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# Root Traits of Perennial $C_4$ Grasses Contribute to Cultivar Variations in Soil Chemistry and Species Patterns in Particulate and Mineral-Associated Carbon Pool Formation

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RESEARCH ARTICLE

# Root traits of perennial $C_4$ grasses contribute to cultivar variations in soil chemistry and species patterns in particulate and mineral-associated carbon pool formation

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## Abstract

Recent studies have indicated that the $C_4$ perennial bioenergy crops switchgrass (*Panicum virgatum*) and big bluestem (*Andropogon gerardii*) accumulate significant amounts of soil carbon (C) owing to their extensive root systems. Soil C accumulation is likely driven by inter- and intraspecific variability in plant traits, but the mechanisms that underpin this variability remain unresolved. In this study we evaluated how inter- and intraspecific variation in root traits of cultivars from switchgrass (Cave-in-Rock, Kanlow, Southlow) and big bluestem (Bonanza, Southlow, Suther) affected the associations of soil C accumulation across soil fractions using stable isotope techniques. Our experimental field site was established in June 2008 at Fermilab in Batavia, IL. In 2018, soil cores were collected (30 cm depth) from all cultivars. We measured root biomass, root diameter, specific root length, bulk soil C, C associated with coarse particulate organic matter (CPOM) and fine particulate organic matter plus silt- and clay-sized fractions, and characterized organic matter chemical class composition in soil using high-resolution Fourier-transform ion cyclotron resonance mass spectrometry. $C_4$ species were established on soils that supported $C_3$ grassland for 36 years before planting, which allowed us to use differences in the natural abundance of stable C isotopes to quantify $C_4$ plant-derived C. We found that big bluestem had 36.9% higher $C_4$ plant-derived C compared to switchgrass in the CPOM fraction in the 0–10 cm depth, while switchgrass had 60.7% higher $C_4$ plant-derived C compared to big bluestem in the clay fraction in the 10–20 cm depth. Our findings suggest that the large root system in big bluestem helps increase POM-C formation quickly, while switchgrass root structure and chemistry build a mineral-bound clay C pool through time. Thus, both species and cultivar selection can help improve bioenergy management to maximize soil carbon gains and lower $CO_2$ emissions.

**KEYWORDS**

perennial $C_4$ grasses, root morphology, soil chemistry, soil fraction carbon

## 1 | INTRODUCTION

Perennial native prairie grasses, such as switchgrass (*Panicum virgatum*) and big bluestem (*Andropogon gerardii*), contribute substantially to soil carbon (C) accumulation due to their extensive rooting systems (Gelfand et al., 2013; Schmer et al., 2008; Tilman et al., 2006). This is significant, because plant roots are the main conduit of atmospheric C to soil (Kong & Six, 2010; Norby & Jackson, 2000; Rasse et al., 2005). Thus, the production of cellulosic bioenergy can contribute to mitigating global carbon dioxide ($CO_2$) concentrations not only by virtue of being a renewable energy source, but also by increasing soil C storage (Gelfand et al., 2013; Murphy & Kendall, 2015; Tilman et al., 2006). In addition, these grasses have low resource requirements, reducing the need for energy use associated with farming operations such as irrigation and fertilization (Griffith et al., 2011; Schmer et al., 2008), creating a low C economy compared to fossil fuels (Larnaudie et al., 2022; Meyer et al., 2020; Murphy & Kendall, 2015). However, species and cultivar impacts on soil C accumulation vary widely (Adkins et al., 2016, 2019; Collins et al., 2010; de Graaff et al., 2013), and the mechanisms that underpin this variability remain unresolved. This complicates our ability to select for species and cultivars that maximize soil C accumulation with bioenergy production. Advancing our understanding of how feedstock root traits contribute to soil C storage will enable selection of bioenergy crops that promote a low C economy, and gain insights into the mechanisms at the root–soil interface that bolster soil C storage.

Soil C accumulation is primarily controlled by plant-derived soil C inputs and by the retention of C in soil. Thus, inter- and intraspecific variability in soil C accumulation is likely linked to variance in root traits that influence the quantity and chemistry of plant-derived C input (Adkins et al., 2016; Ulbrich et al., 2021). The quantity of soil C input is driven by plant litter influx, root exudation (the active release of low molecular weight C substrates through the root tips in living plants), and root turnover (Ma et al., 2018; Merckx et al., 1987; Whipps & Lynch, 1983). Root biomass is typically used as a trait for predicting root C input quantities to soil (Fornara & Tilman, 2008; Robinson, 2007). However, if plant species or cultivars have similar root biomass, specific root length (SRL; a measure of root length relative to weight) might better predict the quantity of root C inputs (Adkins et al., 2016). Indeed, root systems with a higher SRL tend to be composed of more ephemeral roots that contribute significantly to root exudation and root turnover (Czarnota et al., 2003; D. Guo et al., 2008; D.L. Guo et al., 2004; King et al., 2002; Long et al., 2013; Nguyen, 2003; Pineros et al., 2002; Xia et al., 2010). A previous study with switchgrass has shown that SRL can predict plant-derived C inputs in the short term, but it is uncertain whether these trends continue through time (Adkins et al., 2016). Thus, root biomass and SRL may help to explain the quantity of C input, however, other factors such as C chemistry can also influence soil C retention.

The chemical composition of root-derived C influences its processing by microbes. When soil microbes encounter root-derived C, it is either metabolized and retained as microbial biomass, or metabolically respired as $CO_2$ (Frey et al., 2013; Kallenbach et al., 2016; Liang et al., 2017; Malik et al., 2020; Zhalnina et al., 2018). Microbial biomass formation helps to promote soil C retention, because microbial residues are preferentially sorbed and stabilized on soil minerals, whereas microbial respiration leads to C loss (Cotrufo et al., 2013; Kallenbach et al., 2016; Liang et al., 2017). Recent studies have shown that inputs of low molecular weight compounds promote microbial residue formation more so than inputs of structurally complex carbon such as lipids, lignin, and tannins (Cotrufo et al., 2013; Kallenbach et al., 2016). In addition, root litter with a lower C:N ratio enhances microbial residue formation and sorption to soil minerals (Cotrufo et al., 2013, 2019), whereas inputs with a higher C:N ratio contribute more to the particulate organic matter (POM) pool (Angst et al., 2021). The location of C accumulation can indicate relative stability, as POM-C can be quickly lost to environmental disturbances whereas minerally sorbed C is considered more stable (Angst et al., 2021; Villarino et al., 2021). Investigating the links between the chemical composition of C inputs and soil C retention is essential to advancing an understanding of plant effects on soil C sequestration.

With this study comparing big bluestem and switchgrass (three cultivars of each), we aimed to quantify and compare inter- and intraspecific variations in: (1) root structures (SRL, root diameter, root biomass) and (2) soil chemistry (C:N ratio, organic matter functional group or chemical class composition abundance). Importantly, we also sought to determine how these variations in root structures and soil chemistry are related to plant-derived soil C. We hypothesized that (1) species and cultivars with a greater abundance of fine roots are associated with a greater amount of plant-derived C in the soil, and (2) species and cultivars with lower C:N ratios and those that produce more labile or bioavailable compounds in the soil are associated with greater quantities of plant-derived C in the silt and clay soil fractions. In 2018, we collected soils and roots from monoculture plots of three cultivars of switchgrass (Cave-in-Rock, Kanlow, Southlow) and three cultivars of big bluestem (Bonanza, Southlow, Suther). These experimental field plots were established in

2008, 10 years earlier, at the U.S. Department of Energy National Environmental Research Park at Fermilab in Batavia, IL. We measured root biomass, root diameter, SRL, bulk soil C, and C associated with coarse and fine particulate organic matter (CPOM and FPOM) plus silt- and clay-sized fractions. Cultivar monocultures of both $C_4$ species were established on soils that supported $C_3$ grassland for 36 years before planting, which allowed us to use differences in the natural abundance of stable C isotopes to quantify $C_4$ plant-derived C. We also characterized organic matter functional group or chemical class composition abundance of the soil using high-resolution Fourier-transform ion cyclotron resonance (FTICR) mass spectrometry. With this study we will be able to identify inter- and intraspecific variation in root traits and soil chemistry in these perennial grasses and how that impacts soil C accumulation. This can then be used to inform management strategies to help increase soil C accrual and lower $CO_2$ emissions from bioenergy production.

