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Long-term changes in soil carbon and nitrogen fractions in switchgrass, native grasses, and no-till corn bioenergy production systems

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Abstract

Cellulosic bioenergy is a primary land-based climate mitigation strategy, with soil carbon (C) storage and nitrogen (N) conservation as important mitigation elements. Here, we present 13 years of soil C and N change under three cellulosic cropping systems: monoculture switchgrass (*Panicum virgatum* L.), a five native grasses polyculture, and no-till corn (*Zea mays* L.). Soil C and N fractions were measured four times over 12 years. Bulk soil C in the 0–25 cm depth at the end of the study period ranged from 28.4 (± 1.4 se) Mg C ha^{-1} in no-till corn, to 30.8 (± 1.4) Mg C ha^{-1} in switchgrass, and to 34.8 (± 1.4) Mg C ha^{-1} in native grasses. Mineral-associated organic matter (MAOM) ranged from 60% to 90% and particulate organic matter (POM) from 10% to 40% of total soil C. Over 12 years, total C as well as both C fractions persisted under no-till corn and switchgrass and increased under native grasses. In contrast, POM N stocks decreased 33% to 45% across systems, whereas MAOM N decreased only in no-till corn and by less than 13%. Declining POM N stocks likely reflect pre-establishment land use, which included alfalfa and manure in earlier rotations. Root production and large soil aggregate formation explained 69% ($p < 0.001$) and 36% ($p = 0.024$) of total soil C change, respectively, and 60% ($p = 0.020$) and 41% ($p = 0.023$) of soil N change, demonstrating the importance of belowground productivity and soil aggregates for producing and protecting soil C and conserving soil N. Differences between switchgrass and native grasses also indicate a dependence on plant diversity. Soil C and N benefits of bioenergy crops depend strongly on root productivity and pre-establishment land use.

Abbreviations: MAOM, mineral-associated organic matter; POM, particulate organic matter; SOM, soil organic matter.

1 | INTRODUCTION

Bioenergy production is central to all shared socioeconomic pathways scenarios from the United Nations Intergovernmental Panel on Climate Change with a greater than 67% chance of meeting a 2˚C end-of-century target (IPCC, 2018; Robertson et al., 2022), and grass-based cropping systems comprise the greatest source of biomass in all projections. Primarily these include purpose-grown perennial grasses such as switchgrass (*Panicum virgatum* L.), miscanthus (*Miscanthus* × *giganteus*), and sugarcane (*Saccharum officinarum* L.) but also included is biomass from restored prairie and other native species communities and from residues of cereal crops like corn (*Zea mays* L.) and wheat (*Triticum aestivum* L.) (Robertson et al., 2017; U.S. Department of Energy, 2016).

Soil carbon (C) accretion represents an important potential source of carbon sequestration in bioenergy crops (Gelfand et al., 2020), although its magnitude is uncertain due to the limited number of long-term bioenergy cropping system experiments that have measured ecosystem C dynamics (Harris et al., 2015). Especially when perennial bioenergy crops are established on land formerly in annual crops, land use conversion has the potential to re-sequester C in soil organic matter (SOM) via the production, transport, and stabilization of C in soils (Mosier et al., 2021). Many additional benefits of increasing SOM stocks include improved plant nutrient supplies, improved water retention, and reduced soil erosion (Bai & Cotrufo, 2022).

The separation of SOM into physically defined fractions provides insights into stability and persistence not readily inferred from measures of total SOM or from functionally defined fractions (e.g., slow, labile, and passive pools; Robertson & Grandy, 2006a). In particular, separating SOM into particulate and mineral-associated fractions (POM and MAOM, respectively) can yield fractions that contrast in their formation, structure, and function (Lavallee et al., 2020; Spycher et al., 1983). POM is comprised of organic matter particles larger than 50 μm and is created from root and litter fragments that are incorporated into the soil through physical and bioturbation pathways (Cotrufo et al., 2013). POM tends to persist in soil from <10 to 50 years and thus must be regularly replenished in order to maintain long-term C storage (von Lützow et al., 2007). MAOM, on the other hand, is adsorbed to soil colloids and mineral surfaces and is created from microbial metabolites, root exudates, and other sources of dissolved organic matter. MAOM can persist in soil for hundreds of years to millennia (Cotrufo et al., 2013), although sorption and desorption can happen rapidly. Both fractions, but especially POM, can be further protected from decomposition when incorporated into soil aggregates (Grandy & Robertson, 2006a; Tisdall & Oades, 1982; von Lützow et al., 2007).

Soil C accumulation is thus dependent on plant productivity, both above but especially belowground as roots are one of the largest sources of new soil C, especially in systems where aboveground biomass is harvested and removed. Root primary productivity has been shown to affect SOM stocks and stability (Kumar et al., 2006; Norby & Cotrufo, 1998) insofar as root-derived C increases the stability of both MAOM (Austin et al., 2017; Kong & Six, 2010) and POM (Puget & Drinkwater, 2001) as compared to shoot-derived C. Much of the root-derived C mineralizes and becomes part of MAOM due to the close proximity of rhizodeposits to mineral surfaces (Austin et al., 2017; Kong & Six, 2010). Perennial plants tend to have more root stock compared to annual plants, which may make them better suited for soil C sequestration and stabilization when aboveground biomass is harvested (Sprunger et al., 2020).

Nitrogen (N) is also central to sustainable bioenergy production systems. Systems that conserve N mitigate greenhouse gas emissions by reducing crop needs for N fertilizer production with its associated CO_2 costs (Robertson et al., 2000) and by attenuating soil N_2O emissions following fertilizer application (Ruan et al., 2016). Perennial bioenergy crops can conserve more N than annual crops by retranslocating N to roots before harvest (Jach-Smith & Jackson, 2015; Roley et al., 2021; Yang & Udvardi, 2018). Additionally, their longer growing seasons and more extensive root systems can contribute to greater N use efficiency (Mosier et al., 2021; Udvardi et al., 2021).

