui, et al., 2021). Briefly, 1 g of fresh soil was suspended in 50 ml of sterile water. Then, 50 μl aliquot of the soil suspension was pipetted into 96-well microplates, and mixed with 50 μl of buffer and 100 μl of the corresponding substrates. The microplates were measured at 60 and 120 min after substrate addition fluorometrically at an excitation wavelength of 360 nm and an emission

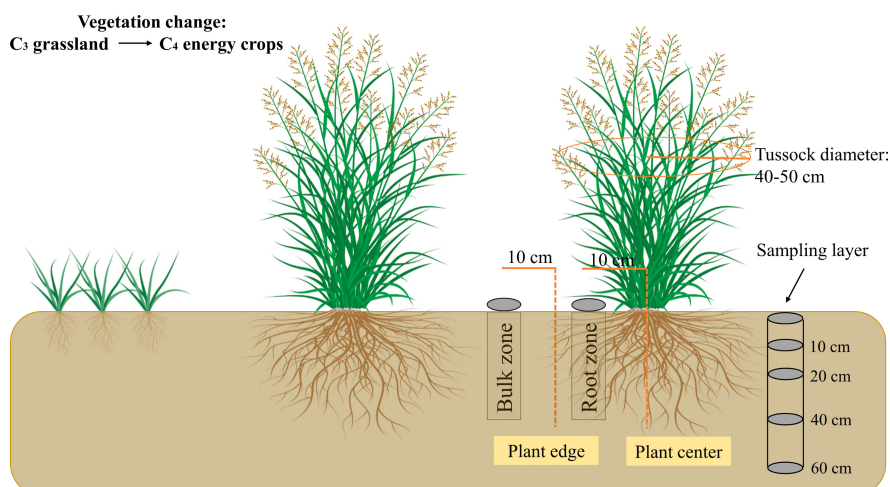


FIGURE 1 Schematic diagram of soil sampling strategy for the perennial energy crops.

wavelength of 450 nm (Thermo Fisher Scientific). The enzyme activities were expressed per SOC unit (i.e., specific activities) as C is an important determinant of below-ground functioning (Blagodatskaya & Kuzyakov, 2013; Sinsabaugh et al., 2009). The three enzyme activities were averaged to represent the C-acquiring enzyme activities (Jia et al., 2022; Luo et al., 2018).

## 2.5 | Incubation experiment

Fresh soil samples from all soil depths (0–20 cm, 20–40 cm, and 40–60 cm) were weighed (equivalent to 10 g dry mass) and placed into 50 ml polypropylene containers with four replicates for each treatment. The soil was adjusted to 60% water-holding capacity by adding distilled water. The bottles were sealed and pre-incubated in the dark at 15 and 25°C for 5 days and then incubated for 40 days at the corresponding temperatures. During the incubation, the CO<sub>2</sub> evolved from the soils was trapped by 1.5 ml NaOH (1 M) in a small beaker which was exchanged at 1, 3, 5, 12, and 20 days. The air inside the bottles was changed at each replacing time via aeration for 30 min to avoid the anaerobic condition. Soil moisture was maintained (not generally decreased by more than 10%) during the incubation by weighing and spraying distilled water evenly over the soil surface. Four bottles at each temperature without soil samples were treated in the same way and used as blanks to correct the CO<sub>2</sub> trapped in the air. The efflux of CO<sub>2</sub> trapped in the NaOH solution was measured by titration with 0.01 M HCl against phenolphthalein after the addition of 1 M BaCl<sub>2</sub> solution (Zang et al., 2016). Finally, the specific mineralization was calculated as CO<sub>2</sub> release per unit of SOC.

## 2.6 | Calculation and statistical analysis

The proportional contributions of the C<sub>3</sub> ( $f_{C_3}$ ) and the C<sub>4</sub> ( $f_{C_4}$ ) sources to total SOC were calculated according to Amelung et al. (2008):

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

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

where  $\delta^{13}C_t$  is the  $\delta^{13}C$  value of the soil under *Miscanthus* or switchgrass and  $\delta^{13}C_3$  is the  $\delta^{13}C$  value of the corresponding layer in the reference soil under C<sub>3</sub> grasses,  $\delta^{13}C_4$  indicates the  $\delta^{13}C$  value of *Miscanthus* or switchgrass root. The C<sub>3</sub>- and C<sub>4</sub>-derived C were considered as old and new C hereafter.

