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Soil total carbon and nitrogen under long-term perennial bioenergy crops receiving various nitrogen fertilization rates

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Abstract

Perennial crops may sequester greater soil carbon (C) and nitrogen (N) than annual crops due to higher root biomass production, but the potential of long-term cultivation of perennial bioenergy crops receiving various N fertilization rates in sequestering C and N needs further exploration. Soil samples (0–120 cm) collected from 2009 to 2019 under perennial bioenergy crops receiving various N fertilization rates and an annual crop were analyzed for soil total C (STC) and N (STN) in the US northern Great Plains. Perennial bioenergy crops were intermediate wheatgrass (IW, *Thinopyrum intermedium* [Host] Barkworth and Dewey), smooth bromegrass (SB, *Bromus inermis* L.), and switchgrass (SG, *Panicum virgatum* L.); N fertilization rates were 0, 28, 56, and 84 kg N ha^{-1}, and annual crop was spring wheat (WH, *Triticum aestivum*, L.). The STC increased, but STN decreased by soil depth, regardless of treatments and years. At 0–120 cm, IW sequestered C at 10.6 Mg C ha^{-1} $year^{-1}$ compared with 5.4–6.8 Mg C ha^{-1} $year^{-1}$ for SB and SG from 2009 to 2019. Nitrogen fertilization rate had limited effect on STC and STN. At 0–30 cm, STC was 2.0–5.4 Mg C ha^{-1} greater and STN 0.10–0.24 Mg N ha^{-1} greater with perennial bioenergy crops than WH. Long-term cultivation of IW can sequester more C in the soil to a greater depth than SB and SG and perennial bioenergy crops can sequester more C and N at the surface layer than an annual crop in the semiarid region of the US northern Great Plains.

1 | INTRODUCTION

Agricultural practices emit significant amounts of greenhouse gases (GHG) that contribute to global warming (Cole et al., 1997; Smith et al., 2007). Improved agricultural management strategies are needed to reduce GHG emissions by sequestering atmospheric CO_2 into the soil because CO_2 is one of the most prominent GHGs (Cole et al., 1997; Smith et al., 2007). One of these strategies is to plant perennial bioenergy crops because they produce more root biomass to a greater soil depth that serves as carbon (C) input to the soil than annual crops (Blanco-Canqui, 2010; Bronson et al., 2004; Post & Kwon, 2000). This, followed by relatively undisturbed soil condition that reduce C and nitrogen (N) mineralization, year-round nutrient cycling, greater adaptability, and higher drought tolerance favor greater C and N sequestration in the whole soil profile under perennial bioenergy crops

Abbreviations: GHG, greenhouse gas; IW, intermediate wheatgrass; SB, smooth bromegrass; SG, switchgrass; SIC, soil inorganic carbon; SOC, soil organic carbon; STC, soil total carbon; STN, soil total nitrogen; WH, spring wheat.

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(Blanco-Canqui, 2010; Bronson et al., 2004; Post & Kwon, 2000). The increased environmental and economic benefits of growing perennial bioenergy crops, such as reduced GHG emissions compared to fossil fuels, less input requirements, increased adaptability on marginal lands, greater biomass production, and higher net income (e.g., C credit) than annual crops, make the production of bioenergy crops more attractive to producers and industrialists (Lemus & Lal, 2005; Pacala & Solocolow, 2004).

Perennial bioenergy crops vary in their potentials to sequester C and N in the soil (Anderson-Teixeiera et al., 2009; Blanco-Canqui & Lal, 2009). Omonode and Vyn (2006) reported that soil organic C (SOC) and soil total N (STN) to a depth of 1 m were greater under switchgrass (SG) than a mixture of Indiana grass (*Sorghastrum nutans* L.), big bluestem (*Andropogan gerardi* L.), and little bluestem (*Andropogan scoparius* L.) in Indiana. In contrast, Johnson and Barbour (2016) found that SOC at 0–5 cm was greater under big blue stem than SG in Minnesota. Both SOC and STN depend on the performance of perennial bioenergy crops, age, soil and climatic conditions, topography, harvest frequency, cutting height, and management type (Johnson & Barbour, 2016; Ma et al., 2000; Sanderson et al., 1996). Several researchers (Anderson-Teixeiera et al., 2009; Blanco-Canqui & Lal, 2009) observed that warm-season grasses, such as SG, can sequester C at 0.3–0.5 Mg C ha^{-1} year^{-1} to a depth of 30 cm. Omonode and Vyn (2006) demonstrated that C and N sequestration rates at 0–60 cm for warm-season grasses were 2.1 Mg C ha^{-1} year^{-1} and 0.14 Mg N ha^{-1} year^{-1}, respectively, after 6–8 years in Indiana. The SG sequestered C at 1.2 Mg C ha^{-1} year^{-1} to a depth of 15 cm after 10 years in Iowa (Al-Kaisi et al., 2005) and at 3.5 Mg C ha^{-1} year^{-1} to a depth of 60 cm after 4 years in eastern Canada (Zan et al., 2001). In Alabama, Ma et al. (2000) found that SG did not increase SOC after 2–3 years, but did so by 28%–45% at 0–30 cm compared to fallow after 10 years. Knaps and Tillman (2000) stated that warm-season grasses can increase SOC, but not STN compared with cool-season grasses.