## 2 | MATERIALS AND METHODS

### 2.1 | Study site and sampling

The study site was established in June 2008 at the U.S. Department of Energy National Environmental Research Park at Fermilab in Batavia, IL (88°13′47″ W, 41°50′29″ N). Prior to plot establishment (1971–2007), the 5.4 ha landscape was dominated by perennial, cool-season $C_3$ grasses—primarily smooth brome (*Bromus inermis*), quackgrass (*Agropyron repens*), and Poa species (Morris et al., 2016; O'Brien et al., 2013). In autumn 2007, all vegetation was removed by a combination of herbicide application (glyphosate) and prescribed burning, and then in June 2008 the switchgrass (*P. virgatum*) and big bluestem (*A. gerardii*) plots were planted. Two experimental designs (agronomic and diversity) were established according to Morris et al. (2016). In brief, switchgrass plots (36 m × 20 m), part of the agronomic experiment, consisted of three unfertilized monoculture treatments (Cave-in-Rock, Kanlow, and Southlow; $n = 3$) that were drill-seeded in 20 cm rows with 6.7 kg ha$^{-1}$ of pure live seed. Big bluestem plots (2 m × 3 m), part of the diversity experiment, consisted of three unfertilized monoculture treatments (Bonanza, Southlow, and Suther; $n = 4$) that were hand sown in 20 cm rows. Weed management of the switchgrass plots consisted of broadcast application of Milestone (aminopyralid) and Garlon (triclopyr) broadleaf herbicides in 2009, and spot application of Milestone, Garlon and Round-Up (glyphosate) in 2010 (Morris et al., 2016). Big bluestem plots were hand-weeded from 2008 to 2010 and received Milestone and Garlon broadleaf herbicides in 2009 (Morris et al., 2016). Plot establishment and yields were monitored through 2014 and details can be found in Morris et al. (2016). Agronomic or switchgrass plots were mowed yearly, and aboveground biomass was harvested up until 2016. Diversity or big bluestem plots were mowed yearly, and aboveground biomass was removed through 2018.

In June 2018, 10 years after the establishment of the plots, we collected soil cores to a depth of 30 cm using a 4.8 cm diameter soil corer. We sampled areas of the plot that had minimal weed invasion and with high cultivar density. For the switchgrass cultivars, three soil cores were collected on randomly selected, separate crowns and composited within each replicate plot ($n = 3$). For the big bluestem cultivars, two soil cores were collected on randomly selected, separate crowns and composited within each replicate plot ($n = 4$). All cores were divided into 10 cm depth increments (0–10, 10–20, 20–30 cm), which were composited separately. The samples were frozen and shipped to the de Graaff Laboratory at Boise State University where they were kept frozen (−20°C) until processed. All soil samples were weighed. Initially for all soil samples, two representative composite 10 g subsamples were immediately removed from the bulk soil for soil chemistry analysis, and soil was transferred back to the freezer (−20°C). After thawing at 4°C for 24 h, the soils were homogenized together, and a 10 g subsample was removed for fractionation and $^{13}C$ analysis.

For root analysis, all switchgrass soils and 0–10 cm big bluestem soils were passed through a 2 mm sieve, and all roots captured on the top of the sieve were removed to represent large roots. A 150 g subsample from each of these homogenized soils was washed through a 250 μm sieve stacked on a 53 μm sieve to collect and represent fine roots. Additionally, a 250 g subsample of all 10–20 cm and 20–30 cm big bluestem soils was collected and washed through a 2 mm sieve stacked on a 250 μm to collect large roots. All collected roots were stored at 4°C.

### 2.2 | Root morphology—SRL, root diameter, and root biomass

Specific root length and root diameter were determined for all 0–10 cm switchgrass and big bluestem samples (Kelly-Slatten et al., 2023). Large roots were cleaned, rhizomes were removed, and then subdivided into 12 subsamples. We tested two switchgrass and two big bluestem 0–10 cm samples and determined that SRL could be calculated accurately from half (6) of the subsamples. Thus, for all other 0–10 cm samples, only six subsamples were analyzed for SRL and diameter size class calculations. For

fine root analysis of SRL and root diameter class, the roots were cleaned, separated into three subsamples, and all were analyzed.

In brief, large roots were placed in a water bath on an EPSON Perfection Scanner, and scans were checked to ensure no root overlap. WinRHIZO software from Regent Instruments Incorporated was used to calculate root length and diameter. These data were then used to calculate SRL (m root $g^{-1}$ root) and root abundance (%) of four root diameter size classes (0–0.5 mm, 0.5–1 mm, 1–2 mm, over 2 mm). This was also done with fine root subsamples. Switchgrass cultivar Cave-in-Rock and big bluestem cultivar Bonanza were assessed for SRL and diameter class size in the 10–20 cm and 20–30 cm depth increments to determine if SRL and diameter class size was consistent across depths. The same trends were observed as reported in the 0–10 cm depth increment.

Upon completion of WinRHIZO analysis, roots of all depths were oven dried for 24 h at 65°C. The roots were then weighed and used to calculate root biomass for each depth increment (g root $kg^{-1}$ soil) (Kelly-Slatten et al., 2023). For fine roots, the 150 g subsample was used to calculate total fine roots for the weight of the entire sample. This method was also applied for large roots for big bluestem 10–20 cm and 20–30 cm depth samples. We determined this subsampling method was accurate by comparing it with the 0–10 cm big bluestem samples.

## 2.3 | Total C and $C_4$ plant-derived C analysis of soil fractions

Subsamples of soil from each of the switchgrass and big bluestem plots and from each of the three depth increments were size fractionated to separate CPOM (>250 μm), FPOM (53–250 μm), silt-sized (2–53 μm), and clay-sized (<2 μm) fractions using a procedure adapted from Cambardella and Elliott (1992). Subsamples (10 g) of the 2 mm sieved soil were oven dried at 65°C, and then dispersed by shaking overnight on a reciprocating shaker in 40 mL of 5 g $L^{-1}$ sodium hexametaphosphate (NaHMP) solution. The soil solution was then rinsed through a 250 μm sieve to collect the CPOM fraction, and then washed through a 53 μm sieve to collect the FPOM fraction. CPOM and FPOM fractions were transferred to aluminum tins and dried. The soil solution that remained after passing through the sieves contained both the silt- and clay-sized fractions. To separate the two remaining fractions, the solution was centrifuged for 82 s at 270 RCF at 20°C in a Sorvall Legend X1R centrifuge fitted with a swinging bucket rotor. The supernatant, containing the clay fraction, was removed and the silt pellet was transferred to an aluminum tin to dry. The clay supernatant was mixed with 10 mL of 0.25 M $CaCl_2 + MgCl_2$ solution and centrifuged for 10 min at 2000 RCF at 20°C in the Sorvall Legend X1R centrifuge. The supernatant was discarded, and the clay pellet was transferred to an aluminum tin to dry. All fractions were oven dried (65°C), and then transferred to glass scintillation vials for storage.

The oven dried samples of each soil fraction were ground to a fine powder using a Spex ball mill for 10 min. The soils were then wrapped in tin capsules and analyzed for total C and stable C isotope ratios ($^{13}C/^{12}C$) on a 2010 ThermoFisher Delta V Plus continuous flow isotope ratio mass spectrometer. Soils did not contain carbonates, so total soil C was equivalent to soil organic C. Bulk soil C concentrations were determined for all soil samples by summing the individual fractions (Kelly-Slatten et al., 2023).

Results of the C isotope analyses were expressed as $\delta^{13}C$. The $^{13}C$ values were determined in relation to Vienna–Pee Dee Belemnite as follows: $\delta^{13}C = \left(\left(R_{sample}/R_{standard}\right) - 1\right) \times 1000$, where $R$ is the stable C isotope ratio.

To calculate the amount of $C_4$ plant-derived C present in each soil fraction, the following mass balance was used (Cheng, 1996; Nottingham et al., 2009):

$$Q_p = Q_t \times \left(\delta^{13}C_t - \delta^{13}C_s\right) / \left(\delta^{13}C_p - \delta^{13}C_s\right) \quad (1)$$

where $Q_t$ is the total amount of soil C, $\delta^{13}C_t$ is its isotopic composition, $Q_p$ is the amount of soil C derived from switchgrass/big bluestem root material, $\delta^{13}C_p$ is the isotopic composition of the switchgrass (−14.15‰ ± 0.27) or big bluestem root material (−12.73‰ ± 0.33) (Adkins et al., 2019), and $\delta^{13}C_s$ is the isotopic composition of the adjacent $C_3$ grassland soil (Table S1). The calculation of $Q_p$ for each soil fraction at each depth increment was based on the measured value of $\delta^{13}C_s$ for the corresponding fraction and depth increment.

To calculate total switchgrass and big bluestem-derived C the following formula was used:

$$Q_b = A_{CPOM} \times Q_{P(CPOM)} + A_{FPOM} \times Q_{P(FPOM)} + A_{Silt} \times Q_{P(Silt)} + A_{Clay} \times Q_{P(Clay)} \quad (2)$$

where $Q_b$ is total C derived from switchgrass/big bluestem material in the bulk soil, $A_{fraction}$ is the relative abundance (proportional mass) of the respective soil fractions, and $Q_{p(fraction)}$ is the amount of soil C derived from switchgrass/big bluestem in the respective soil fractions, as calculated using Equation (1). For the calculation of $Q_b$ for each sample, if switchgrass/big bluestem C was not detected in a fraction, then a value of 0 was assigned to $Q_p$ for that fraction.

