

RESEARCH ARTICLE

Switchgrass (*Panicum virgatum* L.) cultivars have similar impacts on soil carbon and nitrogen stocks and microbial function

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Abstract

Switchgrass (*Panicum virgatum* L.) production for biofuel has the potential to produce reasonable yields on lands not suited for conventional agriculture. We assessed nine switchgrass cultivars representing lowland and upland ecotypes grown for 11 years at a site in the upper Midwest USA for belowground differences in soil carbon and nitrogen stocks, soil organic matter fractions, and standing root biomass to 1 m depth. We also compared potential nitrogen mineralization and carbon substrate use through community-level physiological profiling in surface soils (0–10 cm depth). Average yields and standing root biomass differed among cultivars and between ecotypes, but we found no significant cultivar-related impacts on soil carbon and nitrogen stocks, on the distribution of particulate and mineral-associated soil organic matter fractions, nor on potential nitrogen mineralization or microbial community-level physiological profiles. That these traits did not differ among cultivars suggests that soil carbon and nitrogen gains under switchgrass are likely to be robust with respect to cultivar differences, and to this point not much affected by breeding efforts.

KEYWORDS

bioenergy, lowland switchgrass, microbial community profile, mineral-associated organic matter, particulate organic matter, potential nitrogen mineralization, soil carbon, soil nitrogen, switchgrass ecotypes, upland switchgrass

1 | INTRODUCTION

Switchgrass (*Panicum virgatum* L.) is a potential bioenergy crop noted for its ability to grow on marginal lands and produce reasonable yields without large fertilizer inputs (Casler et al., 2015; Gelfand et al., 2013; McLaughlin et al., 2002; Robertson et al., 2011). As a

perennial crop, switchgrass grows without annual replanting and maintains a substantial rooting system. These traits promote soil carbon (C) accrual, nutrient conservation, and other benefits as compared to annual cropping systems (Frank et al., 2004; Liebig et al., 2008; Mosier et al., 2021; Robertson et al., 2017; Sprunger et al., 2020).

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Cultivars of switchgrass, representing three main ecotypes, are adapted to different environments and selected for certain traits (Casler, 2012; Lovell et al., 2021; Yang et al., 2009). Upland ecotypes are typically adapted to colder, drier conditions and higher elevations, while lowland and coastal ecotypes are generally adapted to warmer conditions and lower elevations (Casler, 2012; Lovell et al., 2021). Ecotype differences in soil C and nitrogen (N) attributes are largely unknown but potentially important given the importance of these traits to the overall sustainability of bioenergy cropping systems.

Rates of C accretion under switchgrass can vary widely; rates from -0.6 to $4.3 \text{ Mg C ha}^{-1} \text{ year}^{-1}$ have been documented (Frank et al., 2004; Garten & Wullschlegel, 2000; Lai et al., 2018; Liebig et al., 2008). For example, while lowland ecotypes can have a higher specific root length and more arbuscular mycorrhizal fungi (Emery et al., 2018; Kinnetz, 2017), and root: shoot ratios can also differ among cultivars (Cordova et al., unpublished results), it is unclear if these root traits have an impact on soil C and N. Additionally, we know little about the distribution of C and N stocks across soil organic matter fractions under different switchgrass cultivars.

Though bulk C and N stocks are an important metric of switchgrass soil impact, soil C and N fractions that are functionally distinct can tell us much more about soil C and N protection and permanence (Lavalley et al., 2020). For example, particulate organic matter (POM) can be protected from decomposition through occlusion within aggregates and typically persists for 1–50 years, whereas mineral-associated organic matter (MAOM) is protected via chemical bonding on minerals and typically persists from 10 to 1000 years (Golchin et al., 1997; Kleber et al., 2015; Lavalley et al., 2020).

There may also be differences in indices of microbial C and N cycling among cultivars. Potential N mineralization rates can provide information about plant-available N and soil N cycling, and potential C substrate use, also known as microbial community-level physiological profiling, can provide information about microbial activity and function by describing how microbial communities are utilizing soil C sources (Sinsabaugh et al., 1999).

In this study, we evaluate differences with respect to soil C and N storage as well as microbial function among nine different switchgrass cultivars from both lowland and upland ecotypes grown at a single location for 11 years. At a single site in SW Michigan, we analyzed soil C and N stocks and their distributions between POM and MAOM fractions for each cultivar, and as well tested for differences in potential N mineralization rates and C substrate use. We hypothesize that differences in aboveground and belowground productivity will be reflected in soil C and N storage and microbial function.

2 | MATERIALS AND METHODS

2.1 | Study site

This study was conducted at the Great Lakes Bioenergy Research Center's Switchgrass Variety Experiment located at the Kellogg Biological Station Long-term Ecological Research Site in southwest Michigan, USA ($42^{\circ}24'18''\text{N}$, $85^{\circ}24'02''\text{W}$). Mean annual precipitation at the site is $\sim 1005 \text{ mm}$ and mean annual temperature is $\sim 10.1^{\circ}\text{C}$ (Robertson & Hamilton, 2015). Soils are in the Kalamazoo soil series and are coarse and fine loamy, mixed, mesic Typic Hapludalfs (Robertson & Hamilton, 2015). Prior to the establishment of the experiment, the land was managed as a rotational cropping system with alfalfa, soybeans, and maize (Perry et al., in review).