The effect of N fertilization rate for perennial bioenergy crops on SOC and STN can also be variable. Several researchers (Ma et al., 2000; Reeder et al., 1998) reported that N fertilization rate for perennial bioenergy crops had no effect on SOC and STN. However, Rice (2000), in Kansas, found that N fertilization to cool-season grasses increased C sequestration rate at 0–30 cm by 1.6 Mg C ha^{-1} year^{-1} compared with no N fertilization after 5 years. Similarly, Bremer et al. (2007) showed that N fertilization to perennial grasses increased C sequestration rate at 0–5 cm by 0.5 Mg C ha^{-1} year^{-1} compared with no N fertilization after 6–12 years in Alberta, Canada. Lee et al. (2007) observed that N fertilization to SG sequestered C at 2.4 Mg C ha^{-1} year^{-1} at 0–90 cm compared to no N fertilization after 4 years in South Dakota.

Core Ideas

- Intermediate wheatgrass had greater soil total carbon (C) at 15–30 cm and 0–120 cm than smooth bromegrass and switchgrass.
- Soil total nitrogen (N) at 15–30 cm varied by perennial bioenergy crop and year.
- N fertilization rate had no effect on soil total C and N.
- Soil total C and N at 0–120 cm were greater under perennial than annual crops.
- Intermediate wheatgrass can sequester more C than smooth bromegrass and switchgrass.

Soil C and N stocks are usually greater under perennial crops due to higher root biomass production and distribution than annual crops (Gebhart et al., 1994; Ma et al., 2000; Omonode & Vyn, 2006). Liebig et al. (2005) reported that SOC at 30–60 and 60–90 cm was 4.3–7.7 Mg C ha^{-1} greater under SG than croplands due to reduced C mineralization in the US northern Great Plains. Lal et al. (1998) found that SOC at 0–15 cm was 17% greater under smooth bromegrass (SB) than corn (*Zea mays* L.)-soybean (*Glycine max* L.) rotation after 5 years in the US Midwest. Zan et al. (2001) demonstrated that SOC was not different between SG and corn after 3 years, but was 45% greater under SG than corn at 0–15 cm and 28% greater at 15–30 cm after 10 years in eastern Canada. Dheri et al. (2022) showed that SG increased SOC at 0–20 cm by 7.94 Mg C ha^{-1} compared to a loss of 8.25 Mg C ha^{-1} under annual crops after 10 years in Ohio. In contrast, Chaterjee et al. (2018) did not observe significant difference in SOC between SG and winter wheat after 6 years in eastern North Dakota. Perennial grasses also increased STN compared with corn-soybean rotation (Al-Kaisi et al., 2005; Omonode & Vyn, 2006; Potter et al., 1999).

We chose soil total C (STC) instead of SOC to measure C sequestration rates under perennial bioenergy crops in dryland farming in the semiarid climatic condition of the US northern Great Plains because STC was equally sensitive to management practices, associated with more soil properties, and related better with crop yields than SOC (Sainju et al., 2021). As dryland soils contain significant amount of soil inorganic C (SIC) mainly in subsoil layers where the potential for C sequestration is greater, using STC to measure C sequestration rate is more meaningful than using SOC, especially in dryland cropping systems in arid and semiarid regions (FAO, 2004; UNEP, 1991). Another reason for using STC instead of SOC is that C sequestration due to management practices occurs both in SOC and SIC and measurement of STC is rapid and as reliable as measurement of SOC in arid and semiarid regions

(FAO, 2004; UNEP, 1991). The simplicity and rapidity of measuring STC compared with SOC, which needs measurements of both STC and SIC, also makes STC an inexpensive and palatable measurement of C stocks and C sequestration rates in dryland cropping systems (Sainju et al., 2021).

There is a need for long-term studies on the effectiveness of perennial bioenergy crops receiving various N fertilization rates on soil C and N sequestration to a greater depth because most studies are focused on the comparison between perennial and annual crops on these parameters at surface soil layers. Our study examined three perennial bioenergy crops and four N fertilization rates on soil bulk density (BD), STC, and STN at 0–120 cm and compared them with an annual crop from 2009 to 2019 in the US northern Great Plains. We hypothesized that (1) intermediate wheatgrass (IW) with 75 kg N ha^{-1} would enhance STC and STN compared to SB and SG with 0–75 kg N ha^{-1} and (2) perennial bioenergy crops would have greater STC and STN than an annual crop from 2009 to 2019. Our objectives were to evaluate (1) if perennial bioenergy crops and N fertilization rates affect BD, STC, and STN at 0–120 cm from 2009 to 2019, (2) how these parameters vary between perennial bioenergy crops and an annual crop, and (3) which perennial bioenergy crops and N fertilization rates enhance STC and STN.

2 | MATERIALS AND METHODS

2.1 | Experimentation

The study was conducted from 2009 to 2019 in a dryland farm at Froid, MT. The soil at the site was a Williams loam (fine-loamy, mixed, superactive, frigid, Typic Argiustoll) characterized by 660 g kg^{-1} sand, 180 g kg^{-1} silt, 160 g kg^{-1} clay, 10.1 g kg^{-1} SOC, 7.2 pH, and 1.27 Mg m^{-3} BD at the 0–15 cm depth. The mean (30-year average) monthly air temperature at the site is 12°C and average annual precipitation is 341 mm, 80% of which occurs during the perennial bioenergy crop growing season (April to October). The site was cropped with no-till continuous spring wheat for 5 years before the experiment was initiated.