## 2.4 | Soil chemistry—Abundance of organic matter chemical class composition or functional groups

A water–methanol–chloroform sequential extraction was preformed, where the water extraction targets polar compounds that are relatively bioavailable in the soil solution. The methanol extraction removes compounds that are polar/semi-polar increasing the amount of organic molecules extracted by water. Finally, the chloroform extraction targets organic compounds that are non-polar and/or a portion of molecules that can be sorbed to soil minerals (e.g., lipids). Due to the large overlap of organic matter represented in the water and methanol extractions (Tfaily et al., 2017), only water and chloroform extractions were analyzed to represent a more bioavailable C pool (water) and a less bioavailable C pool (chloroform).

A frozen subsample of soil from each depth and replicate of all cultivars was lyophilized for 48 h, and then a water–methanol–chloroform sequential extraction adapted from Tfaily et al. (2017) was performed to determine the soil biochemical profile of each sample. From each lyophilized sample, 1 g of soil was transferred to a glass vial, and 2 mL of deionized water was added and vortexed for 30 s, after which the vial was shaken for exactly 2 h on an Eberbach Variable Speed Reciprocal Shaker. The vial was then centrifuged at 1500 *g* for 10 min, and then using a glass Pasteur pipette the water was removed and stored (−20°C). This was repeated with 2 mL of MeOH followed by 2 mL of $CHCl_3$. The water and $CHCl_3$ extraction layer were then shipped on ice to the Environmental Molecular Sciences Laboratory (EMSL), a Department of Energy Biology and Environmental Research (DOE-BER) national user facility located in Richland, WA.

A 12 T Bruker SolariX FTICR spectrometer located at EMSL was used to collect high-resolution mass spectra of the organic material in the extracts. A standard Bruker electrospray ionization (ESI) source was used to generate negatively charged molecular ions. Samples were then introduced directly to the ESI source. The instrument was externally calibrated weekly to a mass accuracy of <0.1 ppm using a tuning solution from Agilent, which contains the following compounds: $C_2F_3O_2$, $C_6HF_9N_3O$, $C_{12}HF_{21}N_3O$, $C_{20}H_{18}F_{27}N_3O_8P_3$, and $C_{26}H_{18}F_{39}N_3O_8P_3$ with an $m/z$ ranging between 112 and 1333. The instrument settings were optimized by tuning on a Suwannee River Fulvic Acid standard. Blanks (high performance liquid chromatography grade MeOH) were also run at the beginning and the end of the day to monitor potential carry over from one sample to another. The instrument was flushed between samples using a mixture of water and methanol. The ion accumulation time was varied (0.1 and 0.3 s) to account for differences in C concentration between samples. Ninety-six individual scans were averaged for each sample and internally calibrated using an organic matter homologous series separated by 14 Da ($CH_2$ groups). The mass measurement accuracy was <1 ppm for singly charged ions across a broad $m/z$ range (i.e., $200 < m/z < 1200$). To further reduce cumulative errors, all sample peak lists for the entire dataset were aligned to each other prior to formula assignment to eliminate possible mass shifts that would impact formula assignment. Putative chemical formulas were assigned using Formularity software (Tolić et al., 2017) based on the following criteria: S/N > 7, and mass measurement error <1 ppm, taking into consideration the presence of C, H, O, N, S, and P and excluding other elements. Peaks with large mass ratios ($m/z$ values > 500 Da) often have multiple possible candidate formulas. These peaks were assigned formulas through propagation of $CH_2$, O, and $H_2$ homologous series. Additionally, to ensure consistent choice of molecular formula when multiple formula candidates are found, the following rules were implemented: we consistently pick the formula with the lowest error with the lowest number of heteroatoms, and the assignment of one phosphorus atom requires the presence of at least four oxygen atoms. Peaks that were present in the blanks were removed from the sample data sets. Additionally, all single peaks, that is, peaks that were present in only one sample were removed and were not included in the downstream analysis. Overall, the organic compounds with assigned peaks from each extraction were classified into one of the potential molecular groups based on their O:C and H:C ratios: lipids, unsaturated hydrocarbons, condensed hydrocarbons, proteins, amino sugars, carbohydrates, lignin, or tannins. Within each sample, the percent abundance of each molecular group was calculated from the total compounds extracted (Kelly-Slatten et al., 2023).

Because the organic matter extraction efficiency of this method varies among soil types (Tfaily et al., 2017), we use the relative abundance of each molecular group as an estimation for the biochemical profile of the entire soil. However, some relative abundances may be over or underestimated due to C that we were unable to extract from the soil samples.

## 2.5 | Statistical analysis

Across all data sets, dependent variables (root biomass, root diameter, SRL, total soil C, $C_4$ plant-derived C in soil fractions, C:N of soil fractions, abundance of organic matter functional groups) were subset by depth and/or species. This allowed us to measure dependent variable impacts across all depths as well as individual soil depths, and to look at impacts between and within species. All

statistical tests were performed in R version 4.1.0 with package *stats* unless otherwise stated. All dependent variables were tested for normality using Shapiro–Wilk, before being assessed for differences between species or among cultivars. If normal a t-test or two-way analysis of variance was conducted, and if not normal either Mann–Whitney or Kruskal–Wallis was used. For each significant result, a Tukey HSD or pairwise Wilcoxon rank sum test was used to determine which groups varied. Using packages *ggbiplot* and *factoextra*, principal component analyses (PCAs) were run and visualized for species and cultivar differences in chloroform-extracted biochemical profiles. Substrates were included in the PCA if they had significantly different abundances between species or cultivars. Finally, using package *ggpubr*, Pearson and Spearman correlations were conducted and visualized to evaluate which traits (abundance of organic matter functional groups, root biomass, C:N of soil fractions) were most strongly related to $C_4$ plant-derived C accumulation in soil fractions. Correlations were corrected for multiple comparisons with a Benjamini and Hochberg method to correct *p*-values for false discovery.

## 3 | RESULTS

### 3.1 | Root morphology—Root biomass, SRL, and root diameter

Species varied in root biomass (g root $kg^{-1}$ soil), SRL (m root $g^{-1}$ root), and relative abundance of roots in a particular size class (%, Tables 1 and 2). Big bluestem had a 48% and 45% higher root biomass than switchgrass in the 10–20 cm and 20–30 cm depth increments, respectively (Table 1). At 0–10 cm, species differences in root biomass were not significant, but big bluestem had a 32% higher SRL compared to switchgrass (Tables 1 and 2). Big bluestem had a 13.0% higher abundance of the fine roots in the 0–0.5 mm class compared to switchgrass, while switchgrass had a 3.8%, 7.0%, and 2.3% higher abundance of larger roots compared to big bluestem in the 0.5–1 mm, 1–2 mm, over 2 mm root diameter classes, respectively (Table 2).

Within switchgrass, cultivars varied in root biomass, SRL, and abundance of roots over 2 mm in diameter. Within the 0–10 cm depth increment switchgrass cultivar Kanlow had a 47% higher root biomass compared to Southlow (Table 1), while Southlow had a 52% higher SRL than Kanlow (Table 2). Kanlow also had a 3.1% and 3.5% higher abundance of roots over 2 mm in diameter compared to Cave-in-Rock and Southlow, respectively (Table 2). Big bluestem cultivars did not vary in root biomass, SRL, or abundance of roots in diameter size classes across depths.

### 3.2 | Total C, $C_4$ plant-derived C, and C:N ratios within soil fractions

Species differed in total soil C (mg C $g^{-1}$ soil) in the bulk soil, FPOM, and clay fractions (Figure 1; Table S2). Within the 0–10 cm depth increment, big bluestem had 10.7% higher bulk soil C compared to switchgrass (Figure 1; Table S2). Switchgrass had 38.8% higher total C in the clay fraction compared to big bluestem in the 10–20 cm soil depth (Figure 1; Table S2). While big bluestem had 54.5% and 49.5% higher total C in the FPOM fraction compared to switchgrass in the 10–20 cm and 20–30 cm depth

**TABLE 1** Dry root biomass (g root $kg^{-1}$ soil) for switchgrass and big bluestem cultivars at 0–10 cm, 10–20 cm, and 20–30 cm soil depths.

| | Root biomass (g root $kg^{-1}$ soil) | | |
|---|---|---|---|
| | **0–10 cm** | **10–20 cm** | **20–30 cm** |
| Switchgrass | 2.71 + 0.33 | 1.51 + 0.15 (A) | 0.93 + 0.10 (A) |
| Big Bluestem | 3.35 + 0.50 | 2.95 + 0.20 (B) | 1.69 + 0.13 (B) |
| Switchgrass | | | |
| Cave-in-Rock | 2.35 + 0.13 (ab) | 1.46 + 0.19 | 0.98 + 0.07 |
| Kanlow | 3.78 + 0.65 (a) | 1.77 + 0.23 | 1.11 + 0.18 |
| Southlow | 1.99 + 0.10 (b) | 1.18 + 0.42 | 0.70 + 0.20 |
| Big Bluestem | | | |
| Bonanza | 3.51 + 1.25 | 3.19 + 0.23 | 1.95 + 0.31 |
| Southlow | 2.71 + 0.89 | 3.12 + 0.46 | 1.61 + 0.08 |
| Suther | 3.84 + 0.48 | 2.54 + 0.33 | 1.51 + 0.19 |

*Note*: Values are means ± standard error. Capital letters indicate significant differences between species (switchgrass $n = 9$, big bluestem $n = 12$) and lowercase letters show differences among cultivars (switchgrass $n = 3$, big bluestem $n = 4$) within a species ($p < 0.05$).