The Switchgrass Variety Experiment began in Spring 2009 when switchgrass was planted at a seeding rate of $6.7\text{--}7.8 \text{ kg/ha}$. The experiment consists of different switchgrass cultivars in $4.6 \text{ m} \times 12.2 \text{ m}$ plots each replicated in four blocks using a randomized complete block design. Each spring post-establishment the switchgrass was fertilized with 78 kg N/ha and harvested in the Fall, leaving $13\text{--}18 \text{ cm}$ of plant height. Harvested biomass was oven-dried before weighing to determine switchgrass yield. We used nine switchgrass cultivars: two lowland (Alamo and Kanlow) and seven upland (Southlow, Cave-in-rock, Trailblazer, Blackwell, Dacotah, NE28, and Shelter), all recommended as cultivars best suitable for southern Michigan.

2.2 | Soil sampling and processing

We sampled soils in November 2020 to a depth of 1 m (7.6 cm diameter) using a hydraulic sampling probe (Geoprobe, Salina, KS, USA). Intact soil cores were then split into four depth increments: $0\text{--}10 \text{ cm}$, $10\text{--}25 \text{ cm}$, $25\text{--}50 \text{ cm}$, and $50\text{--}100 \text{ cm}$. In total we collected soil samples from $9 \text{ treatments} \times 4 \text{ blocks} \times 4 \text{ depths}$ for a total of 144 increment samples. Each soil sample was sieved to 4 mm to exclude gravel. Any roots greater than 4 mm were returned to the remaining soil sample. A subsample of sieved soil was dried at 60°C for gravimetric water content and then a portion was finely ground in an impact mill to $250 \mu\text{m}$ for elemental analysis. All roots were rinsed from the remaining soil using a root hydropneumatics elutriator (Smucker et al., 1982) and then oven-dried to determine root biomass dry weights.

Each 4 mm sieved oven-dried soil sample was fractionated into POM and MAOM by wet sieving (Lavalley et al., 2020). First, 30 mL of deionized water was added to 10 g of oven-dried soil. Soils were then shaken on an orbital

shaker table for 18 h with glass beads to break up aggregates. The soil slurry was then poured through a 53 μm sieve and glass beads were removed. Material caught by the sieve was considered POM and material that passed through, MAOM. After separation, POM and MAOM fractions were dried at 60°C. Recoveries were within $\pm 3\%$ of initial mass. POM and MAOM fractions were then finely ground to 250 μm as above for elemental analysis.

Bulk soil samples as well as POM and MAOM were analyzed for C and N concentrations on a Carlo-Erba Elemental Analyzer (Costech Analytical Technologies, Valencia, CA, USA). Total C and N stocks were determined for each depth increment sample using C and N concentrations and gravel-free bulk density.

2.3 | Microbial analyses

We sampled for microbial attributes in June 2021 by sampling to a depth of 10 cm using a push auger (2 cm diameter). Each soil sample was sieved to 4 mm to remove gravel and large roots. A subsample of sieved soil was dried at 60°C for gravimetric water content.

Potential N mineralization was determined via a 21-day aerobic incubation. First, a subsample of each soil was extracted using 1 M KCl to quantify initial inorganic N concentrations (nitrate + ammonium). To 8 g of fresh soil, we added 100 mL of 1 M KCl and shook briefly by hand. After 24 h, the solution was filtered through a Whatman No. 1 glass fiber filter and analyzed on a Lachat Flow Injection Autoanalyzer (Lachat Instruments, Milwaukee, WI, USA). Another subsample of each soil (8 g) was weighed into 250 mL specimen cups and incubated in an incubator at constant temperature (25°C) and moisture for 21 days. Then the soils were extracted as described above to quantify the change in inorganic N concentrations. The potential net nitrification rate was calculated as $([\text{nitrate on day 21}] - [\text{nitrate on day 0}]) / 21 \text{ days}$. The potential net mineralization rate was calculated as $([\text{nitrate} + \text{ammonium on day 21}] - [\text{nitrate} + \text{ammonium on day 0}]) / 21 \text{ days}$.

We analyzed soils for community-level physiological profiling using Biolog EcoPlates (Biolog, Hayward, CA, USA). Fresh soil samples were each diluted (1:10) in a phosphate buffer solution (8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4 , 0.24 g KH_2PO_4). We then added four 3 mm glass beads and vortexed and centrifuged the sample. The supernatant was then diluted, with 1 mL of the 1:10 dilution added to 9 mL of phosphate buffer solution. Next, 100 μL of the solution was added to a 96-well plate and incubated in the dark at room temperature for 5 days. After 5 days, the color absorbance was measured at 590 and 750 nm using a Biotek Synergy HTX plate reader (BioTek Instruments, Winooski, VT, USA). Substrate utilization for richness,

diversity, and evenness based on well color development follows Sofo and Ricciuti (2019).