Treatments were three perennial bioenergy crops (IW {*Thinopyrum intermedium* [Host] Barkworth and Dewey, IW}), smooth bromegrass (*Bromus inermis* L., SB), and SG (*Panicum virgatum* L., SG), each receiving four N fertilization rates (0, 28, 56, and 84 kg N ha^{-1}). The IW and SB were cool-season grasses and SG was a warm-season grass, which were adaptable to and grow vigorously in the semiarid condition of the US northern Great Plains (Frank et al., 2004; Sainju et al., 2017). While 84 kg N ha^{-1} is the recommended N fertilization rate for perennial grasses in the region, we wanted to examine how perennial grasses affect STC and STN under reduced N fertilization rates. The annual crop was spring wheat (*Triticum aestivum* L., WH). In late April 2009, IW and SG were seeded at 17 kg ha^{-1} each, SB at 22 kg ha^{-1}, and WH at 71 kg ha^{-1} with a no-till drill at 20 cm spacing. Perennial bioenergy crops were planted only once in 2009, but WH was planted in late April every year. At planting, a one-time application of phosphorus (P) as monoammonium phosphate (11% N, 23% P) at 64 kg P ha^{-1} was broadcast which also supplied N at 31 kg N ha^{-1} to perennial bioenergy crops based on soil testing. No potassium (K) fertilizer was applied because the soil was rich in K concentration. Perennial bioenergy crops received broadcast N fertilizer at designated rates from urea (46% N) in late April every year. At the same time, WH received N, P, and K fertilizers at 80 kg N ha^{-1}, 11 kg P ha^{-1}, and 27 kg ha^{-1} from urea, monoammonium phosphate, and muriate of potash (54% K), respectively, as banded application 5 cm below and 5 cm to the side of seed every year. No irrigation was applied. No herbicides or pesticides were applied to perennial bioenergy crops due to lower infestation, but WH received them as needed. From July to October in each year, aboveground biomass of perennial bioenergy crops was harvested at 5 cm above the ground one to two times per year depending on the growth of aboveground biomass from two 0.5 m^2 areas by hand randomly within the plots and composited. Biomass yield was determined on an oven-dried basis after drying a subsample at 65°C for 3 days. Total biomass yield in a year was determined by adding yields from individual cuttings. For WH, grain yield was determined by harvesting grains with a plot combine on an oven-dried basis after drying a subsample at 65 °C for 7 days from late July to early August each year. After harvesting grains, biomass residue was returned to the soil. The experimental design was a split plot with perennial bioenergy crop as the main-plot and N fertilizer rate as the split-plot treatment arranged in randomized block. Each treatment had four replications. The size of the main plot was 12.2 m × 30.5 m and split plot was 3.1 m × 30.5 m. Because of the lack of N fertilization rates to an annual crop, data for WH were analyzed separately by comparing with perennial bioenergy crops receiving 84 kg N ha^{-1} in a randomized block design.

2.2 | Soil sample collection and analysis

Soil samples were collected from the 0- to 120-cm depth using a truck-mounted hydraulic probe (3.5 cm inside diameter) before planting in April 2009 and after crop harvest in October 2013 and 2019. Samples were collected from four random places within a plot, two between plant rows and two in the rows, separated into 0–15, 15–30, 30–60, 60–90, and 90–120 cm depth intervals, and composited within a depth. Samples were air-dried, ground, and sieved to 2 mm. At the same time, an undisturbed soil core (0–120 cm) was collected

from the middle of the split plot using the hydraulic probe to determine BD. The core was separated into 0–15, 15–30, 30–60, 60–90, and 90–120 cm depth intervals as above and BD was determined by dividing the weight of the oven-dried core at 105°C for 24 h by the volume of the core. Because it was difficult to get intact undisturbed soil core and soils fell apart out of core during sampling, it was decided to obtain one mostly undisturbed soil core from each split plot or four per main plot to measure BD.

The STC and STN concentrations (g C or N kg^{-1}) in soil samples at each depth interval were determined by using a C and N analyzer (Elementar Americas Inc.) after further grinding the soil samples to 0.5 mm. The STC and STN stocks (Mg C or N ha^{-1}) at each depth interval were determined by multiplying STC and STN concentrations by BD and the thickness of the soil layer. The STC and STN stocks at 0–120 cm were calculated by adding stocks from individual depth layers. Because STC related similar to or better than SOC with most soil properties and crop yields (Sainju et al., 2021), we used STC instead of SOC to observe changes in C and N stocks under perennial grasses in the semiarid region. Measurement of STC also saves time and cost of analysis because it requires only one-step of determination compared to SOC which requires two-step processes of measuring STC and SIC (Sainju et al., 2021).

2.3 | Statistical analysis of data

The analysis of repeated measures in the MIXED procedure of SAS (Littell et al., 2006) was used to analyze data for BD and STC and STN stocks at a soil depth after testing for normal distribution of residuals. Perennial bioenergy crop was considered as the main plot and N fertilization rate as the split-plot treatment for data analysis. Fixed effects were perennial bioenergy crop, N fertilization rate, and their interaction; repeated measure variable was year; and random effects were replication and replication × perennial bioenergy crop. Data for WH with 80 kg N ha^{-1} and perennial bioenergy crops with 84 kg N ha^{-1} were analyzed separately as above to compare the parameters between annual and perennial crops. It was assumed that N fertilization rate of 80 kg N ha^{-1} applied to WH and of 84 kg N ha^{-1} applied to perennial bioenergy crops will have similar effects on measured parameters. Fixed effect was perennial bioenergy crop, repeated measure variable was year, and random effect was replication. Means were separated by using the least square means test when treatments and their interactions were significant (Littell et al., 2006). Linear regression analysis was used to determine C and N sequestration rates from the slopes of regression lines of STC and STN stocks, respectively, versus year. Statistical significance was evaluated at $P \leq 0.05$ after adjusting for the threshold values, unless otherwise stated.