The MRT was calculated as the reciprocal of the turnover rate as follows (Amelung et al., 2008; Gregorich et al., 1995):

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

where  $k$  means the turnover rate,  $t$  indicates the number of years after vegetation change (10 years in the present study), and  $f_{C_4}$  is the proportional contribution of the C<sub>4</sub> (energy crop-derived) source to the total C pool.

The SOC stock for a specific layer was calculated as follows:

$$C_i = SOC \times BD_i \times H_i, \quad (4)$$

where  $C_i$  is the SOC stock (t ha<sup>-1</sup>) for different soil layers;  $BD_i$  represents the soil bulk density in the corresponding soil layer, and  $H_i$  refers to the thickness of the corresponding soil layer (m).

The temperature sensitivity ( $Q_{10}$ ) of SOC mineralization was determined based on CO<sub>2</sub> efflux rates at two temperatures at the same incubation date [Equation (5)] (Zang et al., 2020).

$$Q_{10} = R_{25} / R_{15}, \quad (5)$$

where  $R_{25}$  and  $R_{15}$  are the specific SOC mineralization rates at 25 and 15°C, respectively.

All statistical analyses were performed with SPSS 25.0 software (SPSS Inc.). Normality and homogeneity of variance (Levene's tests) were confirmed before testing for significant differences. A two-way analysis of variance (ANOVA) was conducted to evaluate the main effects of an energy crop and soil depths as well as their interactions on soil properties. For each dependent variable, additional one-way ANOVAs with Duncan's multiple range tests were conducted to determine significant differences among energy crops or soil depths. The partial least squares path modeling (PLS-PM) was conducted to analyze the direct and indirect effects of energy crop-derived C, SOC mineralization, temperature sensitivity, and C-acquiring enzyme activity on the SOC stocks using the SMARTPLS software (version 3.3.5) after 1000 bootstraps (Barberán et al., 2014).

## 3 | RESULTS

### 3.1 | SOC stock

After 10 years of energy crop cultivation, the SOC stock between 0 and 60 cm was higher under *Miscanthus* and



switchgrass than in the  $C_3$  reference grassland ( $p < 0.05$ ; Figure 2a). The highest SOC stock occurred in the switchgrass ( $57.0 \pm 1.9 \text{ t ha}^{-1}$ ) and *Miscanthus* ( $48.6 \pm 1.2 \text{ t ha}^{-1}$ ) root zone, which were 93.0% and 64.3% higher than the  $C_3$  reference grassland, respectively (Figures 2a and 6). Here, *Miscanthus* and switchgrass cultivation of the root zone increased the SOC stock between 0–40 cm ( $p < 0.05$ , Figure 2). The SOC stock in the bulk zone of *Miscanthus* and switchgrass from 0–60 cm was 28.3%–39.6% lower than the root zone, but 16.5%–17.8% higher in comparison