2.4 | Data analysis

We used a general linear mixed-effects model to assess the effect of switchgrass cultivar and switchgrass ecotype on total, POM, and MAOM soil C and N stocks, standing root biomass, and average yields from years 2010–2020. Each soil core depth increment was analyzed individually in addition to the whole 1 m soil core. Switchgrass cultivar and ecotype were treated as fixed effects and experimental blocks were treated as a random effect. We used the same model to assess the effect of switchgrass cultivar on microbial indices for potential N mineralization rates and potential C substrate use. We used a significant alpha level of $p < 0.05$.

3 | RESULTS

3.1 | Switchgrass yields

Switchgrass cultivars had different yields when averaged across 11 years of cultivation from 2010 to 2020 (Figure 1; Table S1). Dacotah had significantly lower average yield ($4.2 \pm 0.5 \text{ Mg ha}^{-1} \text{ year}^{-1}$) compared to all of the other cultivars (Figure 1). Trailblazer ($6.4 \pm 0.3 \text{ Mg ha}^{-1} \text{ year}^{-1}$) and NE28 ($6.4 \pm 0.1 \text{ Mg ha}^{-1} \text{ year}^{-1}$) also had lower average yields compared with all of the other cultivars (Figure 1). Cave-in-rock had the greatest average yields of $10.3 \pm 0.4 \text{ Mg ha}^{-1} \text{ year}^{-1}$. In general, lowland

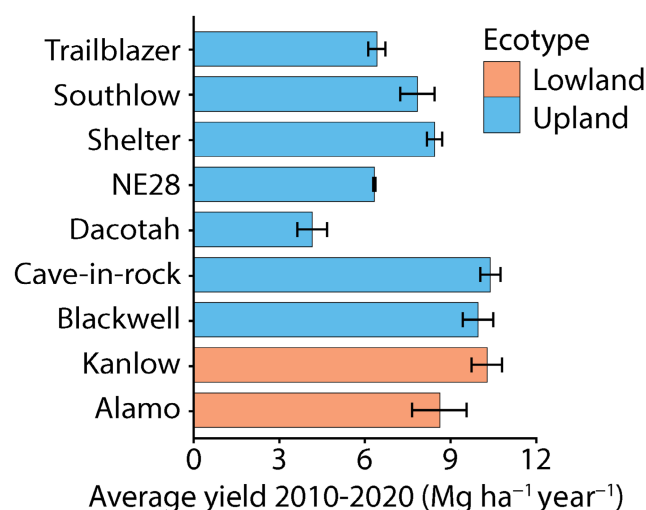


FIGURE 1 Average 11-year yields (2010–2020) for nine different switchgrass cultivars. Error bars represent standard errors ($n = 4 \text{ plots} \times 11 \text{ years}$). ANOVA results appear in Table S1.

ecotypes had greater average yields compared with upland ecotypes ($9.5 \pm 0.6 \text{ Mg ha}^{-1} \text{ year}^{-1}$ compared with $7.7 \pm 0.4 \text{ Mg ha}^{-1} \text{ year}^{-1}$, respectively; Table S2). Switchgrass yields did not correlate with total root biomass, bulk soil C and N stocks, POM C and N stocks, or MAOM C and N stocks.

3.2 | Root biomass

We found differences in standing root biomass among switchgrass cultivars at each depth increment below 10 cm as well as in the entire 1 m depth (Figure 2; Tables S1 and S3). Standing root biomass in the 0–10 cm depth averaged $1.6 \pm 0.3 \text{ SE Mg ha}^{-1}$ across all cultivars. There were no significant differences in standing root biomass among switchgrass cultivars in the 0–10 cm depth (Figure 2; Table S1). In the 10–25 cm depth, standing root biomass averaged $0.9 \pm 0.1 \text{ Mg ha}^{-1}$ across all cultivars and Southlow had greater average standing root biomass ($2.0 \pm 0.5 \text{ Mg ha}^{-1}$) than NE28 ($0.3 \pm 0.2 \text{ Mg ha}^{-1}$), Trailblazer ($0.3 \pm 0.1 \text{ Mg ha}^{-1}$), and Kanlow ($0.4 \pm 0.1 \text{ Mg ha}^{-1}$). In the 25–50 cm depth, standing root biomass averaged $0.7 \pm 0.1 \text{ Mg ha}^{-1}$ across all cultivars and Alamo had greater average standing root biomass ($1.8 \pm 0.4 \text{ Mg ha}^{-1}$) than Kanlow, Cave-in-Rock, Dacotah, NE28, and Trailblazer. In the 50–100 cm depth, standing root biomass averaged $1.2 \pm 0.2 \text{ Mg ha}^{-1}$ across all cultivars and Cave-in-Rock had lower average standing root biomass ($0.01 \pm 0.01 \text{ Mg ha}^{-1}$) than Southlow ($2.8 \pm 1.2 \text{ Mg ha}^{-1}$), Alamo ($1.7 \pm 0.4 \text{ Mg ha}^{-1}$), and Blackwell ($1.9 \pm 0.4 \text{ Mg ha}^{-1}$). Across the entire 1 m depth, we found that Southlow and Alamo had greater average standing root biomass (7.7 ± 1.4 and $6.5 \pm 1.2 \text{ Mg ha}^{-1}$, respectively) than Trailblazer ($1.1 \pm 0.2 \text{ Mg ha}^{-1}$). Additionally, Southlow had greater average standing root biomass than NE28 ($1.9 \pm 0.6 \text{ Mg ha}^{-1}$). We found no other significant differences among cultivars (average of $4.4 \pm 0.5 \text{ Mg ha}^{-1}$ across all cultivars; Table S3). On

average, lowland ecotypes had slightly greater standing root biomass to 1 m than upland ecotypes (4.8 ± 0.9 vs. $4.3 \pm 0.6 \text{ Mg ha}^{-1}$, respectively; Table S3), mostly due to the 25–50 cm depth (Table S2). Additionally, we found no correlations between root biomass and bulk soil C and N stocks, POM C and N stocks, or MAOM C and N stocks.