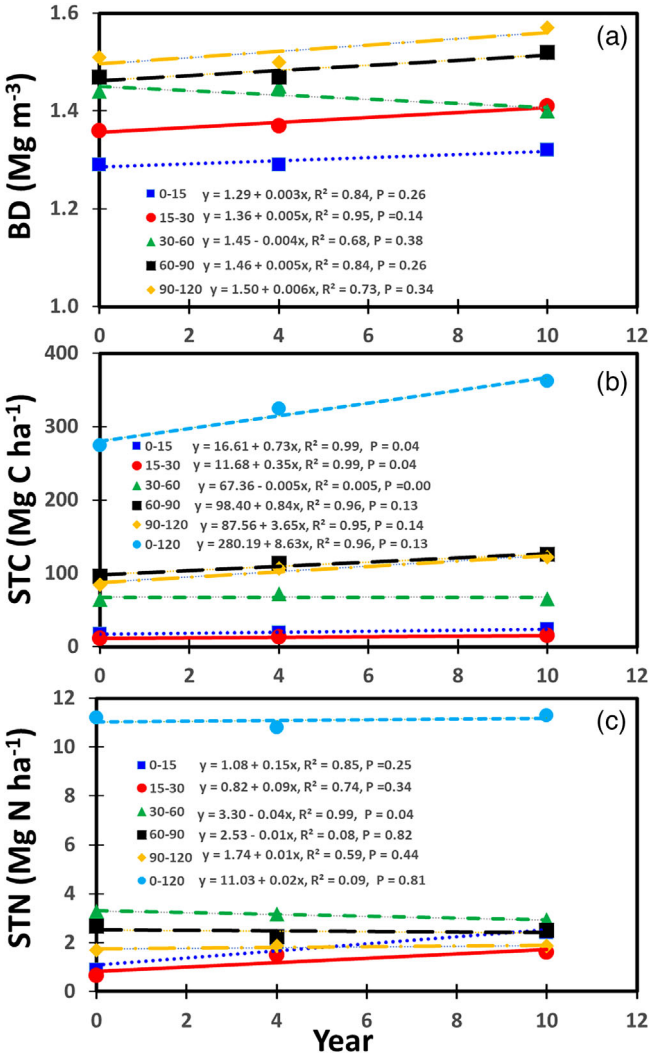


FIGURE 1 Relationships between (a) soil bulk density (BD), (b) soil total carbon (STC), and (c) soil total nitrogen (STN) at 0–15, 15–30, 30–60, 60–90, 90–120, and 0–120 cm depths and year averaged across treatments. Years 0, 4, and 10 represent 2009, 2013, and 2019 respectively.

3 | RESULTS AND DISCUSSION

3.1 | Soil bulk density

The BD was affected by year at all soil depths, with significant interactions for perennial bioenergy crop × N fertilization rate and perennial bioenergy crop × N fertilization rate × year at 0–15 cm (Table 1). The BD at 0–15 cm did not change with treatments in 2009 and 2013 (Table 2). In 2019, BD at 0–15 cm was 7–13% significantly greater for SB with 56 kg N ha^{-1} than other treatments, except for IW and SG with 0 kg N ha^{-1}. Averaged across treatments, BD tended to increase with year at all depths, except at 30–60 cm where it tended to decrease (Figure 1a). When perennial bioenergy crops and an annual crop (WH) were compared, BD was affected by year at

TABLE 1 Analysis of variance for soil bulk density (BD), soil total carbon (STC), and soil total nitrogen (STN) at 0–15, 15–30, 30–60, 60–90, 90–120, and 0–120 cm depths, with sources of variance for perennial bioenergy crop (P), nitrogen fertilization rate (N), and year (Y).

	Soil depth (cm)					
Source	**0–15**	**15–30**	**30–60**	**60–90**	**90–120**	**0–120**
BD[a]			*p*-Values			
P	0.341	0.964	0.591	0.371	0.423	–
N	0.144	0.852	0.951	0.448	0.667	–
P × N	0.045	0.566	0.912	0.554	0.213	–
Y	<0.001	<0.001	<0.001	<0.001	<0.001	–
P × Y	0.182	0.947	0.662	0.055	0.376	–
N × Y	0.100	0.952	0.994	0.503	0.716	–
P × N × Y	0.016	0.639	0.978	0.624	0.171	–
STC						
P	0.791	0.084	0.849	0.693	0.443	0.826
N	0.137	0.326	0.937	0.614	0.671	0.898
P × N	0.304	0.084	0.967	0.944	0.820	0.941
Y	<0.001	<0.001	0.318	<0.001	<0.001	<0.001
P × Y	0.429	<0.001	0.194	0.344	0.098	0.012
N × Y	0.517	0.616	0.624	0.254	0.355	0.667
P × N × Y	0.634	0.554	0.896	0.964	0.722	0.965
STN						
P	0.765	0.239	0.608	0.421	0.828	0.482
N	0.187	0.521	0.378	0.593	0.539	0.369
P × N	0.649	0.318	0.466	0.850	0.639	0.437
Y	<0.001	<0.001	<0.001	<0.001	0.166	0.044
P × Y	0.242	0.022	0.432	0.133	0.881	0.168
N × Y	0.784	0.541	0.647	0.475	0.816	0.508
P × N × Y	0.140	0.639	0.892	0.293	0.368	0.491

[a]BD at 0–120 cm is not available because it cannot be added to individual depth layers.

TABLE 2 Interaction between perennial bioenergy crop, nitrogen (N) fertilization rate, and year on soil bulk density (BD) at the 0–15 cm depth.