Knowing the compositions of POM and MAOM in soil can thus help our understanding of N use efficiency because N cycles within MAOM and POM differently (Sollins et al., 1984). POM recycles N more tightly due to its higher C:N ratio, making N loss less likely. MAOM, on the other hand, better matches microbial C:N ratios with their higher N contents (Lavallee et al., 2020), but this makes N loss more likely

Core Ideas

- Soil carbon (C) and nitrogen (N) changes after bioenergy crop establishment are important components of bioenergy climate mitigation potentials.
- After 13 years, soil C under switchgrass and native grasses was, respectively, 8% and 23% greater than under no-till corn.
- Mineral-associated organic matter ranged from 60% to 90% of total C, and particulate organic matter from 10% to 40%.
- Prior land use, perenniality, and plant diversity were important determinants of soil C and N accretion.
- Rates of soil C and N accretion were associated with root productivity and the formation of large soil aggregates.

as the same amount of C utilization results in a greater release of N.

Here, we evaluate the effect of three grass-based bioenergy cropping systems on soil POM, MAOM, and total C and N stocks for the first 13 years of establishment. Our aim is to contrast rates of C and N accrual among systems that differ in major cropping system traits (monoculture versus polyculture; perennial versus annual) and to determine the degree to which changes in soil C and N stocks can be attributed to differences in fine root production and soil aggregation among systems. We hypothesize that the fine root production of cropping systems will cause changes in soil C and N stocks, especially in the POM fraction, and that soil aggregation protects accumulated SOM from decomposition.

2 | MATERIALS AND METHODS

2.1 | Study site

The Biofuel Cropping System Experiment is located at the W.K. Kellogg Biological Station (KBS) in southwest Michigan (42˚24′18″N, 85˚24′02″W, elevation 288 m; Robertson & Hamilton, 2015). The site is part of the DOE Great Lakes Bioenergy Research Center and is an NSF Long-term Ecological Research site. Soils are loamy Hapludalfs from the Kalamazoo series (Crum & Collins, 1995), developed on glacial outwash with intermixed loess in surface horizons (Luehmann et al., 2016). The climate is humid, continental, and temperate, with a mean annual precipitation of 1005 mm and a mean annual temperature of 10.1˚C (Robertson & Hamilton, 2015).

The study site was established in 2008 on preexisting farmland as a randomized complete block design with five replicate 30 × 40 m plots of 10 different bioenergy cropping systems (Sanford et al., 2016); we examined switchgrass (variety Cave-in-Rock), a five native grasses polyculture including switchgrass (variety Southlow) as noted below, and continuous no-till corn. The switchgrass system was planted as a monculture; the native grasses system was comprised of big bluestem (*Andropogon gerardii* Vitman), Canada wild rye (*Elymus Canadensis* L.), Indiangrass [*Sorghastrum nutans* (L.) Nash], little bluestem [*Schizachyrium scoparium* (Michx.) Nash], and switchgrass (variety Southlow). Prior to 2008, the site produced corn, soybean (*Glycine max* L.) and alfalfa (*Medicago sativa* L.) in a several-decade rotation that from 2003 included fertilization with manure at ~68 kg N ha^{-1} year^{-1} (Brook Wilke, personal communication, 2021). To prepare for initial planting, the field underwent primary tillage using a chisel plow followed by seconary tillage using a soil finisher. Following establishment of the experimental plots in 2008, no-tillage was used. Further details on experimental design, site history and experiment initiation are provided in Sanford et al. (2016).

The switchgrass and native grasses systems were seeded at planting densities of 7–8 kg ha^{-1} and 8–11 kg ha^{-1} respectively, using a drop spreader (Traux Company, Inc). Both of these systems were N fertilized each May at 56 kg N ha^{-1} starting in 2010 and were harvested annually from 2010 postfrost in the fall (October or November), leaving a cutting height of 13–18 cm of plant stubble in the field. The corn system was planted annually at a 76 cm row spacing by a six-row no-till corn planter with a seeding rate of 84,000 seeds ha^{-1}. Preemergence weed control followed planting (Roundup Powermax at 3.7 kg ha^{-1}, ammonium sulfate at 3.8 kg ha^{-1}, and Acuron at 6.7 kg ha^{-1}); N fertilizer averaged 167 kg N ha^{-1} year^{-1}; and phosphorus and potassium application varied among plots based on annual postharvest soil tests. Corn grain was harvested in October, followed by stover harvest that left ~10 cm of corn stubble in the field.

2.2 | Soil sampling

Surface soil samples (0–25 cm depth) were taken in June 2021 from three randomly assigned sampling stations in each plot with a 2 cm diameter push probe. Each soil sample was pushed through a 4 mm sieve (roots and rocks >4 mm were removed), oven-dried at 60˚C until constant weight, and then composited by plot for a total of 15 samples (three systems × five replicate blocks).

2.3 | Archived soil samples

Archived soils were collected in 2008, 2013, and 2017 from the same approximate locations in each plot with a hydraulic probe (Geoprobe model 540MT, Geoprobe Systems; 7.6-cm diameter) to 1-m depth. Cores had been divided into depth intervals (0–10, 10–25, 25–50, and 50–100 cm), processed as above, and archived in glass jars. We composited subsamples of the archived 0–10 and 10–25 cm increments by plot then combined the increments proportionately to create a single composite sample for each plot to represent the 0–25 cm depth. Bulk density was measured for these cores using the dry mass and the volume of each core interval at time of sampling.

2.4 | Fine root production

For fine root production for the 2009–2013 period we used ingrowth core data from Sprunger et al. (2017). Briefly, two ingrowth cores (5-cm diameter by 13-cm depth) comprised of nylon mesh filled with a mixture of soil and sand were buried upright to 13-cm depth between plants at each of three sampling stations per plot prior to the beginning of the growing season (April) and one was retrieved after 3 and 6 months respectively. Fine roots (<2-mm diameter) were extracted from each core by gentle washing over a 1-mm sieve. Fine root production was calculated as total root biomass divided by number of days in the field. Further details appear in Sprunger et al. (2017). For corn, fine root production was estimated as the standing stock of fine roots at the time of peak biomass in late August of each year.