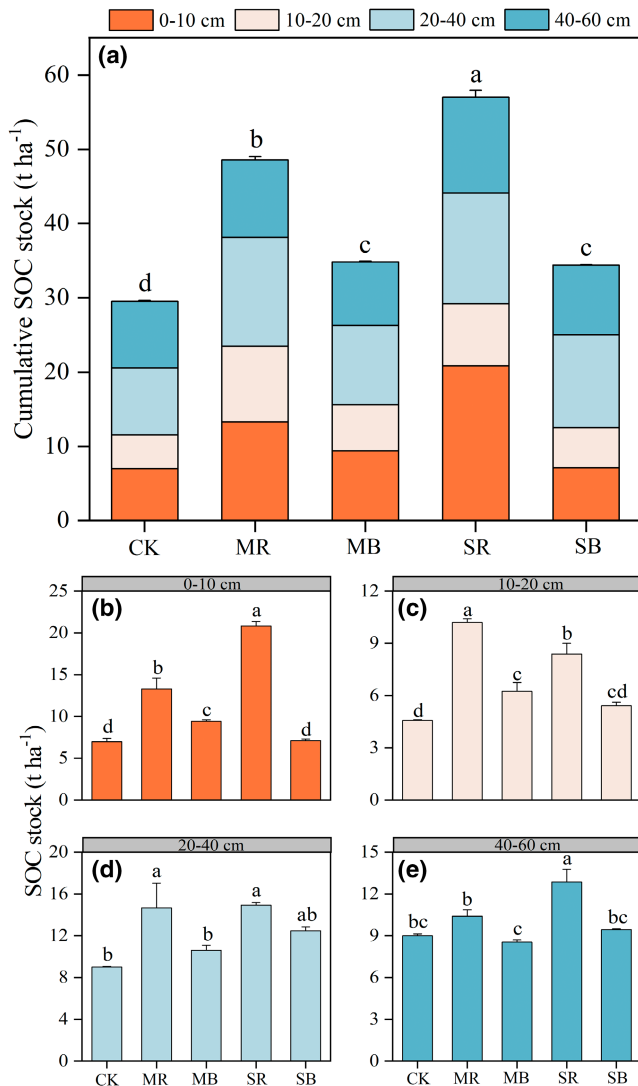
to the  $C_3$  reference grassland (Figure 2). In the bulk zone, *Miscanthus* increased the SOC stock between 0 and 20 cm by 35.2%–37.0% relative to the  $C_3$  reference grassland ( $p < 0.05$ ), whereas there was no effect between 20–60 cm depth (Figure 2). The SOC stock did not statistically differ at all soil depths in the bulk zone of switchgrass as compared to the  $C_3$  reference grassland (Figure 2).

### 3.2 | SOC turnover and derived from energy crops

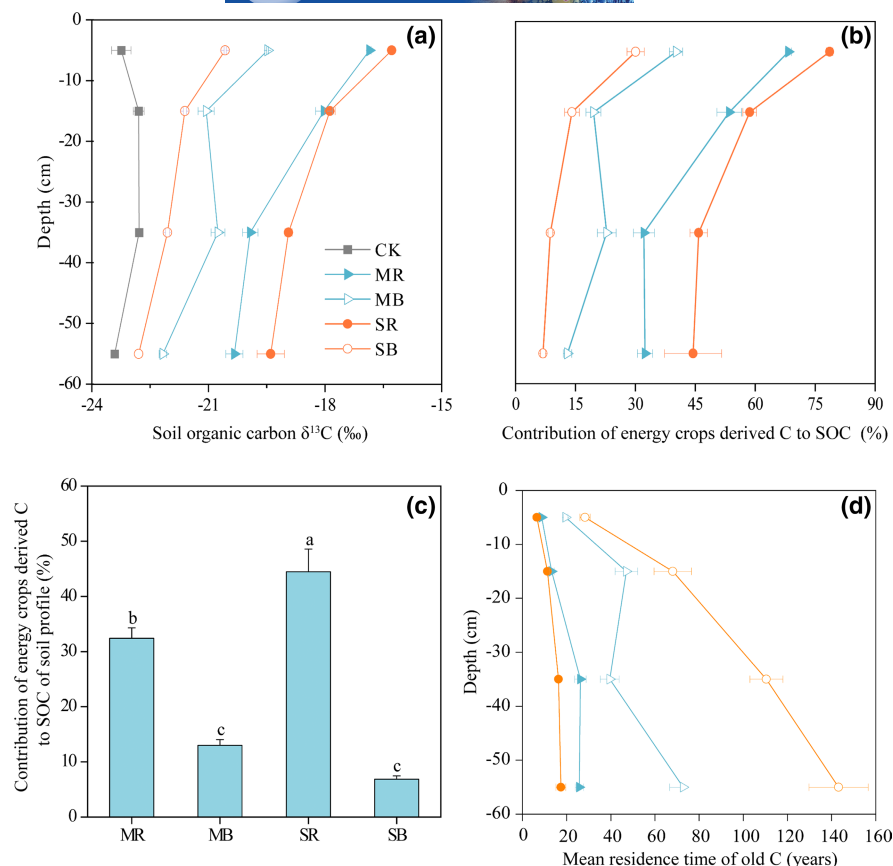
Energy crop cultivation increased  $\delta^{13}\text{C}$  values at all soil depths relative to the  $C_3$  reference grassland ( $p < 0.05$ , Figure 3a). The  $\delta^{13}\text{C}$  value was higher in the root than in the bulk zone for switchgrass and *Miscanthus*. The  $\delta^{13}\text{C}$  value strongly decreased with depth in the root zone both in switchgrass and *Miscanthus* from  $-16.9\text{‰}$  to  $-20.3\text{‰}$  ( $p < 0.05$ ), where it marginally decreased with depth in the bulk zone both in switchgrass and *Miscanthus* from  $-19.5\text{‰}$  to  $-22.8\text{‰}$  ( $p > 0.05$ ). Based on the  $\delta^{13}\text{C}$  values, the contribution of energy crop-derived C in the root zone was 0.4–5.5 times higher than in the bulk zone at all depths. The amount of  $C_4$ -derived C was 9.6%–42.7% higher under switchgrass than under *Miscanthus* in the root zone, where it was 25.2%–61.7% lower under switchgrass relative to *Miscanthus* in the bulk zone ( $p < 0.05$ , Figure 3b). The contribution of  $C_4$ -derived C to SOC between 0 and 60 cm soil profile was around 44.5% and 32.4% C under switchgrass and *Miscanthus* in the root zone, while it was 6.9% and 13.0% in the bulk zone, respectively (Figure 3c). Furthermore, the MRT of old C rapidly increased with soil depth in the bulk zone under *Miscanthus* and switchgrass, where this trend was more pronounced under switchgrass increasing from 28.3 to 143.2 years (Figure 3d). Conversely, the MRT of old C in the root zone remained stable between 0 and 60 cm, regardless of energy crop species ( $p > 0.05$ ).

