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Root and soil total carbon and nitrogen under bioenergy perennial grasses with various nitrogen rates

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Abstract

As aboveground biomass of perennial grasses is harvested for feedstock or bioenergy production, root biomass C and N become primary C and N inputs for enhancing soil C and N sequestration. Information is scanty about root biomass C and N and subsequent soil C and N stocks under bioenergy perennial grasses applied with various N fertilization rates in semiarid regions. We evaluated the effect of perennial grass species and N rates on root biomass C and N and soil total C (STC) and total N (STN) stocks at the 0–120 cm depth from 2011 to 2013, 2–4 yr after grass establishment, in the northern Great Plains, USA. Perennial grasses were intermediate wheatgrass (*Thinopyrum intermedium* [Host] Barkworth and Dewey), smooth bromegrass (*Bromus inermis* L.), and switchgrass (*Panicum* virgatumL.), and N fertilization rates were 0, 28, 56, and 84 kg N ha−1. Root biomass C and N at 0–15 and 0–120 cm were greater with intermediate wheatgrass and switchgrass than smooth bromegrass in 2011, but the trend reversed for root biomass C at 0–15 cm in 2012. Root biomass C at both depths among N rates and years. The STC at 0–15, 30–60, and 0–120 cm also varied among grass species and years. At 30–60 cm, STC increased with increased N rates under intermediate wheatgrass and switchgrass, but decreased under smooth bromegrass. At 60–90 cm, the trend for STC reversed. The STN at 15–30 cm was greater under intermediate wheatgrass than smooth bromegrass and switchgrass and at most depths was greater in 2012 than 2013. Overall root biomass C and N at 0–120 cm were 12–16 times greater and STC and STN 8–9% greater under perennial grasses than adjacent annual spring wheat. Although intermediate wheatgrass returned more root C and N to the soil than other grasses, both STC and STN varied among grass species and N rates. Increased root C and N inputs, however, increased STC and STN under perennial grasses compared with annual spring wheat.

Keywords

Bioenergy grasses, Nitrogen fertilization, Root carbon, Root nitrogen, Soil carbon, Soil nitrogen

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Research paper

Root and soil total carbon and nitrogen under bioenergy perennial grasses with various nitrogen rates

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ABSTRACT

As aboveground biomass of perennial grasses is harvested for feedstock or bioenergy production, root biomass C and N become primary C and N inputs for enhancing soil C and N sequestration. Information is scanty about root biomass C and N and subsequent soil C and N stocks under bioenergy perennial grasses applied with various N fertilization rates in semiarid regions. We evaluated the effect of perennial grass species and N rates on root biomass C and N and soil total C (STC) and total N (STN) stocks at the 0–120 cm depth from 2011 to 2013, 2–4 yr after grass establishment, in the northern Great Plains, USA. Perennial grasses were intermediate wheatgrass (*Thinopyrum intermedium* [Host] Barkworth and Dewey), smooth bromegrass (*Bromus inermis* L.), and switchgrass (*Panicum virgatum* L.), and N fertilization rates were 0, 28, 56, and 84 kg N ha^{-1}. Root biomass C and N at 0–15 and 0–120 cm were greater with intermediate wheatgrass and switchgrass than smooth bromegrass in 2011, but the trend reversed for root biomass C at 0–15 cm in 2012. Root biomass C at both depths among N rates and years. The STC at 0–15, 30–60, and 0–120 cm also varied among grass species and years. At 30–60 cm, STC increased with increased N rates under intermediate wheatgrass and switchgrass, but decreased under smooth bromegrass. At 60–90 cm, the trend for STC reversed. The STN at 15–30 cm was greater under intermediate wheatgrass than smooth bromegrass and switchgrass and at most depths was greater in 2012 than 2013. Overall root biomass C and N at 0–120 cm were 12–16 times greater and STC and STN 8–9% greater under perennial grasses than adjacent annual spring wheat. Although intermediate wheatgrass returned more root C and N to the soil than other grasses, both STC and STN varied among grass species and N rates. Increased root C and N inputs, however, increased STC and STN under perennial grasses compared with annual spring wheat.

