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Global Change Biology Bioenergy
BIOPRODUCTS FOR A SUSTAINABLE BIOECONOMY

RESEARCH ARTICLE OPEN ACCESS

A 13-Year Record Indicates Differences in the Duration and Depth of Soil Carbon Accrual Among Potential Bioenergy Crops

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Received: 14 April 2025 | **Revised:** 31 July 2025 | **Accepted:** 5 August 2025

Funding: This work was supported by Leverhulme Research Centre Award (RC-2015-029) and U.S. Department of Energy (DE-SC0018420).

Keywords: agriculture | bioenergy | carbon isotope | miscanthus | restored prairie | row crop | soil health | switchgrass

ABSTRACT

Six years after replacing a maize/soybean cropping system, perennial grasses miscanthus (*Miscanthus × giganteus*) and switchgrass (*Panicum virgatum*), and a 28-species restored prairie increased particulate organic carbon in surface soils without increasing soil organic carbon (SOC). To resolve potential changes in the quantity and distribution of SOC, soils were resampled after seven to thirteen years to measure bulk density, carbon (C) content, and stable C isotopes to a depth of 1 m. SOC stocks increased between 1.75 and 2.5 Mg ha^{-1} year^{-1} in all perennial crops between 2008 and 2016 (nine growing seasons). Despite relatively low litter inputs and belowground biomass, the highest rate of SOC accrual was in restored prairie (2.5 Mg ha^{-1} year^{-1}), followed by miscanthus (2.0 Mg ha^{-1} year^{-1}) and switchgrass (1.75 Mg ha^{-1} year^{-1}). The change in SOC in maize/soybean was not significant. After 2016, total SOC decreased in maize/soybean and miscanthus, resulting in slower overall rates of SOC accumulation over the full sampling period for miscanthus (0.8 Mg ha^{-1} year^{-1}). The rate of SOC accumulation was greatest below 50 cm depth for restored prairie and switchgrass but in the top 10 cm for miscanthus. Stable isotope analysis showed ^{13}C enrichment in all depths of switchgrass soils, an indication of new organic C accumulation, but mixed results in all other crops. Planting perennial crops on land formerly in an annual maize/soybean cropping system can slow or reverse soil carbon losses, with the greatest increases in SOC from species-rich prairie.

1 | Introduction

More than 36 M hectares of maize and over 32 M hectares of soybeans are planted in the United States each year (USDA NASS 2022; Hatfield et al. 2018), and intensive management of these crops drives substantial losses of soil organic carbon (Wander et al. 1998; Zinn et al. 2005; Johnston et al. 2009; Crews and Rumsey 2017; Moore et al. 2025). Many of the major losses of C attributed to agriculture occurred during a land use change, often the transition from the native ecosystem to a managed agriculture system, perpetuating C loss through crop removal at harvest and soil management techniques that are destructive to soil structure (Six et al. 2000).

In contrast with annual crops, perennial crop management strategies reduce tillage, a major contributor to C loss on annual systems, and differences in biomass allocation, root structure, and soil microbial communities can increase SOC in perennial systems (Jackson et al. 1996; Jastrow 1996; Paustian et al. 1997; Allison and Jastrow 2006; Grandy and Robertson 2007; Anderson-Teixeira et al. 2009; Duo et al. 2013; Chimento et al. 2014; Ontl et al. 2015). The diversification of agriculture in the Midwest United States through planting perennial grasses has the potential to increase belowground carbon (C) storage and improve soil quality through changes in belowground C allocation and litter inputs (McLauchlan et al. 2006; Fornara and Tilman 2008; Blanco-Canqui 2010). The duration of changes in SOC following planting perennial crops and the distribution of SOC in the soil profile, potentially affecting turnover times, remains uncertain.

Though perennial crops are long-lived, many studies in the United States in perennial crop production are short-term (<6 years; Anderson-Teixeira et al. 2013; Chimento et al. 2014; Boersma and Heaton 2014; Zumpf et al. 2019), leading to overestimation of productivity and an incomplete understanding of the dynamics of SOC storage. Additionally, despite being depleted in SOC compared to the pre-agricultural prairie ecosystem, the background SOC stocks in the Mollisols of Illinois remain high and changes in SOC can be difficult to resolve over short periods of time (Anderson-Teixeira et al. 2013; Kantola et al. 2017). Previous work in perennial bioenergy grasses in Illinois confirmed an increase in particulate organic matter (POM), the low-density, least-decomposed component of soil organic matter, after 6 years (Kantola et al. 2017). However, measurements of total SOC were unable to statistically resolve a change over that period (Anderson-Teixeira et al. 2013; Kantola et al. 2017). In this study, SOC under perennial grasses grown for bioenergy (miscanthus and switchgrass) and restored prairie was compared with conventional row crops over a 7- to 13-year period to identify the C sequestration potential of each crop, and to quantify changes in SOC sequestration over time. Miscanthus (*Miscanthus* × *giganteus* Greef & Deuter ex Hodkinson & Renvoize) and switchgrass (*Panicum virgatum* L. var. "Cave in Rock") have been identified as economically viable bioenergy crops for the Midwest (Somerville et al. 2010) and despite relatively low yields, mixed-species prairie has been proposed as a source of biomass that also restores ecological services to the landscape (Tilman et al. 2006).

The objectives of this research were to calculate the change in SOC and its rate of change over the life cycle of each crop. We hypothesize that (1) high-yielding monoculture perennial crops will increase SOC rapidly as the crop establishes, but (2) this rate of change may decline with time as biomass production plateaus, as has been observed in long-term miscanthus production (Arundale et al. 2014; Kantola et al. 2022; Sharma et al. 2022). Time is not expected to affect SOC accumulation rates in prairies after the establishment phase; except for climate-induced variation, NPP in prairie systems may remain stable for prolonged periods. (3) We hypothesize that the increase in belowground biomass in perennial crops will contribute to the increase in SOC, and will be evident through stable isotope analysis of SOC.

2 | Materials and Methods

2.1 | Site Description and Design

In 2008, the University of Illinois Energy Farm was established to investigate bioenergy potential and ecological consequences of two C_4 perennial grasses, miscanthus and switchgrass. These grasses were chosen as potential replacements for maize grain for bioethanol production, as they produce higher yields than maize with fewer management requirements (Conant et al. 2001; Dondini et al. 2009; Chimento et al. 2014). The elimination of annual tillage and the perennial growth form of these crops contribute to the allocation of large amounts of biomass underground as roots and rhizomes, leading to the prediction that belowground C accumulation and storage would increase following the switch from annual row crops to perennial grasses (Fornara and Tilman 2008; Anderson-Teixeira et al. 2009, 2013; Anderson et al. 2011; Chimento et al. 2014; Kantola et al. 2017; Kantola et al. 2022). In addition, a 28-species restored prairie (Zeri et al. 2011) was established on the site to investigate the potential benefits of polyculture versus monoculture and to simulate the species composition of the prairie native to the region prior to the introduction of large-scale row crops. Initial ecosystem models projected that the interruption of annual tillage and the establishment of perennial crops with life cycles similar to those historically found in the area would conserve soil organic carbon (SOC) and begin to rebuild C lost during intensive cultivation over the previous two centuries (Davis et al. 2012; Hudiburg et al. 2014; Duval et al. 2015).