3.3 | Bulk soil carbon and nitrogen stocks

We found no significantly detectable differences in bulk soil C or N stocks among switchgrass cultivars for the entire 1 m core nor for any individual depth increments, including the surface horizon (Figure 3; Table S1). Bulk soil C and N stocks to 1 m depth averaged $56.4 \pm 3.4 \text{ Mg C ha}^{-1}$ and $7.4 \pm 0.3 \text{ Mg N ha}^{-1}$ across all cultivars (Table S3). Soils under Cave-in-Rock had the greatest 1 m deep bulk soil C stocks ($68.9 \pm 17.3 \text{ Mg C ha}^{-1}$), and also the greatest variability (Table S3). Soils under NE28 had the greatest 1 m deep bulk soil N stocks ($7.9 \pm 1.3 \text{ Mg N ha}^{-1}$; Table S3). Soils under Blackwell had the smallest 1 m deep bulk soil C and N stocks ($47.0 \pm 2.9 \text{ Mg C ha}^{-1}$ and $6.4 \pm 0.4 \text{ Mg N ha}^{-1}$, respectively; Table S3). Although not statistically significant, the biggest differences in soil C and N stocks among cultivars was in the top 10 cm depth increment (Figure 3; Table S1), which contained on average $15.9 \pm 0.7 \text{ Mg C ha}^{-1}$ and $1.7 \pm 0.1 \text{ Mg N ha}^{-1}$ ranging from $12.6 \pm 1.3 \text{ Mg C ha}^{-1}$ and $1.4 \pm 0.1 \text{ Mg N ha}^{-1}$ (NE28) to $19.0 \pm 4.1 \text{ Mg C ha}^{-1}$ and $1.99 \pm 0.39 \text{ Mg N ha}^{-1}$ (Trailblazer).

We also did not detect significant differences in the C:N ratios of the bulk soils among switchgrass cultivars for any depth increment (Table S1). Additionally, when we compared upland to lowland switchgrass ecotypes, we saw no significant differences in bulk C and N stocks (Table S2). Upland ecotypes averaged $57.47 \pm 3.97 \text{ Mg C ha}^{-1}$ and $7.45 \pm 0.35 \text{ Mg N ha}^{-1}$ to 1 m soil depth, whereas lowland ecotypes averaged $53.01 \pm 6.77 \text{ Mg C ha}^{-1}$ and $7.18 \pm 0.69 \text{ Mg N ha}^{-1}$.

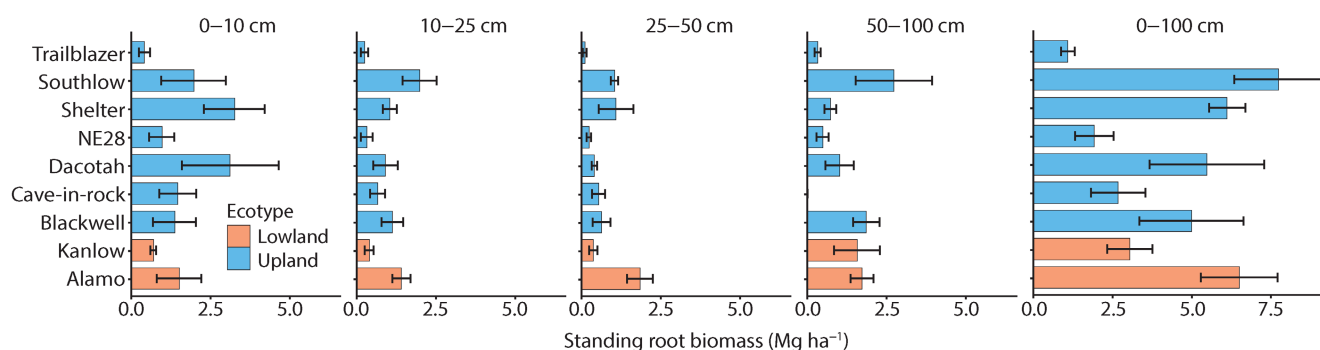


FIGURE 2 Standing root biomass at the time of sampling for nine different switchgrass cultivars separated by sampling depth increment. Error bars represent standard errors ($n=4$ plots). ANOVA results appear in Table S1.