### 3.3 | Soil carbon mineralization and temperature sensitivity

The specific SOC mineralization between 0 and 60 cm was lower under *Miscanthus* and switchgrass than in the  $C_3$  reference grassland at both 15 and 25°C ( $p < 0.05$ ), except for soil under *Miscanthus* in the bulk zone at 15°C (Figure 4). The lower specific SOC mineralization of *Miscanthus* ( $336.1 \text{ mg C kg}^{-1} \text{ SOC}$ ) and switchgrass ( $288.1 \text{ mg C kg}^{-1} \text{ SOC}$ ) in the root zone at 15°C were 26.2% and 36.7% lower than the  $C_3$  reference grassland, respectively (Figure 4a). Similar to the specific SOC mineralization, the  $Q_{10}$  of *Miscanthus* from the root and bulk zone were 25.0% and



**FIGURE 2** Soil organic carbon (SOC) stock between 0 and 60 cm after 10 years of cultivation of *Miscanthus* and switchgrass. SOC stock of the whole profile (a), 0–10 cm (b), 10–20 cm (c), 20–40 cm (d), and 40–60 cm (e). Values are means ( $\pm$ SE) of three replicates. Lower-case letters indicate significant differences among treatments ( $p < 0.05$ ). CK indicates  $C_3$  reference grassland; MR indicates soil under *Miscanthus* in the root zone; MB indicates soil under *Miscanthus* in the bulk zone; SR indicates soil under switchgrass in the root zone; SB indicates soil under switchgrass in the bulk zone.



**FIGURE 3** Soil organic carbon (SOC)  $\delta^{13}\text{C}$  values (a), the contribution of energy crops-derived C to SOC in different soil layers (b), the contribution of energy crops-derived C to SOC in the whole soil profile (c), and the MRT of old C (d) in different soil layers after 10 years of *Miscanthus* and switchgrass cultivation. Values are means ( $\pm$ SE) of three replicates. CK indicates C<sub>3</sub> reference grassland; MR indicates soil under *Miscanthus* in the root zone; MB indicates soil under *Miscanthus* in the bulk zone; SR indicates soil under switchgrass in the root zone; SB indicates soil under switchgrass in the bulk zone.

18.0% lower than the C<sub>3</sub> reference grassland, respectively ( $p < 0.05$ ).