The University of Illinois Energy Farm (40°3′46″ N, 88°11′46″ W) is located in Urbana, IL, USA. Mean annual temperature is 10.9°C, and mean annual precipitation is 1051 mm (Angel 2010, 1981–2010 average). Prior to perennial crop establishment in 2008, the Energy Farm produced row crops, primarily maize and soybean, for more than 100 years. Soils on the site are predominately Argiudolls: Dana silt loam (fine-silty, mixed, superactive, mesic Oxyaquic Argiudolls) with inclusions of Flanagan silt loam (fine smectitic, mesic, Aquic Argiudolls), Blackberry silt loam (fine-silty, mixed superactive, mesic Oxyaquic Argiudolls), and Drummer silty clay loam (fine-silty, mixed superactive, mesic Typic Endoaquolls).

Perennial monoculture crops (miscanthus and switchgrass), a 28-species restored prairie polyculture, and conventional maize-maize-soybean in a 3-year rotation were planted in a complete randomized block design with four 0.7-ha plots of each crop (Zeri et al. 2011; Figure S1). In 2019, the switchgrass plots were tilled for crop replacement and removed from the study (Moore et al. 2020). Prairie management changed after 2016 (incorporating fire instead of harvest removal of biomass) and prairie was removed from the study. Consequently, final sampling for each crop occurred in different years: prairie in 2016, switchgrass in 2019, maize in 2020, and miscanthus in 2021 (Figure S2).

Eddy covariance towers were established in a single neighboring 3.8-ha plot of each crop (Figure S1), the size of which was determined to be the minimum required in this area given crop height and prevailing wind direction and speed. For eddy covariance measurement details see Moore et al. (2021). Biomass was collected in the 3.8-ha plots in addition to the 0.7-ha plots for above- and belowground biomass measurements from 2008 to 2016.

Maize and soybean were planted using conventional local management practices, with pre-planting tillage and post-emergence herbicide and pesticide application. Maize was planted by drill seeding at 76.2 cm centers. Nitrogen fertilizer (28% UAN) was applied prior to all maize plantings at 168 kg N ha^{-1} in first (2008, 2011, 2014) and 202 kg N ha^{-1} in second (2009, 2012, 2015, 2017–2018, 2020–2021) rotational years. After 2016, all maize years were fertilized at 202 kg N ha^{-1}. Soybean was alternated with maize every third year (2010, 2013, 2016, 2019) and was not fertilized. Maize and soybean were harvested for grain using a combine harvester, with all non-grain aboveground biomass returned to the soil surface. For full management details, see Kantola et al. (2022).

Initial planting of perennial crops occurred in 2008. All plots in this study were managed according to the best-known Midwestern US agricultural practices for each species (Zeri et al. 2011). Miscanthus was propagated by locally harvested rhizomes planted on 76.2-cm centers (Zeri et al. 2011). In 2008 and 2009, harsh winter conditions resulted in high mortality of juvenile miscanthus, and plots were replanted in the spring of 2009 and partially replanted in 2010 (Smith et al. 2013). Miscanthus was not fertilized until 2014, when annual spring application of 56 kg N ha^{-1} as granular urea was initiated. Miscanthus was harvested in late winter/early spring of the following year (generally February or March), and either baled or chopped for removal from the fields.

Switchgrass was broadcast seeded in 2008 with oats (*Avena sativa*) as a first-year nurse crop. Switchgrass plots were fertilized at 56 kg N ha^{-1} from 2010 onward. In 2019, switchgrass plots were converted back to maize and soybeans, and sampling was discontinued (Moore et al. 2020). The prairie was also broadcast seeded in 2008 from a seed mix developed to approximate the species composition of the area prior to cultivation. The mixture consisted of five perennial grasses and sedges and 23 forbs and herbaceous plants, including nitrogen-fixing species (for complete species list, see Zeri et al. 2011). Prairie plots were not fertilized. Switchgrass and prairie were harvested in December after senescence using a forage harvester (Case New Holland) and material was baled from 2009 to 2016, and switchgrass was chopped for bagging from 2017 to 2019.

2.2 | Soil Sampling and Analysis

Soil samples were collected with a Giddings probe hydraulic rig with a 3.81-cm diameter coring probe in spring 2008, fall 2015 to spring 2016, and fall 2016 to spring 2017 for all crops, spring 2018 (miscanthus only), spring 2019 (switchgrass only) and fall 2020 to spring 2021 (maize/soybean, miscanthus) (Figure S2). The number of soil cores varied from 4 to 8 per plot, depending on the year, for a total of 16–32 cores per crop at each sampling. Due to management logistics, maize/soybean plots were generally sampled in the fall after harvest (October/November), and perennial biomass plots were sampled in early spring (February–April) after harvest and prior to the emergence of the next year's crop.

Soil cores were stored in plastic tube liners and kept in cold storage until processing, weeks to months after sampling. Cores were cut into 0–10 cm, 10–30 cm, 30–50 cm, and 50–100 cm sections and weighed. A subsample from each sampling location was dried at 105°C to a constant mass to determine remaining moisture for bulk density calculations. After calculating bulk density, the remaining soil from each depth was air dried, crushed (Dynacrush DC-5, Custom Laboratory Equipment Inc., Orange City FL USA) and sieved to 2 mm.

Subsamples were finely ground using a modified coffee grinder (Mr. Coffee, Rhode Island USA) and analyzed for percent C, N, and C stable isotope ratios ($\delta^{13}C$) on a Costech 4010 elemental analyzer (Costech Analytical Technologies, Valencia CA USA) in combination with an isotope ratio mass spectrometer (DeltaV Advantage, Thermo Fisher Scientific, Bremen, Germany). By convention, values of $\delta^{13}C$ were defined relative to the reference ratio of ^{13}C to ^{12}C in Pee Dee Belemnite (PDB) as follows: $\delta^{13}C‰ = [(R_{\text{sample}} - R_{\text{PDB}})/R_{\text{PDB}}] \times 1000$; where $R = {}^{13}C{:}\ {}^{12}C$. Replicates of three laboratory standards ranging from −11.99‰ to −35.35‰ and calibrated relative to International Atomic Energy Agency (IAEA) standards (NBS19, glutamic acids USGS 40 and 41, as well as sucrose) were included with each run to anchor raw IRMS outputs to the international PDB scale.