**TABLE 2** SRL (m root $g^{-1}$ dry root) and root diameter size abundance (%) for switchgrass and big bluestem cultivars in the 0–10 cm soil depth.

| | SRL (m root $g^{-1}$ dry root) | Root diameter size (mm) | | | |
|---|---|---|---|---|---|
| | | 0–0.5 (%) | 0.5–1 (%) | 1–2 (%) | >2 (%) |
| Switchgrass | 56.59 ± 6.55 (A) | 66.3 ± 1.1 (A) | 19.1 ± 0.7 (A) | 12.0 ± 0.9 (A) | 2.6 ± 0.6 (A) |
| Big Bluestem | 82.90 ± 8.15 (B) | 79.3 ± 1.7 (B) | 15.3 ± 1.4 (B) | 5.0 ± 0.4 (B) | 0.3 ± 0.1 (B) |
| Switchgrass | | | | | |
| Cave-in-Rock | 58.59 ± 2.46 (ab) | 63.5 ± 0.6 | 21.2 ± 0.7 | 13.7 ± 1.2 | 1.7 ± 0.4 (a) |
| Kanlow | 36.12 ± 11.07 (a) | 67.0 ± 2.5 | 18.4 ± 1.4 | 9.8 ± 1.2 | 4.8 ± 0.8 (b) |
| Southlow | 75.06 ± 2.15 (b) | 68.5 ± 1.4 | 17.9 ± 0.5 | 12.4 ± 1.6 | 1.3 ± 0.2 (a) |
| Big Bluestem | | | | | |
| Bonanza | 68.44 ± 8.40 | 78.9 ± 4.4 | 15.9 ± 3.4 | 4.8 ± 1.0 | 0.4 ± 0.0 |
| Southlow | 74.41 ± 3.67 | 81.2 ± 0.5 | 13.4 ± 0.7 | 5.0 ± 0.2 | 0.4 ± 0.0 |
| Suther | 105.86 ± 19.44 | 77.9 ± 3.5 | 16.7 ± 2.9 | 5.2 ± 0.9 | 0.2 ± 0.0 |

*Note*: Values are means ± standard error. Capital letters indicate significant differences between species (switchgrass $n = 9$, big bluestem $n = 12$) and lowercase letters show differences among cultivars (switchgrass $n = 3$, big bluestem $n = 4$) within a species ($p < 0.05$).

Abbreviation: SRL, specific root length.

increments, respectively (Figure 1; Table S2). Cultivar variation was not detected in total C of bulk soil or any of the soil fractions.

Species varied in the amount of $C_4$ plant-derived C (mg C $g^{-1}$ soil) across all soil depths (Table 3). Within the 0–10 cm depth increment big bluestem had 31.5% higher bulk $C_4$ plant-derived C compared to switchgrass, which is due to 36.9% and 42.5% higher levels of $C_4$ plant-derived C in the CPOM and FPOM fractions, respectively, for big bluestem compared to switchgrass (Table 3). The CPOM fraction for big bluestem was composed of 91% plant-derived C and the FPOM fraction was 49% plant-derived C compared to 76% and 36% plant-derived C for switchgrass CPOM and FPOM fractions (Table 4). In contrast, switchgrass had 35.4% more $C_4$ plant-derived C in the bulk soil in the 10–20 cm depth increment compared to big bluestem, which was driven by switchgrass having 60.7% higher $C_4$ plant-derived C in the clay fraction compared to big bluestem (Table 3). The clay fraction for switchgrass was composed of 38% plant-derived C compared to 25% plant-derived C for the big bluestem clay fraction in the 10–20 cm soil depth (Table 4). Finally in the 20–30 cm depth increment, switchgrass had 59.3% higher clay $C_4$ plant-derived C compared to big bluestem (Table 3). Variation in $C_4$ plant-derived C per gram of soil was found for switchgrass cultivars in the bulk soil and clay fractions in the 0–10 cm depth increment, while $C_4$ plant-derived C associated with big bluestem cultivars remained similar across all depths (Table 3). Switchgrass cultivars varied in the percentage of $C_4$ plant-derived C found within CPOM and FPOM fractions in the 0–10 cm and 20–30 cm depth increments, while big bluestem cultivars varied in the percentage of $C_4$ plant-derived C found within the CPOM fraction in the 10–20 cm depth increment (Table S5).

Species varied in the ratio of C to N across all soil depths (Table 5). Specifically, big bluestem had 16.6% and 27.7% higher C:N compared to switchgrass in the CPOM fraction in the 0–10 cm and 20–30 cm depth increments, respectively (Table 5). Switchgrass on the other hand had 7.1% and 9.1% higher C:N in the FPOM fraction in the 10–20 cm and 20–30 cm depth increments, respectively (Table 5). Big bluestem cultivars differed in the FPOM C:N ratio below the top 10 cm (Table 5).

## 3.3 | Abundance of soil organic matter functional groups—Water extraction (polar bioavailable compounds)

For the water extractions of the soil, 53%–65% of the mass spectrometer peaks were assigned a molecular formula and thereby a chemical class, while the remaining 36%–48% of the peaks were either unknown and/or could not be classified.

Overall, we did not detect major differences in water extractions. However, species did vary in the relative abundance of unsaturated hydrocarbons and tannins (Table S6). Big bluestem consistently had 0.18%–0.52% higher mean abundance of unsaturated hydrocarbons across depths compared to switchgrass, while switchgrass had 0.43% higher mean abundance of tannins in the 10–20 cm depth increment compared to big bluestem (Table S6). Big bluestem exhibited cultivar variation of lignin abundance in the 10–20 cm depth increment, but

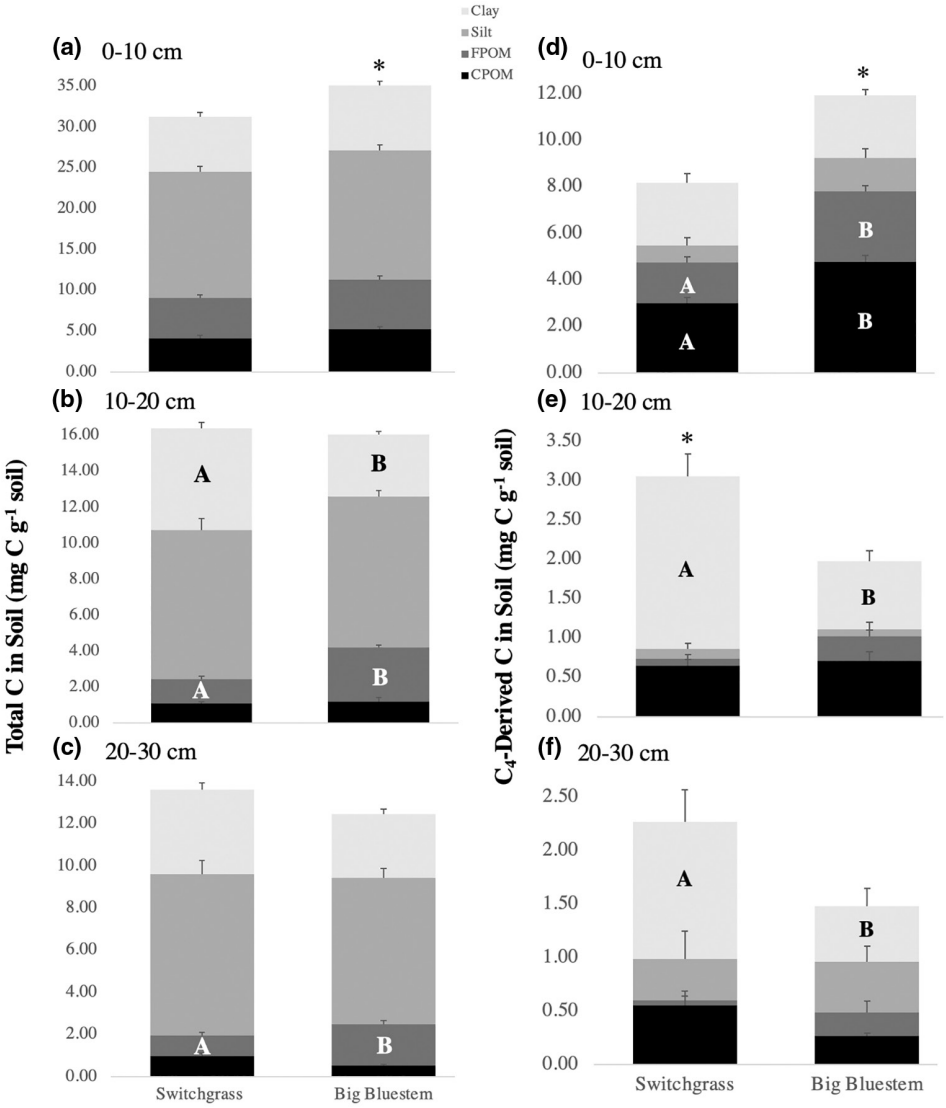


FIGURE 1 Total soil C [mg C $g^{-1}$ soil (a–c)], and $C_4$ plant-derived C [mg C $g^{-1}$ soil (d–f)] for each fraction in the 0–10 cm, 10–20 cm, and 20–30 cm soil depth for switchgrass ($n = 9$) and big bluestem ($n = 12$). Values are means ± standard error, * Indicate significance in bulk C and capital letters indicate differences in fraction layers between species ($p < 0.05$).

otherwise cultivars had similar abundances of water-extractable molecules (Table S6).