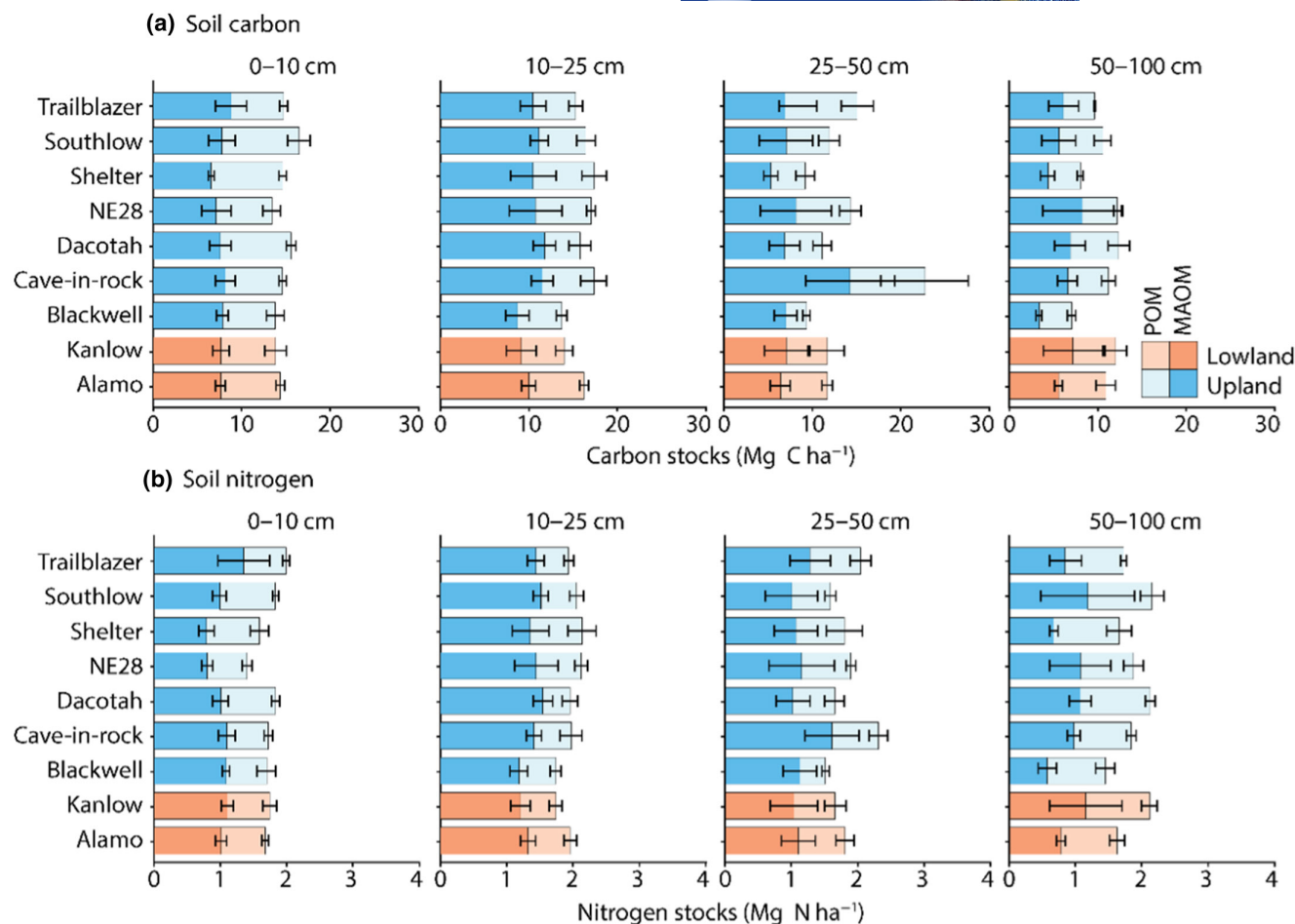


FIGURE 3 Total soil carbon separated by particulate organic matter (POM) and mineral-associated organic matter (MAOM) (a) and total soil nitrogen separated by POM and MAOM (b) after 11-year post-establishment for nine different switchgrass cultivars separated by sampling depth increment. Error bars represent standard errors ($n = 4$ plots). ANOVA results appear in Table S1.

3.4 | Soil organic matter fractionation

We found no differences in POM C and N stocks among switchgrass cultivars. This was the case for the entire 1 m depth as well as for each individual depth increment (Figure 3; Table S1). Average POM C stocks were $22.1 \pm 1.2 \text{ Mg C ha}^{-1}$ and POM N stocks were $2.8 \pm 0.1 \text{ Mg N ha}^{-1}$ to 1 m depth (Table S3). As for bulk soil C stocks, Cave-in-Rock had the greatest 1 m POM C stocks ($25.4 \pm 7.3 \text{ Mg C ha}^{-1}$) and Blackwell the smallest ($17.1 \pm 0.5 \text{ Mg C ha}^{-1}$; Table S3). Blackwell also had the smallest 1 m POM N stocks ($2.4 \pm 0.1 \text{ Mg N ha}^{-1}$), with Shelter having the greatest 1 m POM N stocks ($3.3 \pm 0.4 \text{ Mg N ha}^{-1}$; Table S3). There were also no significant differences in POM stocks between upland and lowland switchgrass ecotypes (Table S2). Upland ecotype POM stocks averaged $22.2 \pm 1.4 \text{ Mg C ha}^{-1}$ and $2.9 \pm 0.1 \text{ Mg N ha}^{-1}$ to 1 m soil depth, and lowland ecotypes averaged $22.0 \pm 2.6 \text{ Mg C ha}^{-1}$ and $2.8 \pm 0.2 \text{ Mg N ha}^{-1}$ (Table S3). Additionally, we compared the C:N ratios of each POM fraction and did not find differences among

switchgrass cultivars (Table S1). The greatest difference in POM C and N stocks among cultivars was in the top 10 cm depth increment, with Southlow having the greatest POM C and N stocks (Figure 3; Table S1).