The presence of pedogenic carbonate, detected by isotope ratio mass spectrometry, was rare. Samples with a $\delta^{13}C$ ratio of $<12.5‰$, which indicates pedogenic carbonate, were fumigated with 38% hydrochloric acid (Harris et al. 2001) and re-analyzed. During QA/QC, remaining samples with $\delta^{13}C$ ratio of $> -10.0‰$ after fumigation were removed from the data set due to suspected inorganic C (carbonate) inclusions, and samples with $\delta^{13}C$ ratio of $< -30‰$ were removed due to likely contamination. Samples with low C:N values (C:N < 2) and shallow samples (0–10 and 10–30 cm) with high C:N values (C:N > 30) were also removed due to the implausibility of the measured values, which may have occurred due to contamination or analysis error. Removed data were gap-filled using the average value for the respective plot.

2.3 | Equivalent Soil Mass Method

Estimating changes in SOC stocks is prone to error caused by changes in bulk density or by potential changes in the depth of soil horizons caused by tillage, compaction, or soil loss to erosion. Historically, soil samples are taken at fixed depths over time to calculate changes in C. Large changes in bulk density, however, cause significant error in estimates of SOC stocks (Von Haden et al. 2020). The ESM method calculates SOC stocks using the mass of soil for a given reference layer, which is unaffected by changes in soil bulk density (Wendt and Hauser 2013; Von Haden et al. 2020; Xiao et al. 2020;

Rovira et al. 2022; Raffeld et al. 2024). The 2008 soil cores were used to establish reference soil masses for cores collected in subsequent years by taking the global median cumulative mineral soil mass for each depth increment. An R script using cubic spline interpolation functions and mineral soil masses was used to calculate ESM-based SOC stocks, SOC mass percentages, and soil $\delta^{13}C$ for each soil core section at each time point (Von Haden et al. 2020). Measured soil depths, apparent bulk densities, SOC mass percentage, and soil organic matter (SOM) mass percentage were inputs to the script. SOM mass percent, which was used to calculate mineral soil mass from the total soil mass, was estimated using an SOC-to-SOM conversion factor of 1.724 g SOM g^{-1} SOC (McBratney and Minasny 2010). SOC stocks and ^{13}C values measured calculated by the fixed depth or equivalent soil mass methods were highly correlated, and no significant differences between the two could be resolved for any cropping system (Figures S3–S6). ESM-calculated results were reported as depth intervals referring to the 2008 reference layers.

2.4 | Carbon Inputs: Biomass, Litter, and Net Ecosystem Carbon Balance

Aboveground and belowground biomass were measured annually at peak biomass (determined by LAI measurements, Zeri et al. 2011), between early August and late September, depending on the crop, as described in Kantola et al. (2017). Litter, the portion of aboveground biomass left behind by harvest, was sampled after mechanical harvest of grain/beans (maize/soybean) or biomass (perennial crops). Aboveground biomass and litter were collected biometrically, with several randomly placed quadrats collected in each field, oven-dried, weighed, and averaged. A 0.5625-m^2 quadrat was used for miscanthus, maize, and soybean; a 0.36-m^2 quadrat was used for switchgrass and prairie. Belowground biomass was averaged from roots and rhizomes collected from three 5.08-cm diameter cores of 30-cm depth collected within the quadrat during the aboveground sampling. Soil cores from each location were combined, and root material was separated from soil by elutriation. Above- and belowground biomass data for each crop from 2008 to 2018 are from Kantola et al. (2022).

Net Ecosystem Carbon Balance (NECB) is calculated as the difference between Net Ecosystem Exchange (NEE), C entering the system by photosynthesis minus losses from plant and soil respiration, and C removed from the system by harvest, fire, or other means (Chapin et al. 2006). NECB represents all fluxes of carbon into and out of a system. Typically, NECB is calculated annually and represents potential gain or loss of C in the system, including SOC and biomass. NECB calculated with eddy covariance does not account for C losses through leaching or lateral transfer (Chapin et al. 2006), minor components of NECB that were not measured separately for this study. NEE was measured by eddy covariance in adjacent 3.8-ha fields for each crop. Eddy covariance was measured in maize/soybean and miscanthus from 2009 to 2021, and towers in prairie and switchgrass were operational from 2009 to 2015. As fire is not a regular occurrence at this location, an accidental fire in spring 2021 in the miscanthus eddy covariance plot was omitted from the NECB calculations. NECB data are from Blakely et al. (2025).

2.5 | Data Processing and Statistical Analysis

SOC content, $\delta^{13}C$, and corresponding rates of change were analyzed for each energy crop and each soil depth layer using a repeated measures ANOVA analysis for a randomized complete block design, with crop, depth, and block as fixed factors. As not all crops were sampled in the same years over the entire duration of the experiment, differences between crops were examined over the comparable period (2008–2016) whereas within-crop depth and time effects included all sampling years over the entire crop-specific sampling period (from 2008 to 2016, 2019, 2020, or 2021, depending on the crop). Therefore, a four-block complete randomized design was considered for the analysis of within-crop differences in SOC content and isotopic signature and change over time. Statistical differences ($p < 0.05$) were further examined with multiple comparisons methods (least square distance, 95%) to examine homogeneous groups. The rate of change over time, where significant, was determined using multiple regression analyses with year as a quantitative factor. To ensure data quality, we examined atypical studentized residues, and observations with >3.5 times absolute deviation from the adjusted model were eliminated. Outliers represented less than 1% of the data included in each individual analysis. All statistical analyses were performed using Statgraphics Centurion, version XV (StatPoint Inc., 2006).

3 | Results

To provide context for changes in SOC, potential soil C sources were estimated either directly as total belowground biomass (i.e., roots and rhizomes) and aboveground litter inputs, or indirectly as NECB (Figure 1). Average aboveground biomass (the source of litter) and belowground peak biomass in miscanthus (2010–2021) was significantly larger than maize/soybean (2009–2020), switchgrass (2010–2019), and prairie (2010–2016) (Figure 1 and Table S1; Kantola et al. 2022). In contrast with maize/soybean, where substantial quantities of stover are left on the field surface as litter after mechanical separation of grain from cobs, stalks, and leaves during harvest (Figure 1), most of the switchgrass and prairie aboveground material was removed via harvest (Anderson-Teixeira et al. 2013), resulting in lower quantities of litter and a higher ratio of belowground-to-litter inputs. A similar ratio of root mass to litter occurs for miscanthus despite larger belowground biomass than other perennial crops (Figure 1) because the additional months the miscanthus crop remains in the field before harvest results in a significant litter layer due to leaf litterfall over the winter (Figure 1). The low N and high Si content (Woli et al. 2011; Masters et al. 2016) in miscanthus litter contribute to slow decomposition.