### 3.4 | Factors controlling variation in SOC stocks

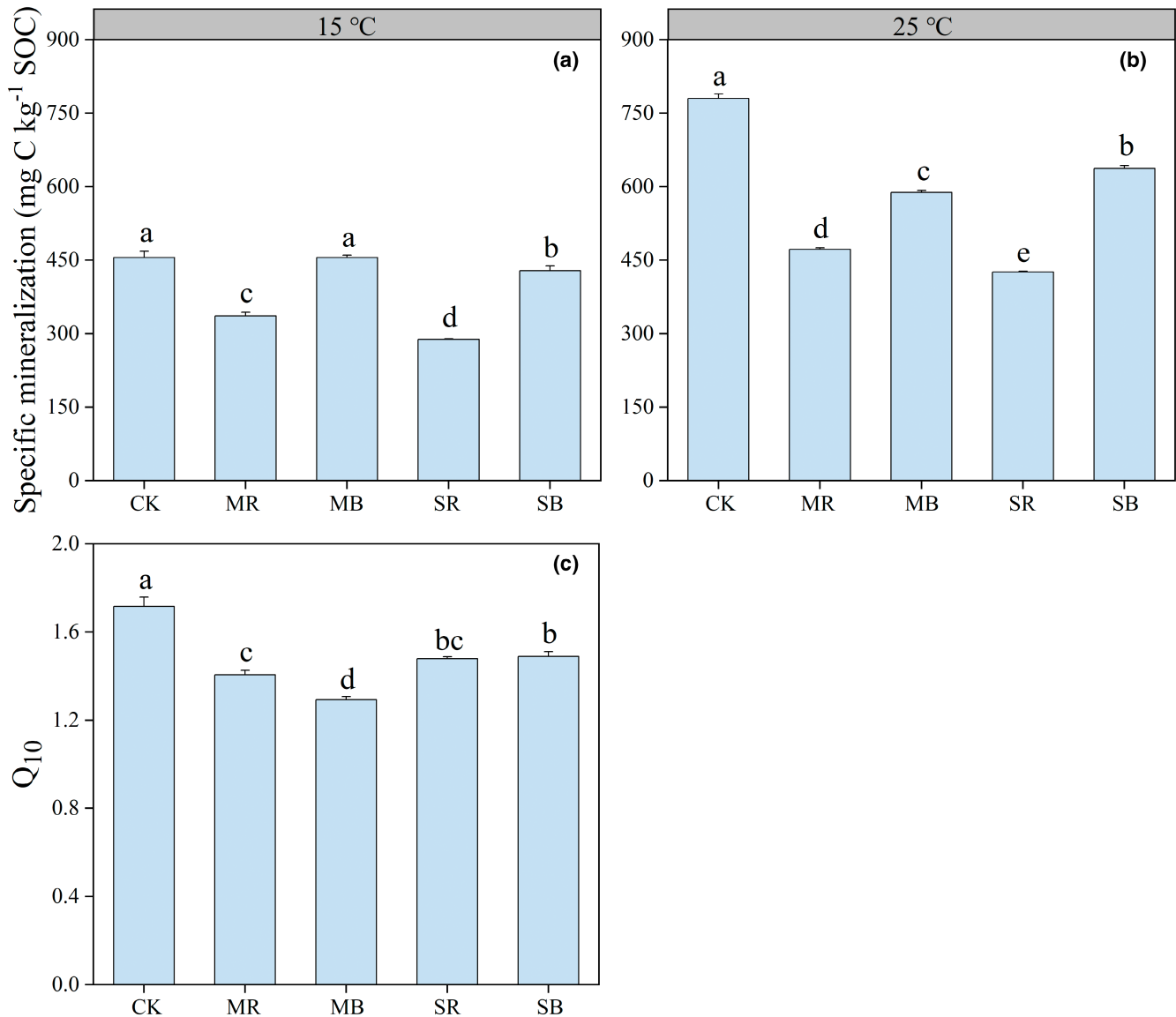
The constructed PLS-PM displayed a good fit ( $\text{GOF} = 0.62$ ) and could explain 46.70% of the variation in the SOC stocks (Figure 5). The PLS-PM also revealed a direct positive effect of C<sub>4</sub>-derived C on SOC (1.016), and a direct negative response of SOC to SOC mineralization ( $-0.623$ ), and  $Q_{10}$  ( $-0.146$ ), as well as C-acquiring enzyme activity ( $-0.300$ ; Figure 5). Overall, energy crops-derived C was the most important regulator for soil C stocks.