		BD at 0–15 cm in various years (Mg m^{-3})		
Perennial bioenergy crop[a]	**N fertilization rate (kg N ha^{-1})**	**2009**	**2013**	**2019**
IW	0	1.29	1.29	1.39ab[b]
	28	1.29	1.29	1.30cd
	56	1.29	1.30	1.32c
	84	1.29	1.30	1.26c
SB	0	1.29	1.30	1.34bc
	28	1.29	1.31	1.34bc
	56	1.29	1.32	1.44a
	84	1.29	1.29	1.31cd
SG	0	1.29	1.31	1.38ab
	28	1.29	1.30	1.28c
	56	1.29	1.32	1.25d
	84	1.29	1.30	1.34bc

[a]Perennial bioenergy crops are IW, intermediate wheatgrass, SB, smooth bromegrass, and SG, switchgrass.

[b]Numbers followed by different letters within a column are significantly different at $p \leq 0.05$ by the least square means test.

TABLE 3 Analysis of variance for the comparison between perennial bioenergy crops and an annual crop for soil bulk density (BD), soil total carbon (STC), and soil total nitrogen (STN) at 0–15, 15–30, 30–60, 60–90, 90–120, and 0–120 cm depths, with sources of variance for crop (C) and year (Y).

	Soil depth (cm)					
Source	**0–15**	**15–30**	**30–60**	**60–90**	**90–120**	**0–120**
BD[a]			*p*-Values			
C	0.199	0.196	0.462	0.066	0.762	–
Y	0.184	<0.001	<0.001	0.139	0.036	–
C × Y	0.170	0.167	0.526	0.036	0.878	–
STC						
C	0.046	0.001	0.844	0.989	0.011	0.199
Y	<0.001	<0.001	0.998	<0.001	0.001	<0.001
C × Y	0.385	0.005	0.928	0.308	0.925	0.258
STN						
C	0.343	0.041	0.485	0.976	0.716	0.601
Y	<0.001	<0.001	<0.001	0.401	0.229	0.171
C × Y	0.597	0.211	0.902	0.798	0.802	0.815

Note: Comparison was made between perennial bioenergy crops receiving N fertilization rate at 84 kg N ha^{-1} and an annual crop (spring wheat) receiving N fertilization rate at 80 kg N ha^{-1}.

[a]BD at 0–120 cm is not available because it cannot be added to individual depth layers.

15–30, 30–60, and 90–120 cm, with a significant interaction for crop × year at 60–90 cm (Table 3). The BD at 60–90 cm tended to increase with year for SG and WH, but tended to decrease for IW and WH (Figure 2a). As a result, BD at 60–90 cm was 7%–8% greater for SG and WH than IW and SB in 2019 (year 10). Averaged across years, BD increased with soil depth for all crops (Table 4).

The nonsignificant difference in BD at 0–15 cm among treatments in 2009 and 2013, but the significant difference in 2019 (Table 2) indicate that BD was most likely affected by root growth and root turnover of perennial bioenergy crops. Initial establishment of perennial bioenergy crops had little impact on BD probably due to limited soil sampling of one core per split plot. The similar values of BD at 0–15 cm among treatments in 2009 was the average values of all plots at the beginning of the experiment. However, continuous root growth and turnover of root biomass from perennial bioenergy crops to soil organic matter for a longer period and soil compaction may have significantly affected BD in 2019. The greater BD at 0–15 cm for SB with 56 kg N ha^{-1} than other treatments in 2019 was likely due to reduced root growth from poor performance of SB and increased soil compaction after three years because SB almost ceased to grow after this period (Sainju et al., 2017). Lower root growth and root biomass production of perennial grasses can enhance BD due to increased soil compaction (Frank et al., 2004; Gebhart et al., 1994). Increased BD with increased soil depth for all crops (Table 4) was also likely a result of reduced root growth and increased soil compaction. However, variable trends of BD with year for various crops (Figures 1a and 2a) indicate that crops affected BD by differently affecting root growth and above- and belowground residue returned to the soil or to random differences due to limited sampling. The reasons for greater BD at 60–90 cm for SG and WH than IW and SB in 2019 (year 10) (Figure 2a) were not known.

3.2 | Soil total carbon

The STC was affected by year at all depths, except at 30–60 cm (Table 1). Significant interactions for STC occurred for perennial bioenergy crop × year at 15–30 and 0–120 cm. Averaged across N fertilization rates, STC at 15–30 cm was 41% greater for IW than SB in 2019 (Figure 3a). The STC at 15–30 cm increased at 0.66 Mg C ha^{-1} $year^{-1}$ for IW and at 0.26 Mg C ha^{-1} $year^{-1}$ for SG. Similarly, STC at 0–120 cm was 14% greater for IW than SG in 2019 (Figure 3b). The STC at 0–120 cm increased at 10.58 Mg C ha^{-1} $year^{-1}$ for IW. The STC at 0–120 cm also tended to increase with year for SB and SG, but the regression coefficients were not significant. Averaged across treatments, STC at 0–15 cm increased at 0.73 Mg C ha^{-1} $year^{-1}$ (Figure 1b). At 15–30 cm, STC increased at 0.35 Mg C ha^{-1} $year^{-1}$. At other depths, STC tended to increase with year, but the regression coefficients were not significant. Averaged across years, STC varied with depth for all perennial bioenergy crops and N fertilization rates (Table 5). Nitrogen fertilization rate did not affect STC at any depth.

When perennial bioenergy crops and an annual crop were compared, STC was affected by crop at 0–15, 15–30, and 90–120 cm and by year at all depths, except at 30–60 cm (Table 3).

TABLE 4 Soil bulk density (BD), soil total carbon (STC), and soil total nitrogen (STN) at 0–15, 15–30, 30–60, 60–90, 90–120, and 0–120 cm depths averaged across years as affected by perennial bioenergy crops and an annual crop.