In all years but one, NECB was positive, and at times strongly positive, for the maize/soybean rotation indicating that this system was losing stored C, presumably from SOC (Figure 1a). In contrast, the three perennial systems consistently had negative

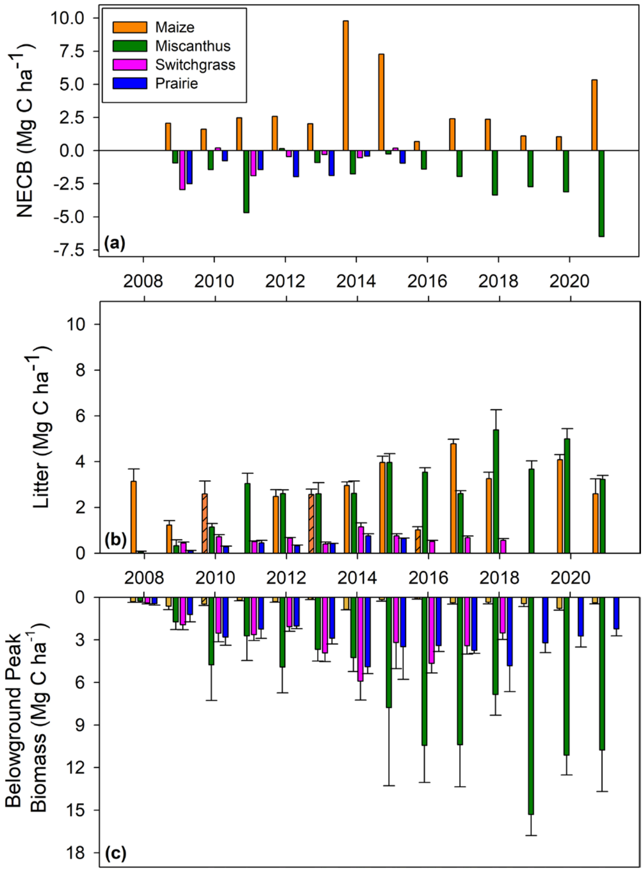

FIGURE 1 | Net ecosystem carbon balance (NECB) (a), post-harvest litter (b), and belowground biomass (c) between 2008 and 2021 show increasing biomass during the establishment phase for perennial grasses. In 2014, fertilization was initiated in established miscanthus, resulting in an increase in belowground biomass as well as litter. NECB data are reproduced from Blakely et al. (2025) and belowground biomass data from 2008 to 2018 are from Kantola et al. 2022. NECB data represent annual totals and bars for litter and belowground biomass are mean values (n = x). Error bars represent standard error of the mean.

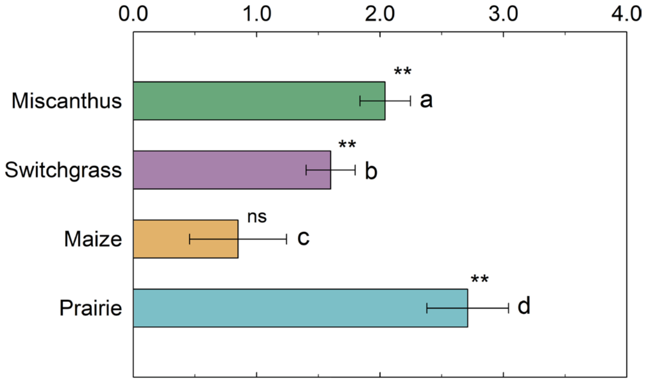

FIGURE 2 | The rate of SOC accrual (Mg C ha^{-1} year^{-1}) in the top 1 m of soil over an 8-year period (2008–2016) for maize, miscanthus, switchgrass, and restored prairie. Bars represent mean values ($n = 5$) and error bars represent standard error of the mean. Asterisks denote that reported rates are statistically significant from zero (*$p \leq 0.1$; **$p \leq 0.05$; ns = not significant) and different letters indicate significant differences among crops.

NECB indicating that these systems were accruing C; miscanthus had the greatest rates of C accrual followed by restored prairie, then switchgrass. Annual variation in NECB for all crops was high. NECB for perennial crops was reduced from 2012 to 2015 following a widespread drought from 2012 through early 2013, which was reflected in aboveground biomass (Joo et al. 2016; Kantola et al. 2022) and litter (Figure 1b). Maize yield in 2012 was affected by the drought, but yields recovered to near-normal in 2013 (soybean) and 2014 (maize). NECB in miscanthus rebounded strongly as the crop increased biomass allocation below ground following the drought, and after fertilization was initiated in 2014.

Both SOC dynamics and the cropping systems included in the analysis were different early and late in the experiment (Figure S2), so we conducted the statistical analysis over two time periods: early period (2008–2016, all crops) and full period (2008 to end-of-experiment, which was different among different crops and did not include prairie). Total SOC to 1-m depth increased for all perennial systems between 2008 and 2016, with significant differences in the rate of change between species ($p < 0.05$, Figure 2). The highest rate of SOC accrual was in prairie (2.5 Mg C ha^{-1} year^{-1}), followed by miscanthus (2.0 Mg C ha^{-1} year^{-1}) and switchgrass (1.75 Mg C ha^{-1} year^{-1}) (Figure 2). The rate of SOC change in maize/soybean between 2008 and 2016 was not significantly different from zero (Figure 2).

The final sampling date varied among crops (prairie, 2016; switchgrass, 2019; maize/soybean, 2020; miscanthus, 2021; Figure S2) and changes in SOC to 1-m depth were also calculated for the entire sampling period for each crop. Switchgrass SOC accumulation slowed after 2016, and the miscanthus soils lost SOC between 2016 and 2021, reducing the overall rate of SOC accumulation for the perennial monoculture crops. SOC in the top meter increased by 1.23 Mg C ha^{-1} yr^{-1} in switchgrass (2008–2019), and 0.8 Mg C ha^{-1} year^{-1} in miscanthus (2008–2021) (Figure 3). The rate of SOC change in maize/soybean (2008–2020) for the full meter depth was not significantly different from zero.

Rates of SOC accrual varied with depth for all crops (Figures 3 and S7). Between 2008 and 2020 in maize/soybean, the total rate of accrual was not significantly different from zero. However, the difference in the rate of change between the soil depths was significant, with the 10–30 and 30–50 cm depths losing more SOC over 13 years than the 0–10 and 50–100 cm depths. All perennial crops accrued more SOC in the 0–10 cm surface depth than in the 10–30 and 30–50 cm depths, though both switchgrass (between 2008 and 2019) and prairie (between 2008 and 2016) accumulated more SOC in the deepest depth (50–100 cm) than the middle depths as well (Figure 3).