## 3.4 | Abundance of soil organic matter functional groups—Chloroform extraction (non-polar compounds)

For the chloroform extractions of soil, 13%–47% of the mass spectrometer peaks were assigned molecular formulas and a specific compound class, while 53%–87% of the peaks were either unknown and/or could not be classified.

Species varied in relative abundance of molecules across all soil depths (Figure 2a; Table S7). Big bluestem had 0.12% higher mean abundance of unsaturated hydrocarbons compared to switchgrass in the 0–10 cm and 20–30 cm depth increments (Table S7). While switchgrass had 0.90% and 0.76% higher mean abundance of lignin compared to big bluestem in the 10–20 cm and 20–30 cm depth increments, respectively (Table S7). Switchgrass also had 7.61% higher mean abundance of lipids in the 10–20 cm depth increment and 0.86% higher mean abundance of proteins in the 20–30 cm depth increment compared to big bluestem (Table S7).

Switchgrass cultivars varied in the relative abundance of condensed hydrocarbons in the 20–30 cm depth increment (Table S7). Across all soil depths combined, switchgrass cultivars showed variation in the relative abundance of lipids, proteins, and lignin, while big bluestem cultivars varied in the relative abundance of proteins and lignin (Figure 2b,c). Within switchgrass, Kanlow had a 5.34%–7.52% higher mean abundance of lipids compared to Cave-in-Rock and

**TABLE 3** $C_4$ plant-derived C (mg C $g^{-1}$ soil) for bulk and individual soil fractions from the switchgrass and big bluestem cultivars at 0–10 cm, 10–20 cm, and 20–30 cm depths.

| | $C_4$ plant-derived C (mg C $g^{-1}$ soil) | | | | |
|---|---|---|---|---|---|
| | **Bulk** | **CPOM** | **FPOM** | **Silt** | **Clay** |
| 0–10 cm | | | | | |
| Switchgrass | 8.17 ± 1.08 (A) | 3.00 ± 0.23 (A) | 1.74 ± 0.24 (A) | 0.74 ± 0.32 | 2.68 ± 0.39 |
| Big Bluestem | 11.94 ± 0.77 (B) | 4.76 ± 0.30 (B) | 3.03 ± 0.24 (B) | 1.45 ± 0.40 | 2.70 ± 0.23 |
| Switchgrass | | | | | |
| Cave-in-Rock | 11.36 ± 1.83 (a) | 3.67 ± 0.39 | 2.44 ± 0.42 | 1.47 ± 0.65 | 3.79 ± 0.49 (a) |
| Kanlow | 5.54 ± 0.39 (b) | 2.70 ± 0.25 | 1.20 ± 0.11 | 0.00 ± 0.00 | 1.64 ± 0.03 (b) |
| Southlow | 7.59 ± 1.33 (ab) | 2.63 ± 0.29 | 1.60 ± 0.28 | 0.76 ± 0.48 | 2.61 ± 0.65 (ab) |
| Big Bluestem | | | | | |
| Bonanza | 11.25 ± 1.01 | 4.71 ± 0.63 | 2.60 ± 0.34 | 1.07 ± 0.42 | 2.87 ± 0.60 |
| Southlow | 12.40 ± 1.80 | 4.68 ± 0.37 | 3.15 ± 0.50 | 2.06 ± 0.91 | 2.52 ± 0.38 |
| Suther | 12.16 ± 1.41 | 4.89 ± 0.65 | 3.34 ± 0.43 | 1.21 ± 0.73 | 2.72 ± 0.23 |
| 10–20 cm | | | | | |
| Switchgrass | 3.05 ± 0.35 (A) | 0.64 ± 0.09 | 0.09 ± 0.05 (A) | 0.13 ± 0.07 | 2.19 ± 0.28 (A) |
| Big Bluestem | 1.97 ± 0.25 (B) | 0.71 ± 0.12 | 0.31 ± 0.08 (B) | 0.09 ± 0.09 | 0.86 ± 0.14 (B) |
| Switchgrass | | | | | |
| Cave-in-Rock | 3.76 ± 0.71 | 0.70 ± 0.22 | 0.24 ± 0.12 | 0.29 ± 0.15 | 2.53 ± 0.74 |
| Kanlow | 2.24 ± 0.37 | 0.57 ± 0.13 | 0.02 ± 0.02 | 0.00 ± 0.00 | 1.66 ± 0.30 |
| Southlow | 3.15 ± 0.52 | 0.67 ± 0.14 | 0.02 ± 0.02 | 0.09 ± 0.09 | 2.38 ± 0.30 |
| Big Bluestem | | | | | |
| Bonanza | 1.86 ± 0.81 | 0.57 ± 0.13 | 0.33 ± 0.18 | 0.31 ± 0.31 | 0.65 ± 0.38 |
| Southlow | 2.05 ± 0.42 | 0.66 ± 0.09 | 0.36 ± 0.13 | 0.00 ± 0.00 | 1.03 ± 0.26 |
| Suther | 1.98 ± 0.24 | 0.86 ± 0.31 | 0.25 ± 0.14 | 0.01 ± 0.01 | 0.85 ± 0.09 |
| 20–30 cm | | | | | |
| Switchgrass | 2.27 ± 0.60 | 0.55 ± 0.13 | 0.05 ± 0.04 | 0.38 ± 0.26 | 1.28 ± 0.31 (A) |
| Big Bluestem | 1.48 ± 0.31 | 0.26 ± 0.03 | 0.22 ± 0.11 | 0.48 ± 0.14 | 0.52 ± 0.17 (B) |
| Switchgrass | | | | | |
| Cave-in-Rock | 1.77 ± 0.83 | 0.68 ± 0.39 | 0.02 ± 0.02 | 0.00 ± 0.00 | 1.07 ± 0.54 |
| Kanlow | 1.46 ± 0.58 | 0.34 ± 0.05 | 0.01 ± 0.01 | 0.00 ± 0.00 | 1.11 ± 0.64 |
| Southlow | 3.57 ± 1.40 | 0.64 ± 0.15 | 0.11 ± 0.11 | 1.15 ± 0.61 | 1.66 ± 0.55 |
| Big Bluestem | | | | | |
| Bonanza | 1.14 ± 0.47 | 0.24 ± 0.04 | 0.29 ± 0.20 | 0.43 ± 0.31 | 0.18 ± 0.13 |
| Southlow | 1.29 ± 0.57 | 0.32 ± 0.06 | 0.26 ± 0.26 | 0.34 ± 0.20 | 0.36 ± 0.17 |
| Suther | 2.08 ± 0.57 | 0.29 ± 0.05 | 0.12 ± 0.12 | 0.65 ± 0.27 | 1.02 ± 0.37 |

*Note*: Values are means ± standard error. Capital letters indicate significant differences between species (switchgrass $n = 9$, big bluestem $n = 12$) and lowercase letters show differences among cultivars (switchgrass $n = 3$, big bluestem $n = 4$) within a species ($p < 0.05$). Calculation: $C_4$ plant-derived C in a fraction = (mg $C_4$ plant-derived C in 1 g fraction) × (fraction abundance in 1 g soil).

Abbreviations: CPOM, coarse particulate organic matter; FPOM, fine particulate organic matter.

Southlow (Figure 2b). Additionally, Southlow had a 1.14% higher mean abundance of proteins and 1.13% higher mean abundance of lignin compared to Kanlow (Figure 2b). Within big bluestem, Suther had 0.69% higher mean abundance of proteins and 0.56% higher mean abundance of lignin compared to Southlow (Figure 2c).