Crop[a]	Soil depth (cm)					
	0–15	15–30	30–60	60–90	90–120	0–120
			BD[b] (Mg m^{-3})			
IW	1.28	1.39	1.42	1.46	1.54	–
SB	1.30	1.39	1.42	1.46	1.53	–
SG	1.31	1.36	1.44	1.50	1.52	–
WH	1.33	1.41	1.42	1.48	1.56	–
			STC (Mg C ha^{-1})			
IW	20.4a[c]	14.8a	71.8	113.9	103.7a	289.4
SB	19.9a	13.7ab	61.0	114.4	108.0a	313.4
SG	19.4ab	12.4bc	67.0	115.0	94.5ab	276.5
WH	18.0b	11.8c	60.0	112.2	86.2b	258.4
			STN (Mg N ha^{-1})			
IW	1.75	1.35a	3.75	2.47	1.83	9.80
SB	1.84	1.27ab	3.95	2.56	1.89	10.24
SG	1.77	1.19b	3.74	2.49	1.76	9.76
WH	1.68	1.18b	3.59	2.44	2.02	9.73

[a]Perennial bioenergy crops are IW, intermediate wheatgrass, SB, smooth bromegrass, and SG, switchgrass. Annual crop is WH, spring wheat. Perennial bioenergy crops received N fertilization rate at 84 kg N ha^{-1} and an annual crop (spring wheat) received N fertilization rate at 80 kg N ha^{-1}.
[b]BD at 0–120 cm is not available because it cannot added to individual depth layers.
[c]Numbers followed by different letters within a column in a set are significantly different at $p \leq 0.05$ by the least square means test.

TABLE 5 Soil total carbon (STC) and soil total nitrogen (STN) at 0–15, 15–30, 30–60, 60–90, 90–120, and 0–120 cm depths as affected by perennial bioenergy crop and N fertilization rate averaged across years.

Perennial bioenergy crop[a]	N fertilization rate (kg N ha^{-1})	Soil depth (cm)					
		0–15	15–30	30–60	60–90	90–120	0–120
STC (Mg C ha^{-1})							
IW		19.9	14.4	71.4	113.0	104.5	323.2
SB		20.5	12.5	61.3	112.5	107.6	314.7
SG		20.0	13.0	69.3	109.5	101.7	313.5
	0	20.2	12.8	66.9	113.4	104.5	317.8
	28	19.5	13.0	66.1	109.0	105.6	313.2
	56	20.9	13.6	69.8	109.8	106.2	320.3
	84	19.9	13.6	66.6	114.4	102.1	316.6
STN (Mg N ha^{-1})							
IW		1.74	1.30	3.77	2.38	1.76	10.95
SB		1.79	1.23	3.88	2.51	1.83	11.24
SG		1.74	1.18	3.13	2.51	1.83	10.39
	0	1.82	1.25	3.78	2.48	1.76	11.09
	28	1.70	1.22	3.64	2.37	1.90	10.83
	56	1.73	1.20	3.94	2.51	1.75	11.13
	84	1.79	1.27	3.82	2.51	1.83	11.22

[a]Perennial bioenergy crops are IW, intermediate wheatgrass, SB, smooth bromegrass, and SG, switchgrass.

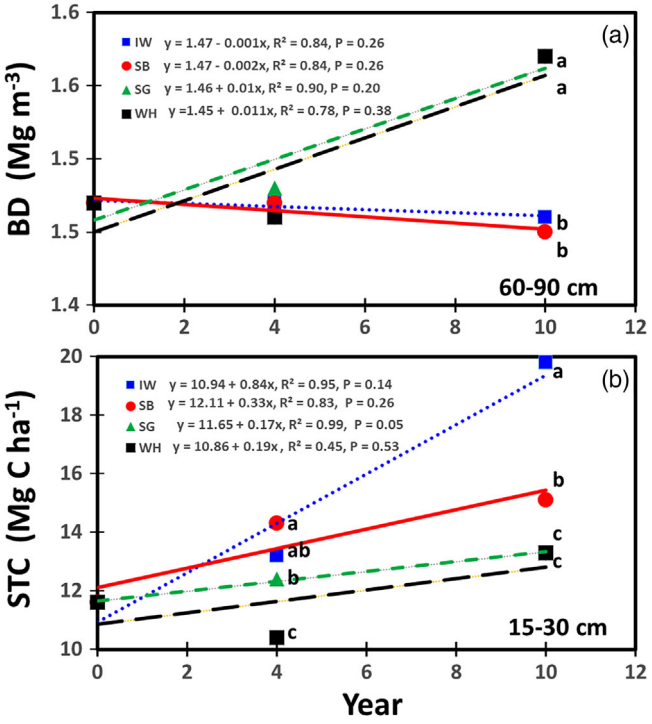


FIGURE 2 Relationships between (a) soil bulk density (BD) at the 0–60 cm depth and (b) soil total C (STC) at the 50–30 cm depth and year when perennial bioenergy crops and an annual crop was compared. Years 0, 4, and 10 represent 2009, 2013, and 2019 respectively. IW, wheatgrass; SB, smooth bromegrass; and SG, switchgrass.