We used the ratio of fine root production to yield for the 2009–2013 period together with yield from 2014 to 2021 to estimate fine root production 20140–2021 based on the yield for each of these years. The estimation model is a linear mixed effect model predicting fine root production with aboveground plant productivity for each cropping system, with block and plot as random effects (see Section 2.9 Statistical Analysis). The resulting mixed effect model was used to predict fine root production by system for the period 2014–2021 (Figure S1; $r^2 = 0.45$, $p < 0.001$).

2.5 | POM and MAOM fractionation

We determined POM and MAOM by size fractionation (Cotrufo et al., 2019). Ten grams of oven-dried soil were shaken with 30 mL of 5% sodium hexametaphosphate and 12 glass beads (4-mm diameter) in 50-mL centrifuge tubes at 120 oscillations min^{-1} for 17 h to disperse aggregates. Samples were then poured over a 53-µm sieve and rinsed with deionized water. Any material that passed through the 53-µm sieve was considered the MAOM fraction and all material trapped by the sieve was considered the POM fraction (Lavallee et al., 2020). Each fraction was oven-dried at 60° C until constant weight. The soil recovery rate was measured by dividing the total mass of POM and MAOM by the original sample mass; recovery rates were between 99% and 103%.

2.6 | Elemental analysis

For each soil sample, oven-dried bulk soils as well as the corresponding MAOM and POM fractions were finely ground to less than 250 µm using a shatterbox mill with a hardened steel grinding container and puck (SPEX SamplePrep 8530 Enclosed ShatterBox 8530). Dry combustion gas chromatography on a Carlo-Erba Elemental Analyzer was used to determine soil C and N concentrations (Costech Analytical Technologies). The analyzer was calibrated with the analytical standard Acetanilide (C_8H_9NO). A blind standard (low organic content soil, elemental microanalysis; 1.55% C, 0.13% N) was run to check the calibration of every 12 samples. If the coefficient of variation of the standards exceeded 10%, or if the blind standard samples were not within 10% of label values, the samples were rerun.

2.7 | Soil C and N stock calculations

C and N in bulk soil, as well as POM and MAOM fractions, were calculated for each system using measured C and N concentrations, the sample depth interval, and bulk density values. Bulk density data were not available for 2008 or 2021, so the mean bulk density values of each system from 2013 and 2017 were used for each depth. Previous work at a nearby site on the same soil series (Syswerda et al., 2011) showed no changes in bulk density upon conversion of similar annual crops to no-till annual or perennial crops in these sandy loam soils, nor were 2013 and 2017 bulk density values statistically distinguishable (Table S1). C and N recovery rates for POM and MAOM were calculated by comparing the sums of the two fractions to the corresponding unfractionated bulk soil; recovery rates were between 96% and 144% and to correct for fractionation bias, we normalized POM and MAOM C and N stocks to their total unfractionated values.

2.8 | Aggregate stability

We wet-sieved 50 g of soil from each plot sampled in 2021 to determine large stable macroaggregates (>2000 µm), small stable macroaggregates (250–2000 µm), microaggregates (53–250 µm), and disaggregated silt and clay fractions (<53 µm; Kemper & Rosenau, 1986). To differentiate size classes we placed a 2-mm sieve into a larger container that was then covered with 2 cm of deionized water. The soil in the sieve was left to slake for 5 min before raising and lowering the sieve about 50 times in 2 min, with the aggregates just surfacing on the upward stroke and without hitting the bottom of the tub on the downward stroke. The aggregates remaining on the sieve were collected and dried in an oven at 60°C until constant weight. The remaining effluent was poured over a 250-µm sieve and the slaking and sieving process repeated. Following this, the effluent was poured over a 53-µm sieve and the slaking and sieving process repeated once more. The disaggregated silt and clay fraction was calculated by subtracting the sum of the dried aggregate mass from the total sample dry mass. Aggregate weights were not corrected for sand content. Mean weight diameter was calculated for each sample and used as a proxy for aggregate stability as follows:

$$\text{MWD} = \sum P_i * X_i,$$

where P_i the proportion of soil in the ith aggregate size class and X_i is the mean diameter of the size above and below the ith aggregate size class.

2.9 | Statistical analysis

Our experiment follows a randomized complete block design. For the objective of comparing the three studied systems in their effects on POM and MAOM, we used the mixed effect model approach. The statistical model included the systems and time (year) and their interaction as fixed effects and the experimental blocks of our field experiment's randomized complete block design as a random effect. Years were treated as a repeatedly measured factor and the effect of experimental units, that is, the field plots, was used as a random effect and as a subject of repeated measurements in the statistical model (Zuur et al., 2009).

We constructed separate models for the C and N stocks as well as for the different soil fractions. Each model had the following form:

$$\text{stock} \sim \text{crop} : \text{year} + \text{r}\,(\text{block}) + \text{r}\,(\text{plot}),$$

where stock is the soil C or N component (e.g., POM C), crop is the cropping system, year is the year that the sample was taken, block is the experimental replicate identifier, plot is the experimental unit identifier, and 'r' denotes a random intercept.

We also used the mixed effect model to test our hypotheses about fine root production and aggregate protection. Here, we used a bivariate form with the change in root C or N stocks as the response variable with block a random effect as above. For the fine root production hypothesis, the mean annual fine root production rate over the study period was used as the predictor variable. For the aggregate protection hypothesis, the proportion of soil found in each aggregate size class was used as the predictor variable.

All linear mixed effects models were diagnosed for normal residuals and homogeneous variance using the Shapiro–Wilks normality test and the Levene homogeneity test (Zuur et al., 2009). All hypothesis test p-values were adjusted for multiple comparisons as per Sidak (1967). A p-value of 0.05 was used to delineate statistical significance. The R software was used for all analyses (R Core Team, 2014).