Similarly, we also found no significant differences in the MAOM C and N stocks among switchgrass cultivars (Figure 3; Table S1). Again, this was apparent for the entire 1 m depth as well as for each individual depth increment. Average MAOM C stocks were $32.5 \pm 2.1 \text{ Mg C ha}^{-1}$ and MAOM N stocks were $4.6 \pm 0.3 \text{ Mg N ha}^{-1}$ to 1 m depth (Table S3). Similar to the bulk soil C stocks and POM C stocks, Cave-in-Rock had the greatest 1 m MAOM C stocks ($40.8 \pm 6.2 \text{ Mg C ha}^{-1}$) and MAOM N stocks ($5.1 \pm 0.4 \text{ Mg N ha}^{-1}$). Shelter had the smallest 1 m MAOM C and N stocks ($26.9 \pm 3.9 \text{ Mg C ha}^{-1}$ and $3.9 \pm 0.4 \text{ Mg N ha}^{-1}$, respectively). We also found no significant differences in MAOM stocks between upland and lowland switchgrass ecotypes (Table S2). Upland ecotype MAOM stocks averaged $33.0 \pm 2.5 \text{ Mg C ha}^{-1}$ and $4.6 \pm 0.3 \text{ Mg N ha}^{-1}$ to 1 m soil depth, and lowland ecotypes averaged $30.6 \pm 3.9 \text{ Mg C ha}^{-1}$ and $4.4 \pm 0.5 \text{ Mg N ha}^{-1}$. The

greatest differences in MAOM C and N stocks among cultivars were in the top 10cm depth increment, with Trailblazer having the greatest MAOM C and N stocks and Shelter having the smallest MAOM C and N stocks (Figure 3; Table S1).

3.5 | Nitrogen cycling

Nitrification and N mineralization potentials were similar among switchgrass cultivars (Table S4). Potential nitrification rates averaged $0.03 \pm 0.004 \mu\text{g N g soil}^{-1} \text{ day}^{-1}$ among all cultivars, with soils under Cave-in-Rock having the highest potential nitrification rate ($0.06 \pm 0.02 \mu\text{g N g soil}^{-1} \text{ day}^{-1}$) and Southlow having the lowest ($0.02 \pm 0.01 \mu\text{g N g soil}^{-1} \text{ day}^{-1}$). We measured average potential mineralization rates of $0.03 \pm 0.002 \mu\text{g N g soil}^{-1} \text{ day}^{-1}$ for all switchgrass cultivars and found very little variation (0.02 ± 0.004 to $0.03 \pm 0.004 \mu\text{g N g soil}^{-1} \text{ day}^{-1}$). We also found no significant differences in N cycling metrics between upland and lowland switchgrass ecotypes (Table S5), with identical potential nitrification and mineralization rates ($0.03 \pm 0.003 \mu\text{g N g soil}^{-1} \text{ day}^{-1}$) for upland and lowland ecotypes.

3.6 | Carbon substrate use

Potential C substrate use richness (the number of C substrates used) averaged 18.8 ± 0.7 among switchgrass cultivar soils, ranging from 15.5 ± 3.2 (NE28) and 22.3 ± 2.2 (Trailblazer), but did not significantly differ among soils under switchgrass cultivars (Table S4). Diversity (averaging 2.8 ± 0.04) and evenness (averaging -0.97 ± 0.001) of C substrates likewise did not significantly differ among soils under different cultivars (Table S4) or ecotypes (Table S4). The upland ecotype used 19.0 ± 0.8 substrates compared to 18.0 ± 1.2 substrates for the lowland ecotype.

Both ecotypes had the same average evenness index (-0.97 ± 0.001) and diversity index (2.8 ± 0.07). Carbon utilization, as indicated by average well color development, differed significantly among switchgrass cultivar soils (Figure 4a; Table S4). Blackwell soils utilized on average 1.3 times more C than NE28 soils (0.9 ± 0.1 compared to 0.6 ± 0.1), with soils under other switchgrass cultivars averaging 0.80 ± 0.05 . There were no significant general differences between upland and lowland switchgrass ecotypes (Table S5).

Most C substrate utilization occurred in the amino acid functional group (on average 1.0 ± 0.04), with a range of 0.7 ± 0.2 in NE28 soils to 1.3 ± 0.1 in Blackwell soils (Figure 4b; Table S4). Blackwell and Alamo cultivar soils used over 1.6 times more amino acid C than NE28 soils (Figure 4b). There were no significant differences in carbohydrate (average 0.8 ± 0.04), polymer (average 0.8 ± 0.03), organic acid (average 0.8 ± 0.03), or amine (average 0.6 ± 0.03) C use among soils (Table S3). There were likewise no significant ecotype differences (Table S5).