Soil C:N ratios were calculated from SOC and total N measurements. The C:N ratios increased in maize/soybean, miscanthus, and prairie over the course of the experiment without significant N loss, indicating that SOC was accumulating in the soil faster than soil N. The largest change in C:N ratio was measured in prairie, followed by miscanthus. A small but significant increase in C:N was measured in maize/soybean, with a similar but not significant difference observed in switchgrass (Figure S10).

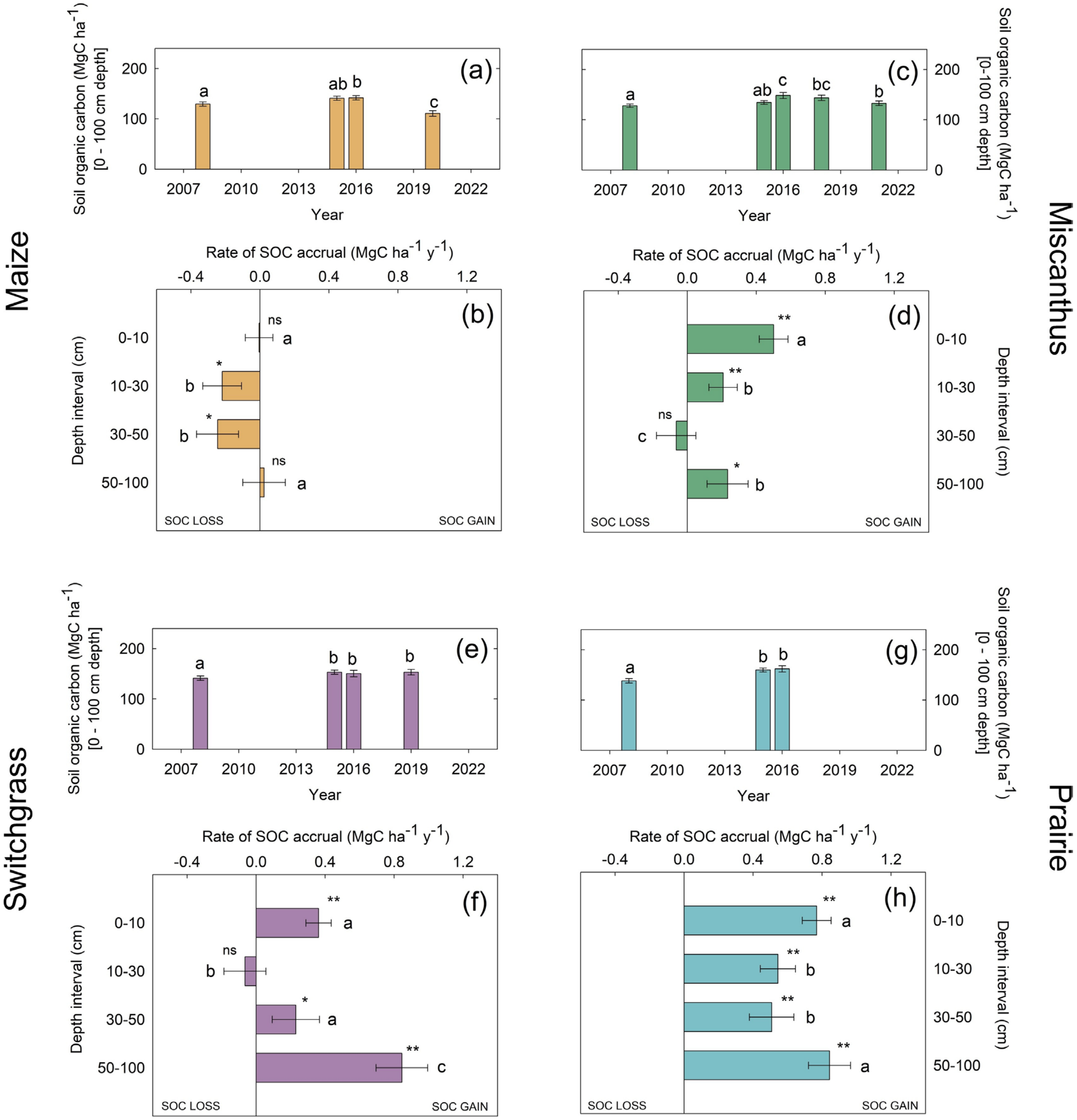

FIGURE 3 | SOC content (Mg C ha^{-1}) in the top 1 m (upper panels) and distribution of SOC accrual rates (Mg C ha^{-1} year^{-1}) by depth (lower panels) in maize (a and b), miscanthus (c and d), switchgrass (e and f), and prairie (g and h). The time covered and sampling years vary by crop. Bars represent mean values ($n=5$) and error bars represent standard error of the mean. Asterisks denote that reported rates are statistically significant from zero (*$p \leq 0.1$; **$p \leq 0.05$; ns = not significant) and different letters indicate significant differences among crops ($p \leq 0.05$; ns = not significant).

At the initiation of this experiment (2008), the ^{13}C isotope value of SOC across all plots from 0 to 30 cm was ~17‰, reflecting past land use and crop rotation between the C4 crop, maize (−12.5‰), and the C3 crop, soybean (−26.5‰) (Ehlringer and Cerling 2002; Schneckenberger and Kuzyakov 2007). In the two C4 perennial monocultures, miscanthus and switchgrass, surface δ^{13}C became less negative after establishment (Figures 4 and S8), indicating the incorporation into the soil of new C from these crops, and this shift to more C4-C was also evident at depth in switchgrass. The restored prairie was a mixture of C3 and C4 plants and presented a mixed effect on soil δ^{13}C, with a small shift to more C4-C at the surface, and more C3-C at greater depths, perhaps responding to differences in rooting depths among plant functional groups. The maize-maize-soybean rotation also received both C3 and C4 inputs, and despite a predominance of C4 input from maize in two out of three years, we observed a shift toward C3-C at all depths below 10 cm (Figure 4).