## 4 | DISCUSSION

### 4.1 | SOC sequestration

Our results revealed that long-term perennial energy crop cultivation increases soil C stocks despite the complete removal of aboveground biomass (Figures 1a and 6). An increased C stock was also found in a broad range of marginal lands including saline-alkaline soil, semi-arid degraded land, and abandoned cropland with biomass

removal (Mi et al., 2014; Xu et al., 2021; Zhao et al., 2020). Although both *Miscanthus* and switchgrass cultivation lead to C sequestration, they differ in sequestration potential and regulatory mechanisms. Specifically, the growth of switchgrass induced higher SOC stocks than *Miscanthus*, particularly in deeper soil layers (40–60 cm; Figure 1). This is irrespective of similar biomass yields (Figure S1). Therefore, the higher potential for SOC sequestration of switchgrass can be attributed to the following two reasons. First, a large proportion of switchgrass fine roots (with a root diameter less than 2 mm) extended into the deeper soil layers with a subsequently greater rhizodeposition than *Miscanthus*, thereby stimulating C<sub>4</sub>-derived C contribution to SOC (Laurent et al., 2015; Powlson et al., 2011). This was confirmed by the higher contribution of switchgrass-derived C to SOC relative to *Miscanthus* in the root zone (44.5% vs. 32.4%; Figure 3c). Second, the higher potential stabilization of SOC in response to less C mineralization under switchgrass (Figure 4) also contributed to the high C sequestration potential under switchgrass. The lower SOC mineralization under switchgrass was confirmed by the lower C-acquiring enzyme activities (Figure S3). The more persistent SOC, reflected by longer MRT, under switchgrass may also contribute to its high SOC sequestration (Sprunger & Robertson, 2018). Here, the MRT of old C<sub>3</sub>-C under switchgrass was significantly higher than *Miscanthus* in the bulk zone (Figure 3). This



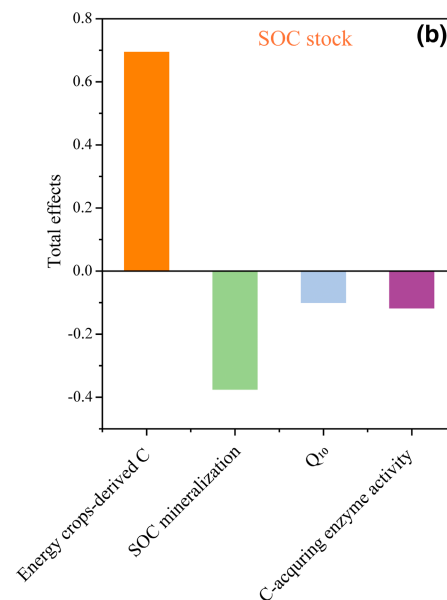
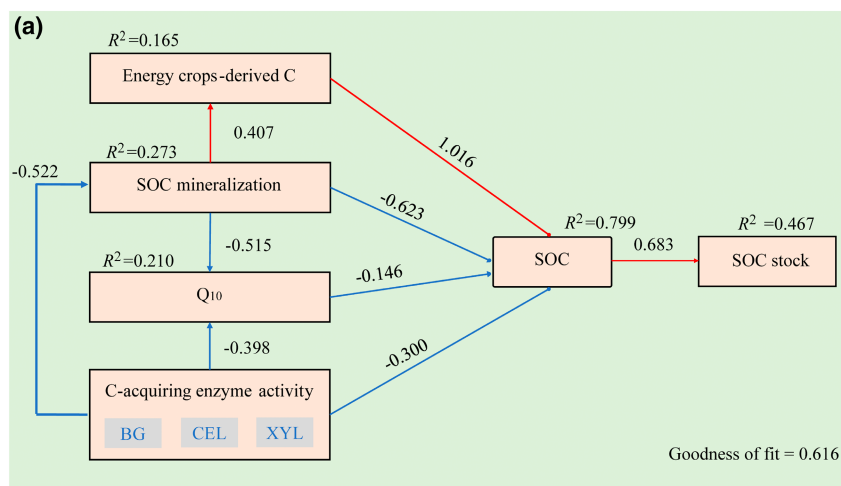
**FIGURE 4** The specific soil organic carbon (SOC) mineralization at 15°C (a) and 25°C (b) and temperature sensitivity (c) between 0 and 60 cm after 10 years of *Miscanthus* and switchgrass cultivation. Values are means ( $\pm$ SE) of three replicates. CK indicates C<sub>3</sub> reference grassland; MR indicates soil under *Miscanthus* in the root zone; MB indicates soil under *Miscanthus* in the bulk zone; SR indicates soil under switchgrass in the root zone; SB indicates soil under switchgrass in the bulk zone.

indicates a lower decomposition rate of old C, thereby facilitating C sequestration (Zang et al., 2018). Collectively, the higher C sequestration under switchgrass was driven by both high C<sub>4</sub>-C accumulation and SOC stability.