3 | RESULTS

3.1 | Soil C stocks

Total soil C stocks responded differently to land use change. The native grasses system accrued C at a rate of 0.36 Mg C ha^{-1} year^{-1} (Figures 1a and 2a, Tables S2, S3, and S6; $p = 0.004$). In contrast, the switchgrass and corn system did not gain C at a statistically significant rate ($p = 0.62$ and 0.28, respectively). By 2021, 13 years post-establishment, the mean total soil C in the upper 25 cm of the no-till corn, switchgrass, and native grasses systems had changed from 30.1 ($\pm$ 0.83 SE) Mg C ha^{-1} to 28.4 ($\pm$ 1.4), 30.8 ($\pm$ 1.4), and 34.8 ($\pm$ 1.4) Mg C ha^{-1}, respectively (Figures 1a and 2a, Table S3; $p = 0.038$ and 0.007, respectively).

The POM and MAOM fractions followed similar trends (Figure 1a): both fractions accrued C in the native grasses system (0.18 Mg C ha^{-1} year^{-1} for POM and 0.22 Mg C ha^{-1} year^{-1} for MAOM; Figure 2a; Table S3), while the switchgrass and corn systems did not gain C at a statistically significant rate in either fraction ($p > 0.30$). For soil C, the two fractions responded in a similar direction and magnitude to that of bulk soil.

In all post-establishment years, the absolute differences (Figure 1) and rates of change (Figure 2) for soil C and N between switchgrass and native grasses increased, though not yet significantly at the $\alpha = 0.05$ level.

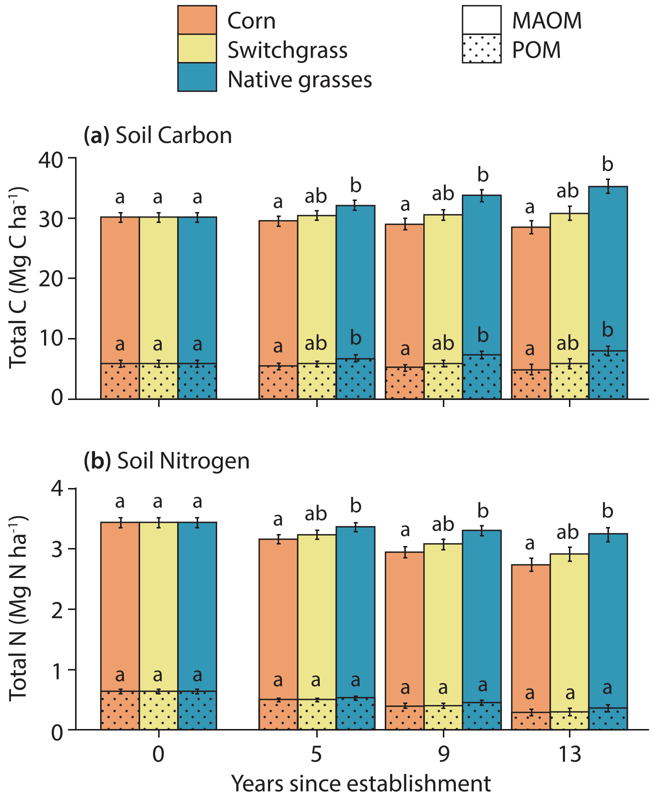

FIGURE 1 Changes in (a) total soil carbon (C) and (b) total soil nitrogen (N) since time of establishment in no-till corn, switchgrass, and native grasses cropping systems based on linear mixed effects model. Within each bar, particulate organic matter (POM) = stippled area, mineral-associated organic matter (MAOM) = non-stippled area. Vertical lines represent standard errors of the POM and MAOM stocks; different letters denote significant differences ($p < 0.05$) within (not across) individual time periods.

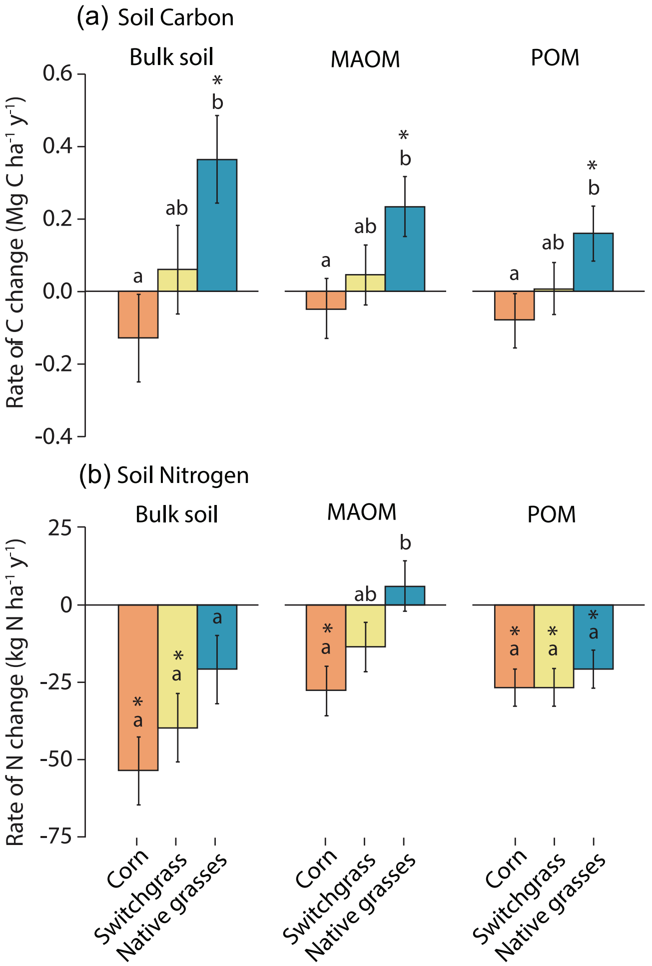

FIGURE 2 Rates of (a) soil carbon (C) and (b) soil nitrogen (N) change in different size fractions of three bioenergy cropping systems based on linear mixed effect models. Vertical lines in each bar denote standard errors. Letters denote statistical differences between slopes within an element and fraction. Asterisks denote rates of change significantly different from zero. MAOM, mineral-associated organic matter; POM, particulate organic matter.