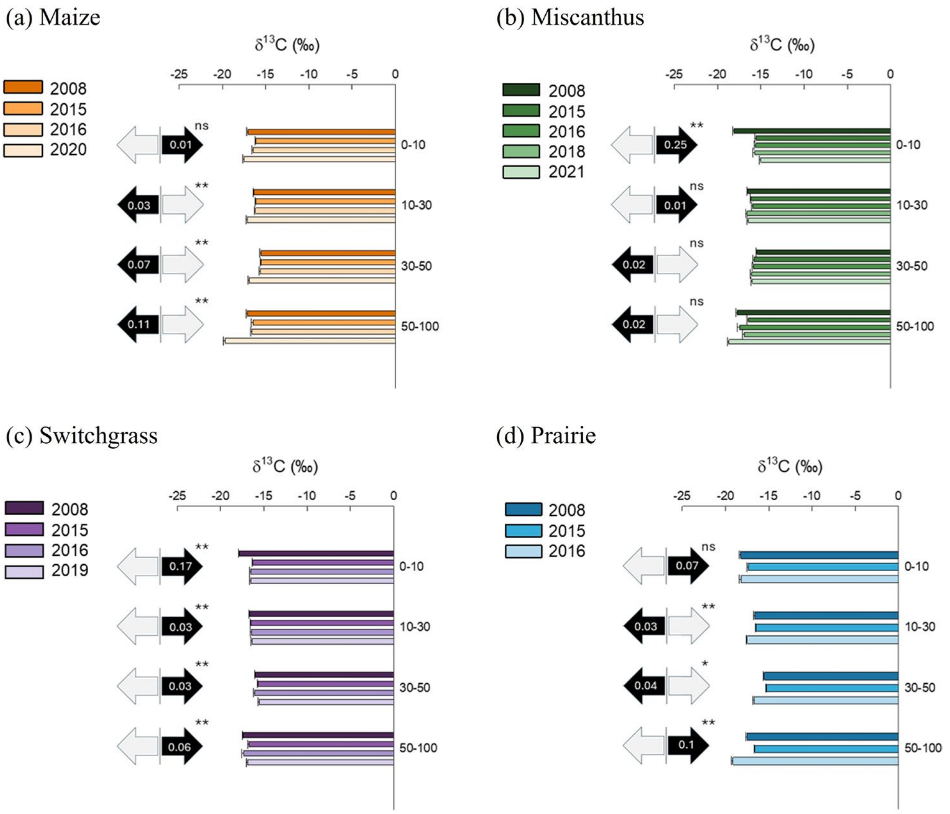

FIGURE 4 | Stable carbon isotope ($\delta^{13}C$) ratio by year and soil depth for maize (a), miscanthus (b), switchgrass (c), and restored prairie (d). Bars represent mean values ($n=5$) and error bars represent standard error of the mean). Arrows indicate the direction of change in $\delta^{13}C$ overtime and the magnitude of change. Asterisks indicate that there was statistically significant change with time ($*p \leq 0.1$; $**p \leq 0.05$; ns = not significant).

4 | Discussion

In this study, samples taken 8 to 13 years after perennial crop establishment statistically resolve that total SOC is increasing in perennial monoculture and polyculture plots, with the highest rate of SOC increase observed in the multi-species restored prairie. SOC declined or remained unchanged in the maize/soybean rotation (Figures 2 and 3). In contrast to our first hypothesis, the rate of SOC accrual was particularly high for the multi-species restored prairie compared to perennial crop monocultures, despite lower biomass production than either the monoculture perennial crops or maize (Figure 1; Kantola et al. 2022). Greater accumulation of SOC for all perennials compared to the row crops was a function of greater belowground C inputs (Figure 1) and decreased losses from organic matter decomposition stimulated by annual crop tillage (Anderson-Teixeira et al. 2009; Kantola et al. 2022). C accumulation varied by depth between crops, with the greatest increase in SOC occurring near the surface (0–10 cm) for miscanthus but at depth (50–100 cm) for switchgrass. C accumulated evenly across all depths in prairie, providing evidence for uniform distribution of roots through the profile from different species. This study demonstrated planting perennial crops on midwestern soils currently under intensive row crop agriculture could restore decades of SOC losses, with the rate and depth of accumulation varying with time.

NECB, representing all fluxes of carbon into and out of a system, was calculated as a proxy for C accumulation or loss. NECB is often driven by disturbance, which is greatly reduced in untilled perennial systems. Throughout the study, the NECB of perennial crops was negative, indicating more C entering the system than leaving, which was reflected in SOC. Despite strongly positive NECB measurements, SOC losses measured in the maize/soybean system were small.

Belowground biomass C stock (as roots) was considerably lower in the maize/soybean rotation than any of the perennial crops (Figure 1), and maize/soybean roots have been shown to concentrate in the upper 20 cm of the soils, with very little biomass at deeper depths (Anderson-Teixeira et al. 2013). These low inputs contributed to small or no change in SOC over the 13 years of this study. Litter inputs for the maize/soybean system were high throughout the study as only grain is removed from this system at harvest, and litter was the dominant C input to soil (Carvalho et al. 2017). However, shoot-derived C may be less chemically recalcitrant and less physically protected than C derived from roots (Rasse et al. 2005; Anderson-Teixeira et al. 2013) which may explain why the large inputs of litter had no discernible effect on SOC for the maize/soybean system. This fast turnover of labile C from aboveground litter is further supported by the greater heterotrophic respiration measured in this maize/soybean system (Anderson-Teixeira et al. 2013; Moore et al. 2021). N contributes to microbial decomposition of C, and in maize years, the maize-soybean system received N fertilizer at 3 times the rate of miscanthus and switchgrass.

In the maize/soybean system, SOC accumulation in the shallowest soil layer was insignificant despite C inputs from roots and the mechanical incorporation of post-harvest litter by tillage at 0 to 10 cm (Figure 3). The belowground biomass of maize/soybean is a small fraction of that observed in the perennial crops (Kantola et al. 2022; Figure 1); however, in an annual system, all of the belowground biomass turns over at the end of the growing season and begins to decompose into SOM. In perennial crops, many perennial roots and rhizomes remain alive for the next season, contributing to the C stock of the system, but not necessarily a gain in SOC. Microbial consumption of both stored C and annual inputs is the likely reason for SOC accrual from 2008 to 2020, similar to previous observations (Bernacchi et al. 2006; West and Post 2002).

The three perennial cropping systems, miscanthus, switchgrass, and restored prairie, had considerably greater belowground C allocation and gains in SOC than maize/soybean, particularly in the first 8 years of observation (Figure 2). Miscanthus SOC increased between 2008 and 2016 (from 128.0 Mg C ha^{-1} to 148.3 Mg C ha^{-1}) and decreased between 2016 and 2021 (from 148.3 Mg C ha^{-1} to 132.8 Mg C ha^{-1}); however, SOC stock in 2021 remained higher than in 2008 despite the loss (Figure 3). This observed SOC loss in miscanthus in the later years of this experiment occurred despite the largest measured belowground biomass in the experiment and contradicted our second hypothesis that SOC would plateau with age in the perennial monocultures. However, there is a difference between annual belowground C inputs and annual belowground biomass, and as previously stated, much of this belowground C held in roots and rhizomes may be turning over slowly or not at all. The root-to-rhizome ratio in miscanthus increased as the total belowground biomass increased in the later years of the experiment (2014–2020, Figures 2 and S9). Switchgrass belowground biomass was lower than miscanthus, but greater than maize/soybean, and SOC increased (from 141.2 Mg C ha^{-1} to 153.2 Mg C ha^{-1}) over 13 years (Figure 3). As in miscanthus, the increase in SOC for switchgrass occurred in the first 8 years, but switchgrass SOC plateaued from 2015 to 2019, suggesting C gain through inputs and losses to microbial consumption had approached equilibrium.