## 3.5 | Correlations of $C_4$ plant-derived C

Significant correlations were found for accumulation of CPOM and clay $C_4$ plant-derived C (Figures 3 and 4). CPOM $C_4$ plant-derived C was correlated with higher levels of root biomass and CPOM C:N as well as higher

**TABLE 4** Total C, $C_4$ plant-derived C, and ratio of total C to $C_4$ plant-derived C (mg C $g^{-1}$ fraction) for individual soil fractions from the switchgrass ($n = 9$) and big bluestem ($n = 12$) cultivars at 0–10 cm, 10–20 cm, and 20–30 cm depths.

| **Total soil C (mg C $g^{-1}$ fraction)** | | | | |
|---|---|---|---|---|
| | **CPOM** | **FPOM** | **Silt** | **Clay** |
| 0–10 cm | | | | |
| Switchgrass | 84.70 ± 5.70 | 55.87 ± 2.42 (A) | 20.87 ± 0.91 | 60.31 ± 1.67 |
| Big Bluestem | 103.10 ± 7.40 | 47.50 ± 1.87 (B) | 23.19 ± 0.99 | 58.18 ± 2.66 |
| 10–20 cm | | | | |
| Switchgrass | 26.97 ± 1.56 | 18.66 ± 1.11 | 11.21 ± 0.89 | 40.93 ± 2.05 (A) |
| Big Bluestem | 24.52 ± 2.33 | 17.37 ± 0.51 | 12.53 ± 0.41 | 33.91 ± 2.21 (B) |
| 20–30 cm | | | | |
| Switchgrass | 19.23 ± 1.90 | 13.53 ± 1.51 | 10.42 ± 0.82 | 29.88 ± 3.33 |
| Big Bluestem | 18.29 ± 1.13 | 12.84 ± 0.58 | 10.13 ± 0.59 | 23.87 ± 2.22 |
| **$C_4$ plant-derived C (mg C $g^{-1}$ fraction)** | | | | |
| | **CPOM** | **FPOM** | **Silt** | **Clay** |
| 0–10 cm | | | | |
| Switchgrass | 66.75 ± 7.82 (A) | 20.55 ± 3.34 | 0.99 ± 0.41 | 23.60 ± 2.92 |
| Big Bluestem | 93.95 ± 6.41 (B) | 23.34 ± 1.07 | 2.09 ± 0.57 | 21.04 ± 2.38 |
| 10–20 cm | | | | |
| Switchgrass | 16.68 ± 2.12 | 1.03 ± 0.61 | 0.17 ± 0.09 | 15.33 ± 1.59 (A) |
| Big Bluestem | 16.03 ± 2.14 | 1.76 ± 0.42 | 0.13 ± 0.12 | 8.73 ± 1.59 (B) |
| 20–30 cm | | | | |
| Switchgrass | 10.91 ± 1.86 | 0.61 ± 0.52 | 0.51 ± 0.34 | 10.73 ± 3.19 |
| Big Bluestem | 9.79 ± 0.97 | 1.26 ± 0.62 | 0.70 ± 0.21 | 4.04 ± 1.32 |
| **$C_4$ plant-derived C:Total C** | | | | |
| | **CPOM** | **FPOM** | **Silt** | **Clay** |
| 0–10 cm | | | | |
| Switchgrass | 0.76 ± 0.06 (A) | 0.36 ± 0.05 (A) | 0.05 ± 0.02 | 0.39 ± 0.05 |
| Big Bluestem | 0.91 ± 0.01 (B) | 0.49 ± 0.02 (B) | 0.09 ± 0.03 | 0.35 ± 0.03 |
| 10–20 cm | | | | |
| Switchgrass | 0.60 ± 0.05 | 0.05 ± 0.03 | 0.02 ± 0.01 | 0.38 ± 0.04 (A) |
| Big Bluestem | 0.63 ± 0.03 | 0.10 ± 0.02 | 0.01 ± 0.01 | 0.25 ± 0.04 (B) |
| 20–30 cm | | | | |
| Switchgrass | 0.54 ± 0.04 | 0.04 ± 0.04 | 0.04 ± 0.03 | 0.33 ± 0.08 |
| Big Bluestem | 0.52 ± 0.03 | 0.09 ± 0.04 | 0.08 ± 0.03 | 0.16 ± 0.04 |

*Note*: Values are means ± standard error. Capital letters indicate significant differences between species ($p < 0.05$).

Abbreviations: CPOM, coarse particulate organic matter; FPOM, fine particulate organic matter.

relative abundances of lignin and proteins (Figure 3). Big bluestem was the only significant relationship for CPOM $C_4$ plant-derived C and relative abundance of lignin and proteins, although switchgrass did show a positive trend. Clay $C_4$ plant-derived C was correlated with higher relative abundances of lignin and proteins only for big bluestem, but switchgrass did show similar trends (Figure 4).

## 4 | DISCUSSION

Our study yielded four main results: (1) big bluestem had a higher SRL and higher percentage of roots in the 0–0.5 mm diameter size class than switchgrass, (2) the 0–10 cm depth increment soils under big bluestem had a higher bulk C concentration and more $C_4$ plant-derived C in the POM fraction compared to switchgrass, (3) soils

**TABLE 5** C:N for individual soil fractions from the switchgrass and big bluestem cultivars at 0–10 cm, 10–20 cm, and 20–30 cm soil depths.

| | C:N | | | |
|---|---|---|---|---|
| | **CPOM** | **FPOM** | **Silt** | **Clay** |
| 0–10 cm | | | | |
| Switchgrass | 26.39 ± 1.41 (A) | 16.02 ± 0.38 | 11.52 ± 0.13 | 10.21 ± 0.09 |
| Big Bluestem | 31.66 ± 1.53 (B) | 16.62 ± 0.32 | 11.90 ± 0.09 | 10.15 ± 0.12 |
| Switchgrass | | | | |
| Cave-in-Rock | 27.83 ± 1.58 | 16.16 ± 0.12 | 11.71 ± 0.26 | 10.30 ± 0.13 |
| Kanlow | 24.10 ± 4.10 | 16.18 ± 1.19 | 11.51 ± 0.28 | 10.27 ± 0.09 |
| Southlow | 27.23 ± 0.81 | 15.72 ± 0.43 | 11.35 ± 0.14 | 10.06 ± 0.22 |
| Big Bluestem | | | | |
| Bonanza | 30.52 ± 3.46 | 16.87 ± 0.68 | 11.88 ± 0.21 | 9.97 ± 0.20 |
| Southlow | 30.40 ± 2.78 | 16.36 ± 0.47 | 11.94 ± 0.20 | 10.33 ± 0.21 |
| Suther | 34.06 ± 1.81 | 16.64 ± 0.63 | 11.89 ± 0.12 | 10.17 ± 0.23 |
| 10–20 cm | | | | |
| Switchgrass | 21.91 ± 1.89 | 14.46 ± 0.26 (A) | 11.82 ± 0.18 | 11.14 ± 0.26 |
| Big Bluestem | 24.74 ± 1.92 | 13.43 ± 0.13 (B) | 12.12 ± 0.14 | 11.28 ± 0.15 |
| Switchgrass | | | | |
| Cave-in-Rock | 26.52 ± 4.35 | 14.37 ± 0.72 | 11.90 ± 0.39 | 11.07 ± 0.70 |
| Kanlow | 17.31 ± 1.27 | 14.49 ± 0.54 | 11.76 ± 0.28 | 11.25 ± 0.39 |
| Southlow | 21.91 ± 1.06 | 14.51 ± 0.10 | 11.81 ± 0.37 | 11.10 ± 0.38 |
| Big Bluestem | | | | |
| Bonanza | 22.49 ± 0.39 | 12.87 ± 0.17 (a) | 11.78 ± 0.15 | 10.99 ± 0.17 |
| Southlow | 30.39 ± 3.38 | 13.60 ± 0.06 (b) | 11.98 ± 0.14 | 11.17 ± 0.27 |
| Suther | 20.77 ± 2.29 | 13.67 ± 0.18 (b) | 12.52 ± 0.23 | 11.60 ± 0.24 |
| 20–30 cm | | | | |
| Switchgrass | 17.93 ± 0.98 (A) | 14.85 ± 0.35 (A) | 11.95 ± 0.19 | 11.09 ± 0.31 |
| Big Bluestem | 24.83 ± 1.39 (B) | 13.49 ± 0.12 (B) | 11.79 ± 0.13 | 11.00 ± 0.14 |
| Switchgrass | | | | |
| Cave-in-Rock | 20.64 ± 0.76 | 14.01 ± 0.48 | 11.77 ± 0.33 | 11.41 ± 0.32 |
| Kanlow | 17.21 ± 0.38 | 14.78 ± 0.15 | 11.98 ± 0.12 | 10.94 ± 0.43 |
| Southlow | 15.95 ± 2.21 | 15.75 ± 0.71 | 12.08 ± 0.55 | 11.93 ± 0.91 |
| Big Bluestem | | | | |
| Bonanza | 26.34 ± 3.82 | 13.20 ± 0.09 (a) | 11.78 ± 0.09 | 10.75 ± 0.05 |
| Southlow | 24.19 ± 1.85 | 13.40 ± 0.19 (ab) | 11.85 ± 0.28 | 11.17 ± 0.27 |
| Suther | 23.95 ± 1.39 | 13.85 ± 0.18 (b) | 11.74 ± 0.31 | 11.07 ± 0.32 |

*Note*: Values are means ± standard error. Capital letters indicate significant differences between species (switchgrass $n = 9$, big bluestem $n = 12$) and lowercase letters show differences among cultivars (switchgrass $n = 3$, big bluestem $n = 4$) within a species ($p < 0.05$).