3.2 | Soil N stocks

In contrast to soil C stocks, soil N stocks tended to decline in all three systems and the two soil fractions responded differently in magnitude and direction following land use change (Figures 1b and 2b, Tables S4–S6). In the bulk soil fraction the corn system lost soil N at a rate of 54 kg N ha^{-1} year^{-1} while the switchgrass system lost N at a rate of 40 kg N ha^{-1} year^{-1} (Table S4, $p < 0.001$). In the native grasses system bulk soil N loss was marginally significant ($p = 0.07$) at a nominal rate of 21 kg N ha^{-1} year^{-1}. Soil N was lost from the POM fraction at similar rates of 27, 27, and 21 kg N ha^{-1} year^{-1} for the corn, switchgrass, and native grasses systems, respectively ($p < 0.001$). MAOM N stocks, on the other hand, declined significantly only in the corn system (28 kg N ha^{-1} year^{-1}; Figure 2b, Table S4; $p = 0.001$).

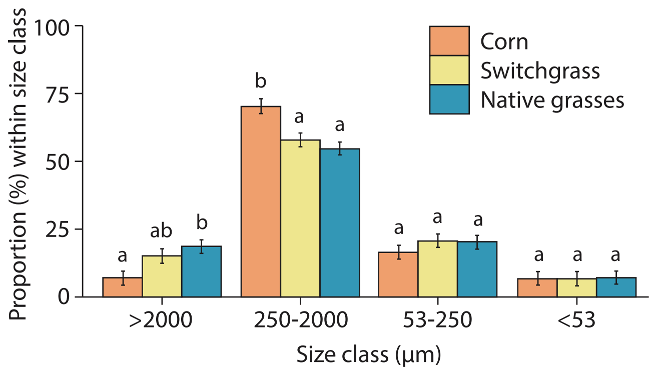

FIGURE 3 Proportion of water-stable soil aggregates in >2000-, 250–2000, 53–250 and <53-µm size classes in corn, switchgrass, and native grasses in 2021. Vertical lines represent standard errors; different letters within size classes denote significant differences among cropping systems ($p < 0.05$).

3.3 | Soil aggregates

In all three systems the 250–2000 µm size class of water-stable aggregates dominated others (Figure 3). The proportion of aggregates in the >2000- and 250–2000 µm aggregate size class responded differently to land use change (Tables S7, S5; $p < 0.001$). The corn, switchgrass, and native grasses systems had 6.9%, 15.1%, and 18.4% of their soil in >2000-µm aggregate size class, respectively. Over the 7-year period, the switchgrass and native grasses systems shifted their aggregate size distributions from the 250–2000 µm into the >2000-µm size class. Systems also differed with respect to the 250–2000 µm aggregate size class: the corn, switchgrass, and native grasses systems had 69.9%, 57.7%, and 54.4% of their soil in this class, respectively. We found no significant differences among systems for the two smaller aggregate size classes (Figure 3).

We found significant associations between aggregate size distributions and total soil C (Figure 4a) and N (Figure 4b) changes across all systems. In particular, there was a positive relationship between the proportion of aggregates in the >2000-µm size class and total C gains and N losses following cropping system establishment ($R^2 \geq 0.35$; $p < 0.025$). The 250–2000 µm size class showed similar relationships to both C and N stock changes, though the direction was negative ($R^2 \geq 0.36$; $p < 0.031$).

3.4 | Fine root production

Fine root production varied year to year (Figure 5), but tended to be highest in the native grasses system, with values typically >2 times higher than in either the corn or switchgrass system. During the 13 years of this study, the corn, switchgrass, and native grasses systems had a mean

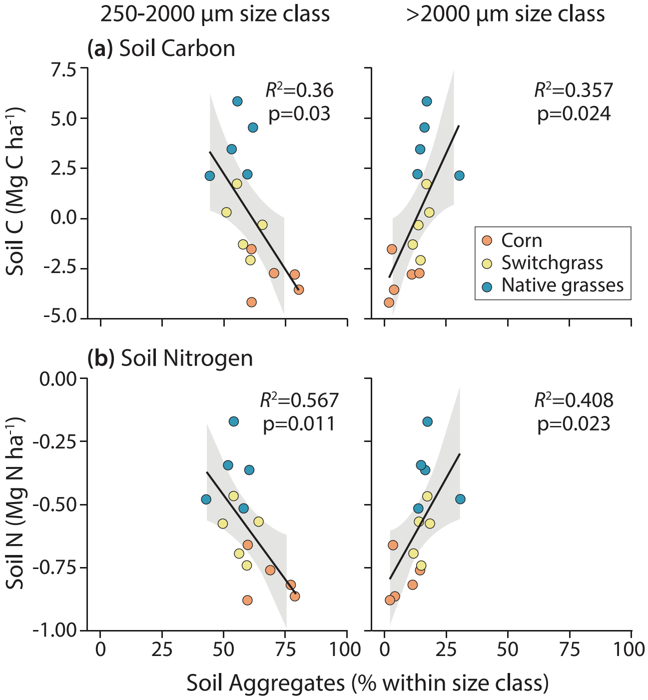

FIGURE 4 Relationship between soil (a) carbon (C) and (b) nitrogen (N) stock change and 2021 aggregate size classes for the two largest size classes. Different cropping systems are represented by different symbol colors; lines represent linear regressions. Shading represents 95% confidence intervals of the regression fits. All p values are less than 0.031.

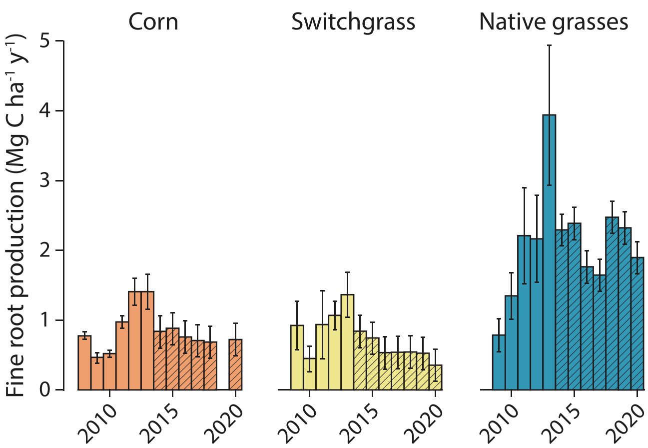

FIGURE 5 Fine root production in ingrowth cores since time of establishment. Values for 2008 to 2013 from Sprunger et al. (2017); values for 2014 to 2020 (hatched) imputed using 2008–2013 fine root production data and 2014–2020 harvest data. Error bars are standard errors.

fine root production rate of 0.85, 0.74, and 2.01 Mg C ha^{-1} year^{-1}, respectively. We found strong positive relationships between the mean rate of fine root production and soil C and N changes following establishment across all systems ($R^2 > 0.60$; Figure 6, Table S5; $p < 0.003$).