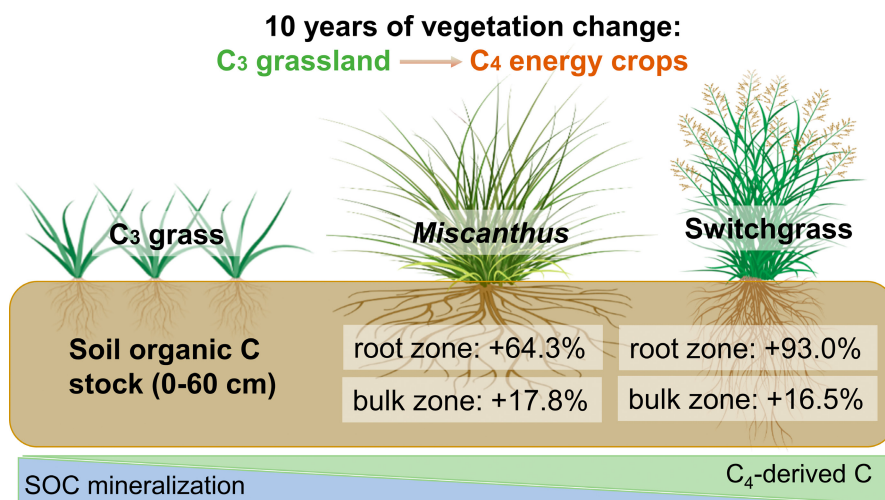
## 4.2 | Underlying mechanisms of SOC sequestration

Our results showed that a greater C<sub>4</sub>-derived C input is the most important factor for increasing SOC stocks (Figure 5). After 10 years of cultivation, the contribution of C<sub>4</sub> derived-C to SOC between 0 and 60 cm was more than one-third in the root zone. Consistent with our results,

Zang et al. (2018) found that 27% of SOC was C<sub>4</sub>-derived C after 9 years of *Miscanthus* cultivation. The PLS-PM model further confirmed that the C<sub>4</sub> derived-C displayed the greatest total effect on the SOC stocks (Figure 5). The increased new C accumulation in the present study can be attributed to the large belowground biomass production and root exudation of perennial energy crops (Clifton et al., 2007). The root to shoot ratio of perennial energy crops is typically greater than 1 and increases with the duration of growth (Xue et al., 2015). Therefore, perennial grasses allocate more C belowground than conventional grasslands (Mi et al., 2014; Zhao et al., 2020). Furthermore, as *Miscanthus* has root crowns, there is a reduction in the proportion of fine roots and consequently



**FIGURE 5** The partial least squares path modeling showing the direct and indirect effects (a) and total effects (b) of energy crops-derived C, soil organic carbon (SOC) mineralization, temperature sensitivity ( $Q_{10}$ ), and C-acquiring enzyme activity, and SOC on the SOC stock. Each box represents an observed (i.e., measured) or latent variable (i.e., constructs). Red and blue arrows indicate positive and negative flows of causality ( $p < 0.05$ ), respectively. Numbers on the arrow indicate significant standardized path coefficients.  $R^2$  indicates the variance of the dependent variable explained by the model.



**FIGURE 6** Graphical abstract illustrating marginal land conversion to perennial energy crops enhances soil carbon sequestration and its underlying mechanisms. *Miscanthus* and switchgrass cultivation increased soil C stocks in both root and bulk zone compared to reference marginal land. New C<sub>4</sub>-C input exceeds old C<sub>3</sub>-C losses via mineralization leading to C sequestration. SOC, soil organic carbon.

reduced C<sub>4</sub>-derived C compared to switchgrass (Bazrgar et al., 2020; Zan et al., 2001). This could be the reason why new C accumulation and subsequent SOC stocks were lower for *Miscanthus* compared to switchgrass in this study.