4 | DISCUSSION

We found surprisingly few differences in the standing stocks of soil C and N pools or soil organic matter fractions among nine switchgrass cultivars grown for 11 years at the same site in SW Michigan, USA despite significant switchgrass productivity differences. Likewise, there were no consistent differences among cultivars with respect to soil N availability or C substrate utilization patterns.

4.1 | Switchgrass above- and belowground productivity

We found significant differences in the productivity of switchgrass cultivars for both average aboveground

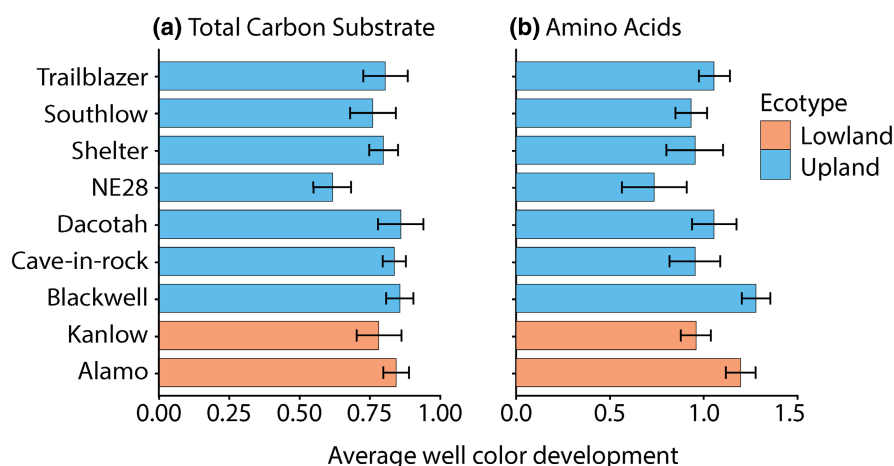


FIGURE 4 Average total carbon substrate well color development (a) and average amino acid carbon substrate well color development (b) for nine different switchgrass cultivars. Error bars represent standard errors ($n=4$ plots). ANOVA results appear in Table S1.

yields and standing root biomass below 10 cm at the time of sampling. Average 11-year yields differed by a factor >2.5 , but differences across years were as large within ecotypes as between: Among the upland ecotypes, average yields ranged from $4.2 \pm 0.5 \text{ Mg ha}^{-1} \text{ year}^{-1}$ for Dacotah to $10.4 \pm 0.4 \text{ Mg ha}^{-1} \text{ year}^{-1}$ for Cave-in-Rock. The lowland cultivar Kanlow was just as productive as Cave-in-Rock (Figure 1).

Root biomass below 10 cm depth was similar among all cultivars, though we found significant differences among several cultivars in both the 10–25 cm depth (Southlow $>$ Trailblazer, Kanlow, and NE28) and the 25–50 cm depth (Alamo $>$ Trailblazer, Cave-in-Rock, Dacotah, NE28, and Trailblazer). In the 50–100 cm depth, several cultivars had significantly greater standing root biomass than the Cave-in-Rock cultivar, including Alamo, Blackwell, and Southlow cultivars. In general, lowland cultivars had slightly greater standing root biomass below 10 cm. Our failure to find root biomass differences among switchgrass cultivars is in contrast to differences among different perennial bioenergy crop species. For example, Sprunger et al. (2017) showed significant differences in fine root production among switchgrass, giant miscanthus (*Miscanthus \times giganteus*), hybrid poplar (*Populus nigra* \times *P. maximowiczii* 'NM6'), and mixed species grasses (*Andropogon gerardii*, *Elymus canadensis*, *P. virgatum*, *Schizachrium scoparium*, and *Sorghastrum nutans*) at the same location. It is also notable that cultivars with the greatest belowground standing root biomass did not correspond to cultivars with greater (or less) aboveground productivity.

Cultivars appeared to differ in root depth distributions. For some cultivars, e.g. Trailblazer, Alamo, and Southlow, roots were distributed fairly evenly with depth, with about the same amount of root biomass in the 50–100 cm depth interval as in any of the shallower horizons (Figure 2). For the other cultivars, roots were more concentrated in surface horizons. Cave-in-rock for example had virtually no roots below 50 cm.

4.2 | Soil carbon and nitrogen stocks

We found no significant differences in soil C or N stocks among our nine switchgrass cultivars. In a comparison of four lowland switchgrass cultivars in Tennessee 3 years after establishment, Garten and Wullschleger (2000) also failed to detect significant bulk soil C and N stock differences, as did Roosendaal et al. (2016) in a two switchgrass cultivar (upland vs. lowland) comparison in Nebraska. That the present study included nine cultivars (upland and lowland) grown for 11 years and still failed to detect significant differences corroborates these findings in a more comprehensive way.

While there is some evidence that surface soil (0–10 cm) differences may result from root architecture differences among switchgrass cultivars, at least early in stand development (Adkins et al., 2016), we detected no 0–10 cm depth differences after 11 years. This may be because differences in the soil C stocks among cultivars is more apparent in younger stands and then equilibrates as switchgrass matures (Garten, 2012).