Despite relatively low litter and belowground biomass inputs compared to miscanthus, restored prairie had the greatest increase in SOC: 2.5 Mg C ha^{-1} year^{-1}. The ratio of fine roots to both rhizomes and coarse roots is greater in prairie than either miscanthus or switchgrass (Black et al. 2017; Figure S3), and it is likely that fine roots have faster rates of turnover, contributing more immediately to increases in SOC. It has also been shown that different prairie species have different rooting profiles and root size classes (Weaver et al. 1935; Weaver 1958; Jackson et al. 1996), and this 28-species assemblage is likely changing in species composition and root distribution over time, with greater root turnover than the other perennials (Tilman et al. 2006). The increase in SOC in prairie occurred in the first 8 years of observation, and measurements were discontinued after 2016. However, at 8 years, SOC stock (162.0 Mg C ha^{-1}) remained below that of native grasslands in the area (David et al. 2009), suggesting that these soils are not yet C-saturated (Six et al. 2002). Though restored prairie and grasslands may not offer enough aboveground biomass to be profitable to farmers for hay production or as bioenergy feedstocks, these perennial systems offer several ecological benefits (Tilman et al. 2006; Fargione et al. 2009) including rebuilding SOC faster than monoculture alternatives.

C:N ratios are frequently used as a proxy for soil health, and C and N sequestration are intrinsically linked (Conant et al. 2005). N may be lost from the system through biomass harvest and leaching or gained through the presence of legumes or fertilization. In this experiment, we measured total N, as opposed to available N, which does not directly provide an estimate of N available to plants and microbes. When soils are low in N compared to C, decomposition will be slowed as microbes require adequate N to break down C. High C:N residues, like maize stover and miscanthus litter, contribute to the persistence of SOC in the soil due to their low nutritional quality for microbes; however, maize litter is less chemically recalcitrant than miscanthus litter (Ridgeway et al. 2022). Generally, N fertilizer rates are calculated to maintain crop yields; the increase in C:N ratio in this experiment showed that maize/soybean fields, with non-significant or low rates of C loss, are also losing N despite fertilization (Figure S10). In miscanthus and prairie, where SOC is increasing with low or no N fertilization, an increase in C:N ratio may be due to the increased mass of lignin and suberin-rich roots compared to maize/soybean (Figure 4). The presence of N-fixing legumes in the prairie mixture did not increase soil N at a proportional rate to SOC. In switchgrass, the non-significant trend in C:N ratio indicates that N fertilization is adequate for the losses to harvest and leaching.

Changes in δ^{13}C of the surface soils of the perennial monoculture crops (miscanthus and switchgrass) support the hypothesis that new C4 material from the perennial crops is contributing to SOC in these systems (Figure 4). The difference in the isotope ratio of soil C at different timepoints in this experiment reflected the balance of new C entering the system through photosynthesis and older C leaving the system through mineralization (Ehlringer and Cerling 2002; Schneckenberger and Kuzyakov 2007; Zang et al. 2017; Blanc-Betes, Gomez-Casanovas, Yang, et al. 2023; Choi et al. 2023). The isotopic composition of surface soil C at the beginning of the experiment for all crops (−15.5‰ to −19.5‰) represented the net effect of C inputs from the historic maize (−11.5‰ to −13‰) and soybean (−27.5‰ to −29.5‰) rotation. After planting miscanthus, its rapid belowground biomass allocation caused a substantial increase (positive change) in the δ^{13}C ratio in the surface soil (Figure 4). Though not statistically significant, the opposite occurred below 10-cm depth. The large input of fresh root C and root exudates (Von Haden et al. 2024) in miscanthus may have contributed to preferential decomposition or priming (Kuzyakov et al. 2000) of old C4-derived C in the soil at these depths. Despite the lower belowground inputs, C4 switchgrass increased the δ^{13}C ratio across the entire depth profile, likely a combination of higher C inputs at depth and less C loss to microbial consumption.

The pattern was more difficult to interpret for the multi-species prairie and maize/soybean rotation. In the prairie, the C isotope ratio became more positive (C4-like) in surface soils and more negative (C3-like) at depths from 10 to 50 cm This may represent differences in the depth of root production between graminoids (C4) and herbaceous species (C3) grown at this site. In prairie, only three of the 28 prairie species use C4 photosynthesis, though the proportion of biomass contributed by C4 species was much higher than the species ratio when calculated in 2013 (Feng and Dietze 2013). The benefit of plant species diversity in the prairie was evident from the combination of high rates of SOC accumulation in all depths of prairie soils, while the isotope ratios demonstrate differences in the species contributing to SOC at different depths.

Except for a non-significant trend in surface soil, the soil isotopic C signal shifted more negative at depth in the maize/soybean rotation (Figure 4). NECB and directly measured SOC from 10 to 50 cm confirm that this system lost C from 2008 to 2020, and a shift toward C3-derived C suggests that C4-derived C was preferentially mineralized, as C3 (soybean) inputs in this system only occurred in 1 year of each 3-year rotation. Wynn and Bird (2007) found that the active pool of C4 SOC decomposed nearly twice as fast as C3 SOC, which may account for the persistence of the C3 signal in these soils. The largest δ^{13}C changes in both multi-species systems were observed from 50 to 100 cm, although in opposite directions and with different implications for SOC accrual.

The ability of perennial crops to sequester C and increase SOC stocks depends on the mechanism of C stabilization, which is often depth dependent. C may be stabilized in soil because it is chemically resistant to microbial consumption or because incorporation into the mineral soil matrix physically protects organic matter from microbial decomposition or creates an anoxic environment in which microbial activity is reduced (Oades and Waters 1991; Six et al. 2002; Allison and Jastrow 2006). Organic C sources (plant roots, root exudates, and plant litter) tend to concentrate near the soil surface (Anderson-Teixeira et al. 2013; Black et al. 2017), but are susceptible to microbial decomposition, wind, water, and mechanical disturbance. We observed increases in SOC accrual from 0 to 10 cm with time in all perennial systems, indicating that the rate of C input is exceeding C loss in the perennial crops.