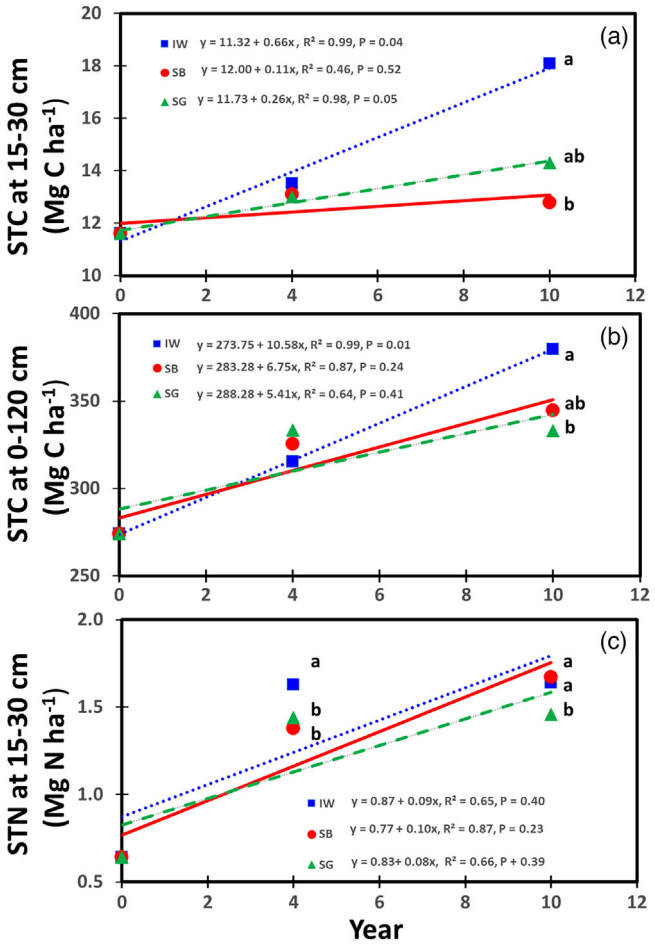


FIGURE 3 Relationships between (a) soil total C (STC) at the 15–30 cm depth, (b) soil total C (STC) at the 0–120 cm depth, and (c) soil total N (STN) at the 15–30 cm depth averaged across N fertilization rates for perennial bioenergy crops. Years 0, 4, and 10 represent 2009, 2013, and 2019 respectively. IW, wheatgrass; SB, smooth bromegrass; SG, switchgrass; WH, spring wheat.

Significant interaction for STC at 15–30 cm occurred for crop × year. The STC at 15–30 cm was 15% –38% greater for SB than SG and WH and 12%–27% greater for IW and SG than WH in 2013 (year 4) (Figure 2b). In 2019 (year 10), STC at 15–30 cm was 31%–49% greater for IW than all crops and 14% greater for SB than SG and WH. The STC at 15–30 cm increased at 0.17 Mg C ha^{-1} $year^{-1}$ for SG. The STC at 15–30 cm also tended to increase with year for other crops, but the regression coefficients were not significant. Averaged across years, STC at 0–15 cm was 11%–13% greater for IW and SB than WH (Table 4). At 15–30 cm, STC was 16%–25% greater for IW and SB than WH. At 90–120 cm, STC was 20%–25% greater for IW and SB than WH.

The increased STC below the 30 cm depth for all treatments (Table 5) was probably due to the presence of large amount of SIC at subsoil layers. While SOC decreases with depth, SIC increases in dryland soils in arid and semiarid regions (Lal, 2004; Sainju et al., 2020). Sainju et al. (2020) from a different experiment in a nearby site reported that SIC contributed from 0% of STC at 0–15 cm to 78% at 60–90 cm. Total STC of 313 to 323 Mg C ha^{-1} to a depth of 120 cm for various treatments observed in our study was, however, greater than 210 Mg C ha^{-1} for the same depth reported by Sainju et al. (2020) in a different experiment. Spatial variability of the deposition of calcite and magnesite that contribute to SIC may have resulted in the variations of STC between our study and that reported by Sainju et al. (2020). The variation in STC by depth was likely due to differences in SOC and SIC concentrations, bulk density, and thickness of the soil layer.

The increased STC with year at all depths, except at 30–60 cm, for all treatments (Figure 1b) suggests that increased C inputs from root residue during continuous growth of perennial bioenergy crops that enhanced SOC and dissolution of CO_2 leading to the accumulation of carbonates and bicarbonates that enhanced SIC likely increased STC over time (Cihacek & Ulmer, 2002; Lal, 2004; Liebig et al., 2005). The greater STC for IW than SB at 15–30 cm (Figure 3a) and greater for IW than SG at 0–120 cm (Figure 3b) in 2019 (year 10) was probably due to increased root growth. Sainju et al. (2017) from the same experiment reported that root biomass to a depth of 120 cm was 21% greater for IW than SB and SG. Our C sequestration rate of 5.4 Mg C ha^{-1} $year^{-1}$ at 0–120 cm was lower than 10.1 Mg C ha^{-1} $year^{-1}$ at 0–90 cm

for SG reported in North Dakota (Frank et al., 2004). Our C sequestration rate of 1.1 Mg C ha^{-1} $year^{-1}$ at 0-30 cm for perennial bioenergy crops (Figure 1b) was slightly greater than 0.8 Mg C ha^{-1} $year^{-1}$ at 0–0.3 m for SG reported in Italy (Zegada-Lizarazu et al., 2022). Differences in soil and climatic conditions may have influenced C sequestration rates of perennial grasses among the regions. North Dakota receives 50–75 mm more precipitation than our site in eastern Montana which may have enhanced the growth of above- and belowground biomass of SG and there may have higher C sequestration rate compared to our site. In contrast, soil and climatic conditions were almost identical between our site and Italy, resulting in nearly similar C sequestration rates for perennial grasses for both sites. Carbon sequestration rates of perennial grasses usually increase with the age of establishment (Ma et al., 2000). Several researchers (Ma et al., 2000; Reeder et al., 1998; Siri-Prieto et al., 2021) reported that N fertilization had little effect on STC under perennial grasses, a case similar to that observed with our findings.