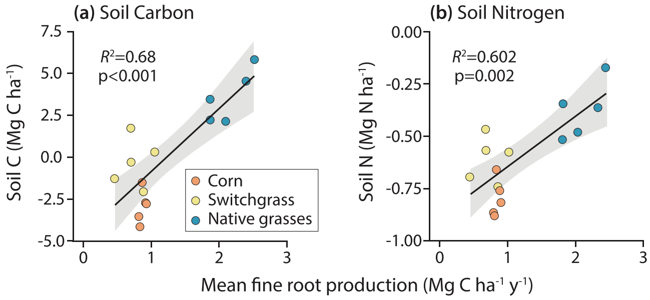

FIGURE 6 Relationship between soil (a) carbon (C) and (b) nitrogen (N) stock changes and mean fine root production (see Figure 5 legend). Different cropping systems are represented by different symbol colors; lines represent linear regressions. Shading represents 95% confidence intervals of the regression fits. All p values are less than 0.003.

4 | DISCUSSION

In general, our results show that for the 13 years following cropping system establishment, soil C stocks persisted under continuous no-till corn and switchgrass, but accrued significantly under native grasses. Soil N declined in all systems but followed a similar pattern: the most declined under corn, less under switchgrass, and least under grasses. POM and MAOM did not change proportionately in any system throughout the study period; MAOM made up ~80% of total C and N stocks while POM made up the difference. These findings reinforce the notion that soil C and N stock changes are dependent on current and previous land use and that the magnitude and direction of these stock changes are associated with both root productivity and aggregate stability.

Consistent with our first hypothesis, soils under native grasses, which had the greatest fine root production, also had higher soil C stocks following establishment when compared to switchgrass and continuous corn, each of which had lower fine root production. Soil C accrual in the native grasses system occurred in both POM and MAOM fractions, suggesting that both particulate residue from recent plant inputs and associated microbial by-products and soluble plant material (as primary contributors to MAOM) contributed to total soil C accumulation (Kutsch et al., 2009). Prior studies have also attributed soil C sequestration following land use change to increased root productivity (Cates et al., 2016; Liebig et al., 2005; Mosier et al., 2021; Puget & Drinkwater, 2001; Stewart et al., 2016; Stockmann et al., 2013).

We were surprised that switchgrass did not significantly accrue surface soil C because other studies have observed C accrual in perennial bioenergy cropping systems in general, including switchgrass (e.g., Liebig et al., 2005). Perennial crops often accrue C because of longer lived roots (Anderson-Teixeira et al., 2013) and low soil disturbance (Mosier et al., 2021). Quantitative models also predict soil C accretion under switchgrass; Martinez-Feria and Basso (2020), for example, modeled the impact of establishing switchgrass on soil C stocks at our site and estimated an increase of ~10 Mg C ha^{-1} to 1 m depth after 13 years of production. In contrast, we measured a nonsignificant increase of 0.78 Mg C ha^{-1} in surface soil. Our switchgrass C accrual rates to date (0.06 Mg C ha^{-1} $year^{-1}$) are lower than most found in the Great Plains (Liebig et al., 2008) and Texas (Dou et al., 2013), where accrual rates in surface soil were 0.8 to 1.2 Mg C ha^{-1} $year^{-1}$ and 1.4 Mg C ha^{-1} $year^{-1}$, respectively. These sites also report switchgrass aboveground productivity ranges (3.2–12.6 Mg ha^{-1} $year^{-1}$) similar to those observed at our site (1.8–10.6 Mg ha^{-1} $year^{-1}$). In shorter term studies, others (e.g., Schmer et al., 2011) have reported lower accrual rates in the Great Plains, including C loss attributed to landscape variability. Likewise, in early work at our site, Sprunger and Robertson (2018) noted no increase in active C pools five years after establishmentexcept when switchgrass was in more diverse assemblages (mixed native grasses and restored prairie), consistent with more recent findings that plant diversity promotes C accrual (Furey and Tilman, 2021; Kravchenko et al., 2019, 2021).The lower SOC sequestration rates observed at our site may additionally be due to higher baseline C stocks contributed by prior manure applications, as Sanford et al. (2012) concluded was the cause for their observed SOC loss over time in a similar land use change experiment in Wisconsin.

We expected that soil N stocks would largely mirror C increases especially in the perennial grasses because of traits that favor N conservation—belowground translocation prior to senescence, long growing seasons, and large root systems (Mosier et al., 2021). Instead, soil N stocks largely declined following establishment, probably because the prior corn-alfalfa rotation had reduced soil C:N stocks due to N fixation (alfalfa) and N fertilizer and manure inputs (corn). Hussain et al. (2021) found a similar pattern for legacy phosphorus loss at our site, where soluble phosphorus from the prior cropping history continued to leach from all systems consistently for 7 years after bioenergy crop establishment. Soil N retention at our site (i.e., no significant loss) only occurred in the MAOM fractions of the switchgrass and the native grasses systems.

We observed a positive association between soil C and N stock gains and the amount of soil shifted from the 250–2000 to the >2000-μm aggregate size class, consistent with our second hypothesis which predicted greater C and N accumulation in systems with larger soil aggregates. Thirteen years after establishment the proportion of aggregates in the >2000-μm size class of the native grasses system was 12% greater than the proportion in the corn system, associated with a faster rate of soil C accumulation. There was a corresponding loss of aggregates from the 250- to 2000-μm size class in the native grasses system. In the corn system neither the proportion of large aggregates nor soil C increased significantly. Trends in the switchgrass system were intermediate to these.