We were nevertheless surprised to find no significant differences in soil C fractions. We would expect to see differences in standing root biomass and average aboveground yield in the POM C stocks because POM stocks are more plant derived (Christensen, 2001). Processes such as root fragmentation and decomposition as well as aboveground litter incorporation are known to contribute to this soil fraction (Cotrufo et al., 2015). However, there were no differences in POM C or N stocks among switchgrass cultivars even when there were small changes in standing root biomass and average aboveground yield. MAOM C stocks typically correlate with soil N stocks because MAOM requires more N to form than does POM (Averill & Waring, 2018; Cotrufo et al., 2013). That we did not see differences in soil N stocks among switchgrass cultivars across any sampling depths is consistent with the absence of MAOM stock differences.

4.3 | Switchgrass productivity and soil C correlations

We found no correlation between average aboveground yields over 11 years (2010–2020) and soil C and N stocks. This could be because aboveground C typically has less impact on soil C than belowground C (Austin et al., 2017; Mosier et al., 2021) or because the aboveground biomass is harvested each year and not returned to the soil. In addition, yield differences were only apparent among a few switchgrass cultivars. It appears that these small differences in average aboveground yield among a few cultivars have had little effect to date on soil C and N stocks.

We would expect soil C to correlate with standing root biomass as root C has been shown to correlate with soil C accrual (Austin et al., 2017; Cates et al., 2016; King et al., 2020; Kong & Six, 2010; Puget & Drinkwater, 2001). However, standing root biomass and soil C were not correlated in this experiment. This finding is similar to Roosendaal et al.'s (2016), who found two times greater root biomass under the lowland ecotype compared to the upland ecotype, but did not observe differences in soil C stocks. One explanation why our standing root biomass and soil C stocks were not correlated could be that the differences in standing root biomass among switchgrass cultivars were small and only apparent below 10 cm.

4.4 | N availability and C substrate utilization

Neither net nitrification nor N mineralization potentials differed among soils from different switchgrass cultivars. Although rates were similar to those from other perennial cropping systems in this area (Millar & Robertson, 2015), we found no significant differences in this proxy for microbial N cycle function. This could be one explanation for why we did not see any differences in soil N stocks among switchgrass cultivars. Our bulk soil N estimates take into account both inorganic and organic pools of soil N, whereas the potential N mineralization rates only quantify inorganic soil N changes. Since we did not see differences in inorganic soil N nor bulk soil N, we can assume that there were also no differences in organic N among cultivars. Soil organic N can come from processes such as plant decomposition and microbial turnover, which are likely unaffected by switchgrass cultivar in this system.

We found only one small difference (between two cultivars) in community-level physiological profiling as assessed via C substrate utilization assays. The lack of differences helps to explain why we did not see any differences in C stocks among switchgrass cultivars. Microbial transformation is important for MAOM formation (Kallenbach et al., 2016; Miltner et al., 2012), and in our soils it appears that microbes are utilizing C similarly among all cultivars, with richness, evenness, and average well color development being largely indistinguishable among cultivars. Although soils under NE28 had slightly lower potential C substrate use than soils under other cultivars, this appeared limited to amino acid C use with no larger impact on soil C stocks or fractions.

We did not normalize our microbial indices for microbial biomass, which could explain why we did not detect differences. Others have found differences in microbial biomass and community composition among switchgrass cultivars (Roosendaal et al., 2016; Stahlheber et al., 2020; Ulbrich et al., 2021), although in at least one case differences 3 years after establishment disappeared as the switchgrass matured (Stewart et al., 2017). Additionally, some studies have shown that certain microbial communities are associated with higher yields (Sawyer et al., 2019). Though we did not measure microbial communities per se, we did not observe significant correlations between yield and functional microbial indices.

5 | CONCLUSIONS

Cultivating different cultivars of switchgrass for 11 years did not significantly impact soil C accrual into different soil organic matter fractions, measured microbial community

function, or soil N cycling despite differences in average yields and standing root biomass among switchgrass cultivars and between switchgrass ecotypes. Results suggest that contemporary switchgrass cultivars have equivalent impacts on soil C and N cycling, suggesting that soil C and N gains under switchgrass are likely to be unaffected by cultivar differences.

AUTHOR CONTRIBUTIONS

Samantha Mosier: Conceptualization; data curation; formal analysis; investigation; methodology; visualization; writing – original draft. **Lauren Kelly:** Conceptualization; data curation; investigation; writing – review and editing. **Ekrem Ozlu:** Conceptualization; data curation; formal analysis; investigation; methodology; writing – review and editing. **G. Philip Robertson:** Conceptualization; funding acquisition; investigation; project administration; supervision; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. Samantha Mosier is a co-founder, board member, and the Executive Director of Cquester Analytics, LLC.

DATA AVAILABILITY STATEMENT

Data will be made available by corresponding author upon reasonable request or can be requested through the GLBRC data manager at https://www.glbrc.org/data-and-tools/glbrc-data-sets?combine-datasets=&field_dataset_category_target_id=All&field_focus_area_target_id=All&page=2

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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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