In comparison between perennial bioenergy crops and an annual crop, greater root biomass returned to the soil also may have increased STC at 15–30 cm for SB than SG and WH in 2013 (year 4) and for IW than SB, SG, and WH in 2019 (year 10) (Figure 2b). Root biomass at 15–30 cm was 0.53 Mg ha^{-1} greater for IW than SG and 1.12–1.65 Mg ha^{-1} greater for perennial bioenergy crops than WH (Sainju et al., 2017). This was also true for greater STC at 0–15 and 90–120 cm for IW and SB than WH (Table 4), as root biomass at 0–15 cm was 8.77 to 9.68 Mg ha^{-1} greater and at 90–120 cm was 0.38–0.64 Mg ha^{-1} greater for perennial bioenergy crops than WH (Sainju et al., 2017). Carbon sequestration rate of 0.17 Mg C ha^{-1} $year^{-1}$ at 15–30 cm for SG observed during the comparison between perennial bioenergy crops and an annual crop (Figure 2b) was slightly less than 0.26 Mg C ha^{-1} $year^{-1}$ at 15–30 cm for the same crop when the comparison was not made (Figure 3a). It is likely that lower C sequestration rate for WH may have reduced the sequestration rate for SG during comparison between perennial bioenergy crops and an annual crop versus increased sequestration rate when no comparison was made. Johnson and Barbour (2016) reported that C sequestration under SG occurred only at 0–5 cm even after 10 years in Minnesota due to large spatial variability, but the sequestration under perennial grasses were much greater than under annual crops.

3.3 | Soil total nitrogen

The STN was affected by year at all depths, except at 90–120 cm (Table 1). A significant perennial bioenergy crop × year interaction occurred for STN at 15–30 cm. Averaged across N fertilization rates, STN at 15–30 cm was 13%–18% greater for IW than SB and SG in 2013 (year 4) and 12%–14% greater for IW and SB than SG in 2019 (year 10) (Figure 3c). The STN tended to increase with year for perennial bioenergy crops, but regression coefficients were not significant. Averaged across treatments, STN at 30–60 cm decreased by 0.04 Mg N ha^{-1} $year^{-1}$ (Figure 1c). Averaged across years, STN also varied with depth for all treatments (Table 5). Similar to STC, N fertilization rate had no effect on STN.

In the comparison between perennial bioenergy crops and an annual crop, STN was affected by crop at 15–30 cm and by year at 0–15, 15–30, and 30–60 cm (Table 3). Averaged across years, STN at 15–30 cm was 13%–14% greater for IW than SG and WH (Table 4). Averaged across treatments, STN at 0–15, 15–30, and 30–60 cm also tended to increase with year, but their regression coefficients were not significant (data not shown).

In contrast to STC, variable STN with depth for all treatments (Table 5) was likely due to decreased STN concentration, but increased thickness of the depth layer as well as greater BD with depth. The STN concentration decreases with depth due to reduced root growth (Al-Kaisi et al., 2005; Omonode & Vyn, 2006). While decreased STN from 0–15 to 15–30 cm was probably due to reduced STN concentration, increased STN from 15–30 to 30–60 cm was due to increased thickness of the soil layer. Decreased STN concentration, however, resulted in reduced STN from 30–60 to 90–120 cm. The reduced STN at 30–60 cm with year for all perennial bioenergy crops (Figure 1c) was likely due to increased N removal by aboveground biomass because perennial bioenergy crops remove more N in aboveground biomass than N applied from the fertilizer (Johnson & Barbour, 2016; McGowan et al., 2019). Greater STN at 15–30 cm for IW than SB and SG in 2013 (year 4) and greater for IW and SB than SG in 2019 (year 10) (Figure 3c) were likely due increased root growth, a case similar to that observed for STC. Because C and N concentrations in the root of nonlegumes are related to each other (Sainju et al., 2017), we assumed that increased root residue from IW will also increase N input and therefore greater STN for IW than SB or SG. Our nonsignificant effect of N fertilization rate on STN under perennial grasses was similar to those reported by some researchers (Ma et al., 2000; Reeder et al., 1998; Siri-Prieto et al., 2021).

A similar result of greater STN at 15–30 for IW than SG and WH was also observed when perennial bioenergy crops and an annual crop was compared, possibly resulting from increased N input due to enhanced root growth of perennial grasses (Table 4). Several researchers (Al-Kaisi et al., 2005; Zegada-Lizarazu et al., 2022) also reported that perennial bioenergy crops increased STN compared to annual crops. The patterns of STN at 0–5, 15–30, and 30–60 cm with year (Table 3) when perennial bioenergy crops were compared with an annual crop followed the same trends as when the comparison was not made (data not shown).

4 | CONCLUSIONS

Perennial bioenergy crops affected variably in STC and STN. Carbon sequestration rate was greater for IW than SB and SG both at the surface layer and for the whole soil profile due to increased STC stock after 10 years. The STC for all perennial bioenergy crops either increased or tended to increase with year all depths, except at 30–60 cm. The IW and SB have higher STC stocks and C sequestration rates than WH. The STN also followed trends similar to STC, but STN was less impacted by perennial bioenergy crops than STC. Nitrogen fertilization rate had no effect on STC and STN. The IW can sequester and store more C than SB and SG in the whole soil profile and perennial bioenergy crops can store more C and N than an annual crop (WH) during the long-term cultivation of perennial bioenergy crops in the semiarid dryland condition of the US northern Great Plains.

AUTHOR CONTRIBUTIONS

Upendra M. Sainju: Conceptualization; data curation; formal analysis; investigation; methodology; writing—original draft; writing—review and editing. **Brett L. Allen**: Investigation; methodology; project administration; supervision; writing—review and editing. **Andrew W. Lenssen**: Conceptualization; investigation; methodology; project administration; resources; supervision; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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