The relationship between aggregate size distribution and soil C is well established in the literature (e.g., Six et al., 1999; Tisdall & Oades, 1982) and has been observed in other field experiments at KBS (Grandy & Robertson, 2006b; Ruan & Robertson, 2013). The build up of air pressure during inundation, so-called "slaking," breaks apart aggregates and is a key process in making POM accessible for microbial decomposition. In the present experiment, increased soil cover in perennial systems may have dampened the impact of rainfall and lengthened the period of infiltration, thus reducing the breakdown of large aggregates from slaking, which typically occurs overwinter in our soils when untilled (Ruan & Robertson, 2017). A reduction in the slaking process in the perennial systems may have contributed to the observation of higher rates of macroaggregation in this study.

Our data also identify a possible role of plant diversity for increasing soil C and N, perhaps in concert with increased root productivity and aggregate formation. In all post-establishment years, both the absolute differences (Figure 1) and rates of change (Figure 2) for soil C and N increased between switchgrass and native grasses. Though the changes are not yet significant at the $a = 0.05$ level, the trend suggests an emerging role for plant diversity, as noted above and documented elsewhere (e.g., Furey & Tilman, 2021; Kravchenko et al. 2019, 2021; Robertson & Sprunger et al., 2018; Sprunger et al., 2017; Tiemann et al., 2015).

With a changing climate, research that improves our ability to estimate the impacts of land-based negative emission technologies such as bioenergy with carbon capture and storage (BECCS), whereby the C in biomass is captured and stored in geologic formations when biomass is converted to fuel or electricity, are becoming increasingly salient (Robertson et al., 2022). Our study suggests that bioenergy systems with high root productivity and the tendency to form large aggregates that persist following the cessation of tillage may provide the largest soil C and N accretion gains. However, the influence of previous land use is also important insofar as it can dictate the magnitude and even direction of soil C and N changes, as is in this study's findings of little or no carbon gains at a site with a modest history of manure applications prior to establishment. Carbon debt created by bioenergy systems established on land in set-aside programs, forests, and wetlands is well known (Fargione et al., 2008; Gelfand et al., 2011); however, the importance of cropland management history such as prior manure application was a surprising result. Results suggest that careful site selection will be a crucial determinant of the soil-based benefits of BECCS on even current agricultural land. We found that differentiating SOM into POM and MAOM can help to identify the likely consequences of such establishment, especially for soil N change. Separating SOM into two physically defined fractions with distinct formation and loss mechanisms may help to identify which sites have room for MAOM formation and POM accumulation and which sites may be MAOM saturated (Cotrufo et al., 2019).

While we found support for both of our hypotheses—that increased organic matter supply from root production and decreased organic matter loss from aggregate occlusion can lead to overall C accrual and N conservation—we are not able to ascertain their relative importance and interactions. Future research is needed to further disentangle the effects of previous land use, root production, and aggregate occlusion on soil C and N dynamics following land conversion, as well as a greater breadth of study sites and environmental contexts where bioenergy production systems are likely to be deployed.

AUTHOR CONTRIBUTIONS

Sophie Perry: Conceptualization; investigation; methodology; writing—original draft; writing—review and editing. **Grant Falvo**: Conceptualization; formal analysis; methodology; writing—review and editing. **Samantha Mosier**: Conceptualization; methodology; writing—review and editing. **G. Philip Robertson**: Conceptualization; supervision; writing—review and editing.

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DATA AVAILABILITY STATEMENT

Data in this contribution are available at Dryad (https://doi.org/10.5061/dryad.547d7wmf3).

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SUPPORTING INFORMATION

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

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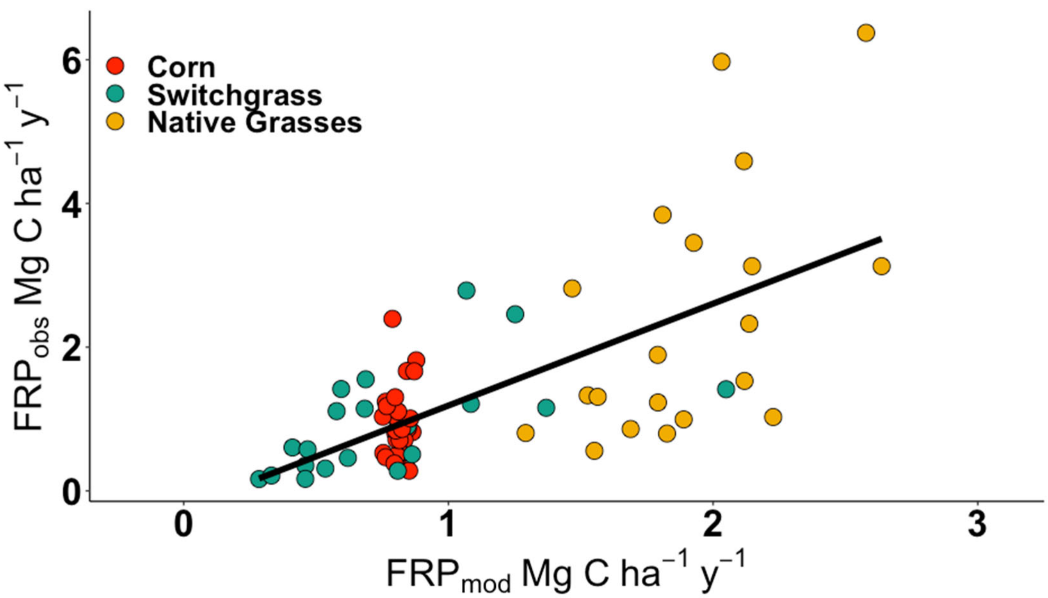
Supplemental Material

Supplemental Figures and Tables

Supplemental Figures



Supplemental Figure S1. Relationships between fine root production observed (FRP_{obs}) versus modeled (FRP_{mod}) as estimated by peak standing stock (corn) or ingrowth cores (switchgrass, native grasses) for the period 2009-2014. $R^2 = 0.45$ ($p < 0.001$). The relationship for corn suggests that fine root peak standing stocks are independent of aboveground production for the range of yields encountered over the sampling period.