Abbreviations: CPOM, coarse particulate organic matter; FPOM, fine particulate organic matter.

under switchgrass tended to have lower C:N ratios in the CPOM fraction compared to big bluestem, and more $C_4$ plant-derived C in the clay fraction in the 10–30 cm depth increments leading to higher bulk C concentration in the 10–20 cm depth increment, and finally (4) both switchgrass and big bluestem showed intraspecific differences in the abundances of organic matter functional groups (chemical class composition), and there was intraspecific variation in root traits in switchgrass. In agreement with our hypotheses, these data indicate a higher SRL and a higher percentage of 0–0.5 mm diameter roots contributes to more $C_4$ plant-derived C, particularly in the POM fraction. Additionally, lower C:N ratios in CPOM may indicate more labile plant-derived

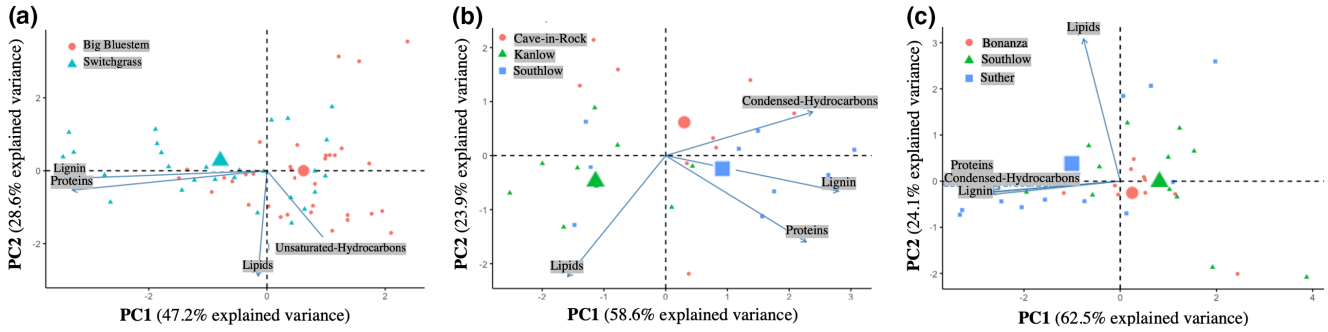


**FIGURE 2** Principal component analysis (PCA) of the significantly different chemical class abundances in the chloroform extracted soil pool across all soil depths for (a) species, (b) switchgrass cultivars, and (c) big bluestem cultivars. Symbol shape represents species or cultivar, while large symbols represent the mean for the group. For species, significantly different chloroform extracted chemical abundances explain 75.8% of the variance [PCA axis 1 and 2 (a)]. For switchgrass cultivars, significantly different chloroform extracted chemical abundances explain 82.5% of the variance [PCA axis 1 and 2 (b)]. For big bluestem cultivars, significantly different chloroform extracted chemical abundances explain 86.6% of the variance [PCA axis 1 and 2 (c)].

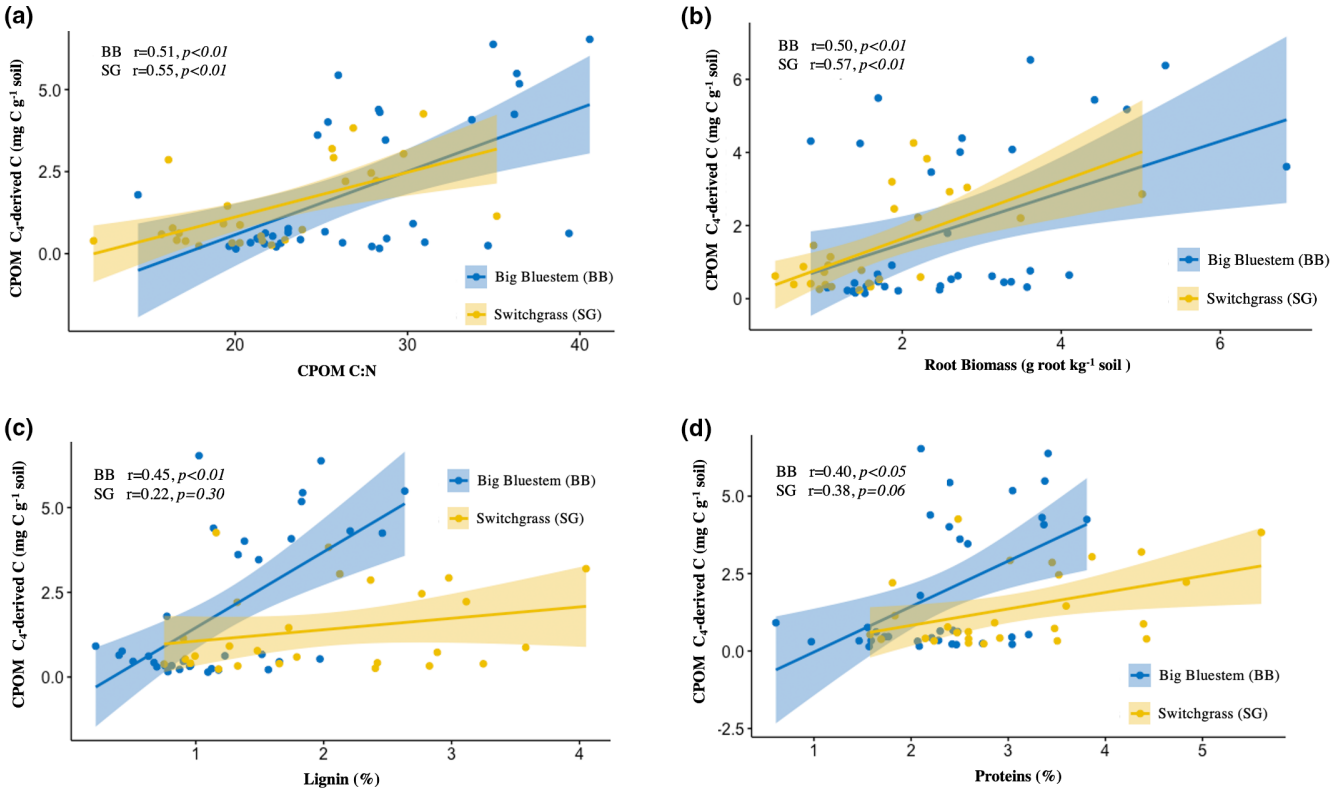


**FIGURE 3** Correlations for CPOM $C_4$ plant-derived C (mg C $g^{-1}$ soil) and (a) CPOM C:N, (b) root biomass (g root $kg^{-1}$ soil), and the relative abundance of (c) lignin (%) and (d) proteins (%) in the chloroform extraction. Blue points and lines indicate big bluestem cultivars, while yellow points and lines indicate switchgrass cultivars. The shading represents a 95% confidence interval. CPOM, coarse particulate organic matter.

C inputs which contribute to more $C_4$ plant-derived C in the clay fraction. Within switchgrass, cultivars showed a diversity of root traits and soil organic matter functional group abundances which may explain different mechanisms of $C_4$ plant-derived C accumulation in the clay fraction.

Soils supporting big bluestem had a greater quantity of $C_4$ plant-derived C and total C in the 0–10 cm depth increment than soils supporting switchgrass, and the majority of big bluestem-derived C in the 0–10 cm soil depth increment was associated with the CPOM and FPOM fractions. Within the 0–10 cm soil depth increment, 90% of

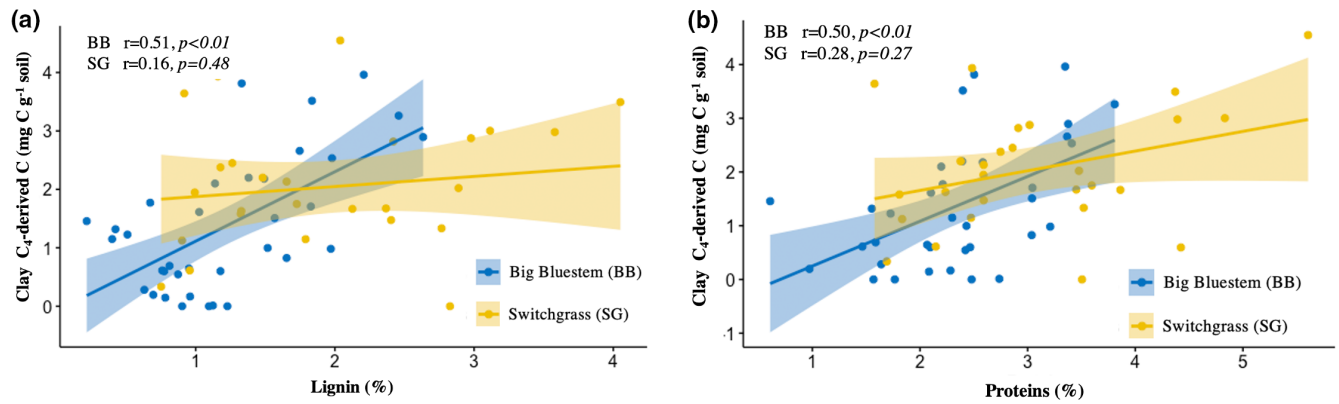


**FIGURE 4** Correlations for clay $C_4$ plant-derived C (mg C $g^{-1}$ soil) and the relative abundance of (a) lignin (%) and (b) proteins (%) in the chloroform extraction. Blue points and lines indicate big bluestem cultivars, while yellow points and lines indicate switchgrass cultivars. The shading represents a 95% confidence interval.