In a previous study, SOC change was unresolved in surface (0–30 cm) samples taken at this site between 2008 and 2014 in the perennial crops due to high background SOC and high spatial variability (Necpálová et al. 2014; Kantola et al. 2017). In this study, SOC accrual was significant when measured for all perennial crops between 2008 and 2016 when the same plots were sampled to 1 m depth. Soil sampling of deeper depths is less common than the 0–30 cm depths due to equipment requirements, and the potential for C storage in deeper soils is less well understood (Von Haden et al. 2020; Raffeld et al. 2024); however, the significant change when deeper depths are included indicates that the interpretation of SOC patterns without deeper depths would underestimate gains from deep-rooted perennials.

In miscanthus, the 2012 drought coincided with a decline in harvest yield, which was remedied in 2014 with the return of more typical rainfall patterns and the addition of N fertilizer (Kantola et al. 2022). The addition of N fertilization stimulated yield recovery, and belowground biomass measurements were not significantly affected by the drought period (Figure 1). Because of this, the observed SOC decline from 2016 to 2021 in miscanthus was unexpected. Miscanthus fertilization was initiated in 2014, and the addition of N fertilizer to an N-limited system can result in increased microbial consumption of C (Kuzyakov et al. 2000). The loss of SOC may also be the result of an ecosystem sink limitation, where the mature miscanthus root system has become growth-limited, and the microbial community has been stimulated to consume C inputs from litter and root turnover.

Modeled estimates of changes in soil C over time in Midwestern soils indicate that SOC would increase significantly in the first five years after planting perennials as plants reach maximum growth and yield (Anderson-Teixeira et al. 2013; Hudiburg et al. 2014; Duval et al. 2015; Blanc-Betes, Gomez-Casanovas, Hartman, et al. 2023), and NECB values for perennial crops were strongly negative from 2008 to 2013, showing large inputs of C to the belowground ecosystem (Figure 1; Zeri et al. 2011; Joo et al. 2016; Moore et al. 2020). However, 0–30 cm samples collected in 2008 and 2016 in miscanthus, switchgrass, and prairie showed significant increases in the particulate organic matter C (POM-C), a component of SOC, while the change in total SOC was unresolved due to spatial variability and high background concentrations of SOC in the soil (Kantola et al. 2017). In contrast, a significant rate of SOC accrual was measured for all perennial crops between 2008 and 2016 when the same plots were sampled to 1 m depth, showing that deep soil SOC accrual is a critical component of SOC storage.

5 | Conclusions

This study emphasizes the need for long-term data sets by demonstrating the dynamic nature of SOC accrual and retention. Modeled estimates of SOC change through time in Midwestern soils showed an increase in the first 5 years after planting perennial crops as plants reached maximum yields (Anderson-Teixeira et al. 2013; Hudiburg et al. 2014; Duval et al. 2015; Blanc-Betes, Gomez-Casanovas, Hartman, et al. 2023), and NECB values in this experiment for perennial crops were strongly negative from 2008 to 2013, with large belowground C inputs (Figure 1; Zeri et al. 2011; Joo et al. 2016; Moore et al. 2020). However, increasing SOC during perennial crop establishment was swamped by the background SOC in these soils, resulting in a lag before significant differences could be resolved. In addition, early modeling efforts did not predict the observed miscanthus yield declines at the Energy Farm after 2012, likely a combination of drought, crop aging, and N depletion (Kantola et al. 2022). The lifespan and turnover of individual perennial roots as this ecosystem ages are not well understood, and the difference between belowground biomass and annual C inputs must be established to elucidate these SOC mechanism dynamics.

Author Contributions

I. B. Kantola: conceptualization, data curation, formal analysis, investigation, methodology, writing – original draft. **E. Blanc-Betes:** conceptualization, formal analysis, investigation, writing – original draft. **A. von Haden:** data curation, methodology, writing – review and editing. **M. D. Masters:** conceptualization, data curation, investigation, writing – review and editing. **B. Blakely:** data curation, formal

analysis, writing – review and editing. **C. J. Bernacchi:** conceptualization, data curation, formal analysis, methodology, writing – review and editing. **E. H. DeLucia:** conceptualization, formal analysis, funding acquisition, investigation, methodology, project administration, writing – original draft.

Acknowledgments

The authors thank Tim Mies for management of the Energy Farm. The Energy Farm was established by the Energy Biosciences Institute, University of Illinois. Continuing research in these plots is funded by DOE Center for Advanced Bioenergy and Bioproducts Innovation (U.S. Department of Energy, Office of Science, Biological and Environmental Research Program under Award Number DE-SC0018420), and the Leverhulme Research Centre Award (RC-2015–029) to David J. Beerling. Any opinions, findings, and conclusions or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the views of the funding agencies. This manuscript represents 12 years of data collection on the site, for which we are grateful for the dedication of our farm staff, student workers, summer workers, and special help: Micah Sweeney, Chris Novatny, Mannan Shah, Lauren Segal, Owen Cofie, Haley DeVries, Mike DeLucia, Nick DeLucia, Brad Danner, Michael Donovan, Jeremy Pillow, Emmanuel Sanders, James Lee, Alex Kent, Ji Soo Kim, Wes Maurer, Victor Wan, Robert Cachro, Krishna Woli, Abhishek Pal, Luke Freyfogle, Taylor Wright, Edwin Albarran, Lance Rodriguez, Jack Rosenthal, Taylor Pederson, Chris Sligar, Konrad Taube, Haley Ware, Megan Gaddy, Cody Randolph, Trace Elliot, Josephine Duffy, Katherine Travia, Nora Danaher, Grace Sharich, Dominic Reztuzek, Chris Montes, Chris Moeller, and Janith Chandrasoma.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Map of energy farm. **Figure S2:** Sampling timeline. Maize, miscanthus, switchgrass, and prairie were planted in four replicate plots (1–4) in 2008. In 2016, management of the prairie plots changed, and the crop was removed from the study. Subsequently, switchgrass plots were sampled prior to conversion to maize-maize-soybean rotation in 2019. Final samplings of extant maize and miscanthus plots were in 2020 and 2021, respectively. **Figure S3:** Comparison of fixed depth and equivalent soil mass for maize. **Figure S4:** Comparison of fixed depth and equivalent soil mass for miscanthus. **Figure S5:** Comparison of fixed depth and equivalent soil mass for switchgrass. **Figure S6:** Comparison of fixed depth and equivalent soil mass for prairie. **Figure S7:** SOC stats 2008–2016. **Figure S8:** $\delta^{13}C$ stats 2008–2016. **Table S1:** Average aboveground biomass, belowground biomass, litter by crop. **Table S2:** Rates of change for all SOC and $\delta^{13}C$ to 100 cm.