Supplemental Tables

Supplemental Table S1. Soil bulk density (means and standard errors) for the 0 - 25 cm soil depth increment in 2013 and 2017.

System	Year	Bulk density	Standard error
		g cm^3	
Corn	2013	1.48	0.03
Switchgrass		1.50	0.03
Native Grasses		1.51	0.03
Corn	2017	1.40	0.03
Switchgrass		1.49	0.01
Native Grasses		1.48	0.02

Supplemental Table S2. Carbon accrual rates for different fractions in different systems based on linear mixed effects model results through time. Bolded p-values denote a rate significantly different from 0. Letters denote statistical differences between systems within each soil fraction.

Soil fraction	System	C accrual rate	Std. error	df	t-value	p-value	Pairwise diff.
Total C	Corn	-0.129	0.120	53	-1.078	0.286	a
	Switchgrass	0.060	0.120	53	0.496	0.621	ab
	Native grasses	0.364	0.120	53	3.034	**0.004**	b
POM C	Corn	-0.079	0.075	53	-1.045	0.301	a
	Switchgrass	0.005	0.075	53	0.061	0.952	ab
	Native grasses	0.160	0.075	53	2.133	**0.038**	b
MAOM C	Corn	-0.048	0.083	53	-0.580	0.564	a
	Switchgrass	0.045	0.083	53	0.539	0.592	ab
	Native grasses	0.234	0.083	53	2.820	**0.007**	b

Supplemental Table S3. Nitrogen accrual rates for different fractions in different systems based on linear mixed effects model results through time. Bolded p-values denote a rate significantly different from 0. Letters denote statistical differences between systems within each soil fraction.

Soil fraction	System	N accrual rate	Std. error	df	t-value	p-value	Pairwise diff.
Total N	Corn	-0.054	0.011	53	-4.743	**<0.001**	a
	Switchgrass	-0.040	0.011	53	-3.529	**<0.001**	a
	Native grasses	-0.021	0.011	53	-1.844	0.071	a
POM N	Corn	-0.027	0.006	53	-4.602	**<0.001**	a
	Switchgrass	-0.027	0.006	53	-4.544	**<0.001**	a
	Native grasses	-0.021	0.006	53	-3.644	**<0.001**	a
MAOM N	Corn	-0.028	0.008	53	-3.432	**0.001**	a
	Switchgrass	-0.014	0.008	53	-1.752	0.086	ab
	Native grasses	0.006	0.008	53	0.745	0.460	b

Supplemental Table S4. Linear mixed effects model results for soil aggregate size distributions. The bolded p-value denotes a significant main effect.

Predictor variable	df	F-ratio	p.value
Size class	44	280.946	**< 0.001**
Size class*System	44	5.682	**< 0.001**

Supplemental Table S5. Linear mixed effect model results for aggregate and fine root production models.

Model formula	**R^2**	**df**	**F-ratio**	**p-value**
Δ Bulk C ~ > 2000 um	0.357	13	6.866	**0.024**
Δ Bulk C ~ 250-2000 um	0.360	13	5.904	**0.030**
Δ Bulk C ~ Root Production	0.680	13	28.519	**<0.001**
Δ Bulk N ~ > 2000 um	0.408	13	7.153	**0.023**
Δ Bulk N ~ 250-2000 um	0.567	13	9.002	**0.011**
Δ Bulk N ~ Root Production	0.602	13	19.724	**0.002**

Supplemental Table S6. Soil C and N stocks by soil fraction in each cropping system. Values are means (standard errors).

System	Year	Bulk C	POM C	MAOM C	Bulk N	POM N	MAOM N
Corn	2008	29.39 (1.55)	5.89 (0.37)	23.5 (1.48)	3.43 (0.14)	0.61 (0.05)	2.81 (0.15)
	2013	30.01 (1.47)	5.84 (0.17)	24.17 (1.34)	3.4 (0.16)	0.58 (0.05)	2.82 (0.13)
	2017	26.97 (0.69)	3.13 (0.2)	23.84 (0.77)	2.84 (0.06)	0.23 (0.02)	2.6 (0.07)
	2021	29.25 (1.13)	6.03 (0.73)	23.22 (0.81)	2.83 (0.08)	0.37 (0.05)	2.46 (0.07)
Switchgrass	2008	32.03 (2.58)	7.04 (0.83)	24.99 (1.8)	3.64 (0.22)	0.71 (0.05)	2.92 (0.17)
	2013	29.06 (1.05)	4.76 (0.39)	24.3 (1.11)	3.26 (0.1)	0.49 (0.09)	2.77 (0.13)
	2017	28.2 (0.92)	3.86 (0.37)	24.34 (0.6)	2.92 (0.07)	0.26 (0.02)	2.66 (0.07)
	2021	33.11 (2.21)	7.7 (0.64)	25.41 (2.36)	3.13 (0.19)	0.39 (0.03)	2.74 (0.2)
Native Grasses	2008	32.24 (1.13)	7.36 (0.98)	24.88 (0.62)	3.67 (0.1)	0.78 (0.11)	2.9 (0.08)
	2013	30.26 (0.4)	5.92 (1.24)	24.34 (1.35)	3.05 (0.16)	0.37 (0.03)	2.67 (0.18)
	2017	31.03 (1.64)	4.97 (0.56)	26.99 (0.82)	3.09 (0.19)	0.3 (0.03)	2.95 (0.08)
	2021	37.13 (0.67)	9.78 (1.21)	27.36 (1)	3.47 (0.04)	0.52 (0.08)	2.95 (0.07)

Supplemental Table S7. Proportion (%) of aggregates in different size class fractions for each cropping system. Values are means (standard errors).

System	> 2000 µm	250-2000 µm	53-250 µm	< 53 µm
Corn	6.89 (2.45)	69.92 (4.11)	16.4 (4.42)	6.78 (1.22)
Switchgrass	15.08 (1.19)	57.68 (2.45)	20.57 (1.69)	6.67 (0.66)
Native Grasses	18.44 (3.08)	54.41 (3.03)	20.17 (1.5)	6.98 (0.22)