CPOM and 49% of FPOM was $C_4$ plant derived, showing a high incorporation of big bluestem plant material into the POM fraction. Additionally, in the 10–30 cm depth increment, we observed a greater quantity of C associated with the FPOM fraction compared to switchgrass. Across all soil depths, $C_4$ plant-derived C in CPOM was found to positively correlate with root biomass and C:N of CPOM. These results suggest that big bluestem contributed more root litter to soil than switchgrass, or that this litter was retained to a greater extent. Greater litter input in the 0–10 cm layer may be explained by big bluestem root systems having a higher SRL than switchgrass root systems. This was caused by a higher percentage of roots in the 0–0.5 mm diameter size class, which represent first-order roots that have higher turnover rates than other roots in a root system (Pregitzer et al., 1998, 2002). In the 10–30 cm depth increments, the higher FPOM-C may be linked to higher root biomass in soils supporting big bluestem compared to switchgrass. Additionally, the CPOM fractions in big bluestem had a higher C:N ratio compared to switchgrass, suggesting that big bluestem roots have a slower decomposition rate than switchgrass roots (Angst et al., 2021; Enríquez et al., 1993; Villarino et al., 2021). We assert that the combination of a higher quantity of root litter inputs and a lower rate of root litter decomposition resulted in greater POM-C accumulation in soils supporting big bluestem, than in soils supporting switchgrass.

Within big bluestem, cultivars had similar root morphology and $C_4$ plant-derived C accumulation. Despite these similarities, big bluestem cultivars did vary in molecular abundances in the non-polar less bioavailable and potentially sorbed C pool. Cultivar Suther showed higher concentrations of both protein- and lignin-like compounds compared to Southlow, and Bonanza consistently fell in the middle. These differences are notable but small, as big bluestem cultivar variability between molecular abundances ranged from 0.4% to 0.7%. We also found that the relative abundance of both protein and lignin were positively correlated with CPOM and clay $C_4$ plant-derived C accumulation. Although differences in protein and lignin abundances were minimal, through time they could lead to differences in C accumulation among big bluestem cultivars.

In the 10–20 cm and 20–30 cm depth increments, switchgrass had more $C_4$ plant-derived C in the clay fraction compared to big bluestem. This accumulation of $C_4$ plant-derived C in the clay fraction in switchgrass may be the result of lower C:N ratios in the CPOM and higher decomposition rates of root C inputs compared to big bluestem, leading to faster transfer of this C from the CPOM fraction to the clay fraction. The quantity of $C_4$ plant-derived C in the clay fraction varied among switchgrass cultivars in the 0–10 cm depth increment with Kanlow having lower levels of $C_4$ plant-derived C, and although statistically this did not vary in deeper depths the trends remained similar. Interestingly, switchgrass cultivars did have significantly different root morphology and molecular abundances of C, which may help explain these differences in clay C accumulation. Notably, Kanlow exhibited lower SRL, higher lipid-like compounds, and lower protein- and lignin-like compounds than Southlow and Cave-in-Rock. This variability may reflect differences in genotypic and phenotypic morphologic and chemical traits in the switchgrass cultivars owing to differences in their geographical distribution (Emery et al., 2018; Lowry et al., 2014; Yang & Udvardi, 2018). Kanlow represents a lowland ecotype while Cave-in-Rock and Southlow are both upland ecotypes (Roley et al., 2021). The diverse root traits and molecular abundances in soils dominated by switchgrass cultivars in concert with differences in $C_4$ plant-derived C in the clay fraction, highlight that C accumulation in the clay fraction may be driven by different stabilizing mechanisms.

We found that switchgrass cultivar Kanlow had a higher concentration of $C_4$ plant-derived C in clay compared to big bluestem cultivars in the 10–30 cm depth increment but had statistically significant lower levels in the 0–10 cm compared to other switchgrass cultivars and this trend remained in the 10–20 cm soil depth. Higher rates of C formation at depths below 15 cm in soils supporting Kanlow relative to other perennial grasses have been corroborated by others (Ferchaud et al., 2016), and this may be caused by higher microbial C use efficiency (CUE) due to high N use efficiency (Keiblinger et al., 2010; Li et al., 2021; Liu et al., 2018). Lowland ecotypes typically have low nitrogen demand (Lowry et al., 2014), and Roley et al. (2021) found that out of 12 switchgrass cultivars, Kanlow performed in the top 3 for fixing and acquiring nitrogen. In addition, greater arbuscular mycorrhizal fungi (AMF) colonization in lowland ecotypes compared to upland ecotypes (Emery et al., 2018) may enhance CUE (De Vries & Shade, 2013). Kanlow's root morphology may have promoted AMF colonization, given that it was dominated by a high percent of roots in the diameter size class that was greater than 2 mm, which may increase colonization by AMF (Valverde-Barrantes et al., 2016). Additionally, we found that Kanlow had a 5%–7% higher percent abundance of lipid-like compounds in the non-polar less bioavailable and potentially sorbed chemical profile across all soil depths as well as lower SRL compared to the other switchgrass cultivars. This accumulation of lipid-like compounds may be from Kanlow having root byproducts (exudates, mucilages) with higher concentrations of lipids, or that the Kanlow microbial community has high CUE and releases more lipid byproducts that have affinity to sorb to the clay (Kögel-Knabner, 2017). Overall, Kanlow seems to benefit from a high CUE that increases accumulation of $C_4$ plant-derived C in clay compared to big bluestem. Despite the efficient microbial community, Kanlow may be limited with its low SRL which may lower root C inputs preventing Kanlow from maintaining similar levels of $C_4$ plant-derived C in the clay compared to the other switchgrass cultivars.

Higher accumulation of $C_4$ plant-derived C in the clay fraction under the upland ecotypes relative to the lowland ecotype Kanlow may be explained by a greater abundance of fine roots. Cave-in-Rock and Southlow have 38%–52% higher SRL compared to Kanlow, suggesting that their root systems have a higher root surface area from which rhizodeposition and root turnover could occur. Higher rhizodeposition and root turnover in upland ecotypes compared to Kanlow is supported by Southlow having higher amounts of protein- and lignin-like compounds in the non-polar C pool compared to Kanlow, and while Cave-in-Rock is not significantly different it tends to trend more like Southlow. A higher abundance of protein-like compounds may promote microbial residue formation that sorbs to C in clay (Kögel-Knabner, 2017), and high lignin-like abundances may be the result of higher fine root turnover. Together these results indicate that more fine roots associated with the upland ecotypes increase rhizodeposition that contributes to C accumulation in the clay. These trends were also corroborated with positive correlations between $C_4$ plant-derived C in the clay and abundances of proteins and lignin. However, switchgrass correlations were not significant although the trends were positive, possibly due to smaller switchgrass sample size and variance among cultivar mechanisms.

Overall, big bluestem increases soil C in the 0–10 cm depth increment by contributing to soil C formation via their extensive rooting systems that lead to the formation of a large POM-C pool dominated by $C_4$ plant-derived C. Switchgrass contributes to soil C accumulation in the 10–30 cm depth increments by increasing mineral sorbed C in the clay fraction via differences in root morphology and chemistry of C inputs. Often, non-polar C that has become sorbed to minerals is considered to be more stable than C found in the POM fraction because C that is mineral sorbed is less likely to be respired by microbes (Angst et al., 2021; Villarino et al., 2021). POM-C on the other hand is more sensitive to environmental disturbances which can lead to increases in respiration (Angst et al., 2021; Villarino et al., 2021). However, since the majority of POM-C comes from plant inputs, it has a larger capacity to grow, whereas mineral sorbed C is limited by the sorption capacity of the minerals in the soil (Angst et al., 2021). This study indicates the importance of both POM-C and mineral C in building soil C pools.

The need for biofuels that maximize net soil C gains and curb atmospheric $CO_2$ concentrations is quickly growing. Our findings show that species and cultivar type of native perennial grasses both impact how soil C is accrued. Big bluestem has a large rooting system that helps increase POM-C formation quickly, while switchgrass root structure and chemistry help to build a mineral-bound clay C pool through time. Depending on management strategy both species have the potential to offset $CO_2$ emissions and increase soil C storage.

## AUTHOR CONTRIBUTIONS

Megan J. Kelly-Slatten, Marie-Anne de Graaff, and Julie D. Jastrow designed the study. Megan J. Kelly-Slatten, Marie-Anne de Graaff, Julie D. Jastrow, Catherine E. Stewart, Malak M. Tfaily, and Abigail Sasso conducted the field and laboratory analyses. Data analysis was conducted by Megan J. Kelly-Slatten and Malak M. Tfaily. The paper was written by Megan J. Kelly-Slatten with input from all authors.

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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.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in figshare at 10.6084/m9.figshare.21984179.v2.

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