Perennial energy crop cultivation increased C stocks by reducing SOC mineralization and temperature sensitivity (Figures 4 and 5). Here these crops produce a large amount of belowground biomass resulting in enhanced root exudation facilitating the aggregate formation and enhancing the physical protection against microbial decomposition (Gioacchini et al., 2016; Tiemann & Grandy, 2015; Yan et al., 2022). This was supported by a large proportion

of macroaggregates under *Miscanthus* and switchgrass compared with the C<sub>3</sub> grassland (Figure S4). Specifically, the reduced mineralization was more pronounced in the root zone than in the bulk zone under both *Miscanthus* and switchgrass, which may be due to the negative priming effect caused by perennial energy crop cultivation (Gauder et al., 2016). The lower pH in the root zone under *Miscanthus* and switchgrass relative to the bulk zone between 0 and 60 cm (Table S1) could constrain microbial functioning, thereby decreasing SOC mineralization (Malik et al., 2018). Furthermore, the lower temperature sensitivity of SOC mineralization under energy crop cultivation suggests a more stable and resistant SOC in the



context of global warming (Kan et al., 2020). This suggests that the accumulated SOC under perennial energy crops can be retained in the soil over longer time frames and thereby increasing the SOC sequestration.

The distinct driving pathway of SOC sequestration by these perennial energy crops was presented in the root zone and bulk zone. In the root zone, perennial energy crops sequestered SOC via a fast SOC turnover rate where new  $C_4$ -derived C replaced the old  $C_3$ -C at a rate sufficient to offset losses. In general, the root zone is characterized as a microbial hotspot where enhanced plant-derived C inputs are likely easily metabolized by microorganisms, resulting in an intense turnover of microbial biomass and a larger accumulation of necromass (Angst et al., 2021; Kuzyakov & Blagodatskaya, 2015). This was supported by higher C-acquiring enzyme activities and higher  $C_4$ -derived C in the root zone relative to the bulk zone under switchgrass and *Miscanthus* (Figures S1 and S3). In contrast to the root zone, the increased C stock in the bulk zone was mainly ascribed to the increased stability of old C. The high stability of old C was reflected by the higher MRT of old C in the bulk zone relative to the root zone (Figure 3 and Figure S2), which indicated that the low decomposition rate of old C leads to higher persistence in soil (Rahmati et al., 2020). Consistent with this, the  $C_3$ -derived SOC was lower in the root zone relative to the bulk zone under perennial energy crops (Figure S2). In addition, the  $C_4$ -derived C was relatively low (<15%) in the bulk zone, especially in the subsoil (20–60 cm), suggesting less contribution of  $C_4$ -derived C for SOC sequestration in the bulk zone. Collectively, the increased stability of old C was the dominant driving factor for C sequestration in the bulk soil.

The present study revealed the main controlling factors for soil C sequestration under energy crops in marginal land, which advances our knowledge of C dynamics within bioenergy systems. An improved understanding of C dynamics within bioenergy systems will also help to project C sequestration globally. Additionally, this knowledge could support environmental management strategies associated with biofuel production, while also informing policy development and financial incentives available to landowners (e.g., C offsets), which can encourage farmers to convert degraded agricultural lands into more sustainable and environmentally benign biomass production systems.

## 5 | CONCLUSION

Overall, our results showed that switchgrass had a higher C sequestration potential in deep soil layers within the root zone, while *Miscanthus* had a broader effect

on C sequestration, particularly between 0 and 20 cm. Switchgrass is thereby preferred over *Miscanthus* as an energy crop for marginal land cultivation due to comparable biomass yield but much higher C sequestration. The large proportion of energy crop-derived C was the most important factor contributing to increased soil C stock. In addition, the increased C stability and reduced C mineralization were also important factors influencing C sequestration. The pathway of C sequestration within this system was mainly via the fast replacement of old C with  $C_4$ -derived C in the root zone. In conclusion, marginal land conversion to perennial energy crops has the potential to provide biomass feedstocks for renewable energy and contribute to climate change mitigation.

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## CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## DATA AVAILABILITY STATEMENT


Data openly available in a public repository. The data that support the findings of this study are openly available in researchgate (<https://www.researchgate.net/>). The DOI of the data is <https://doi.org/10.13140/RG.2.2.12463.00163>.

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Additional supporting information can be found online in the Supporting Information section at the end of this article.

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