1. Introduction

Increasing attention is being paid to the production of bioenergy perennial grasses with ligno-cellulosic feedstock materials used as alternative sources of energy [1,2]. Aboveground biomass of grasses can be used either to produce ethanol or generate electricity [3]. Bioenergy from grass biomass can meet 14% of the total energy globally and 4% in US [4], with a potential to supply up to 20% due to increased availability of marginal lands for growing grasses [5]. There are numerous environmental and economic benefits of perennial grasses for producing bioenergy compared with food crops, such as corn (*Zea mays* L.). These benefits are (1) reduced pressure for using food crops for bioenergy production, (2) decreased amounts of chemicals, such as fertilizers, herbicides, and pesticides applied to crops, (3) high biomass production, (4) easily grown on marginal lands, (5) substantially reduced net greenhouse gas emissions, (6) reduced wind and water erosion, (7) reduced airborne particulate organic matter, (8) increased water infiltration and water holding capacity of the soil, (9) increased biodiversity, and (10) increased net economic returns [1,5–8]. When used as animal feed, increased biomass production can substantially reduce feed cost [9]. Removal of aboveground biomass for bioenergy, however, can adversely affect soil and environmental quality [10]. Therefore, improved management practices, such as ideal grass species and N fertilization rates, are needed to enhance soil and environmental quality while sustaining aboveground biomass yield.

Grassland covers 26% of world's land area and 70% of world's agricultural area [11]. Grassland contains 20% of world's soil C stocks (343 billion ton), nearly 50% more than in the forest [12]. About 16% of grassland is under severe degradation due to overgrazing and conversion to other land uses [13]. Conversion of grassland to other land

Abbreviation: IW, intermediate wheatgrass; SB, smooth bromegrass; SG, switchgrass; STC, soil total C; STN, soil total N; SW, spring wheat

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uses can result in up to 60% of soil organic C loss [10]. Management practices, such as planting proper grass species that are adapted to local soil and climatic conditions, resistant to drought, and able to enhance soil fertility e.g. mixtures of legumes and nonlegumes, fertilization, irrigation, and rotational grazing, can increase C input and soil C stocks [6,14]. Globally, about 0.2–0.8 Gt of C yr^{-1} of atmospheric C can be sequestered in grassland soils by 2030 [15]. About 270 million ha^{-1} of world's degraded land can be planted with grasses to sequester C [16].

One of the main benefits of planting perennial grasses is to sequester C and N in the soil, thereby reducing C pollution in the atmosphere, enhancing nutrient cycling, and reducing N leaching [2,17,18]. Perennial grasses have greater root biomass, some of which can extend to > 3 m depth, thereby allocating more C and N to deeper soil layers than annual cereal crops [19,20]. Switchgrass root C input ranged from 1.7 to 2.2 Mg C ha^{-1} at 0–60 cm [21] to as much as 3.5 Mg C ha^{-1} at 0–100 cm [22]. Relatively undisturbed soil condition that reduces C and N mineralization, extensive root system, year-round nutrient cycling, greater adaptability, and higher drought tolerance in the perennial grass system favor increased C and N sequestration in the soil profile compared with annual crops [2,17,18]. Warm-season grasses, such as switchgrass, can sequester C at 0.3–0.5 Mg C ha^{-1} yr^{-1} to a depth of 30 cm [23,24]. Because of reduced mineralization of soil organic matter, deep accumulation of C and N in the soil profile is beneficial for increased C and N sequestration [25,26]. The performance of these grasses, however, depends on soil and climatic conditions, topography, harvest frequency, cutting height, management practices, and grass species [27]. Additional benefits of C sequestration include improved soil health and quality, increased crop yields, and additional income from C credit markets [28].

The potential for C and N sequestration by perennial grasses also depends on their age of stand establishment. Omenode and Vyn [26] reported that warm-season grasses sequestered C at 2.1 Mg C ha^{-1} yr^{-1} and N at 14 kg N ha^{-1} yr^{-1} to a depth of 60 cm after 6–8 yr in Indiana. Al-Kaisi et al. [29] observed that C sequestration rate of switchgrass at 0–15 cm was 1.2 Mg C ha^{-1} yr^{-1} after 10 yr in Iowa. In eastern Canada, Zan et al. [22] found that switchgrass sequestered C at 3.5 Mg C ha^{-1} yr^{-1} at 0–60 cm after 4 yr. Ma et al. [20] reported that switchgrass did not increase soil organic C (SOC) after 2–3 yr of establishment, but SOC at 0–30 cm was 28–45% greater under switchgrass than under fallow after 10 yr in Alabama. Smooth bromegrass increased SOC at 0–15 cm by 17% compared with corn (*Zea mays* L.)-soybean (*Glycine* max L.) rotation after 5 yr in Ohio [28]. Bioenergy perennial grasses have the potential to sequester about 318 Tg C in USA and 1631 Tg C worldwide [5]. Perennial grasses also increased soil total N (STN) compared with corn-soybean rotation [29,30]. Liebig et al. [25], working on data from 42 different locations in Minnesota, North Dakota, and South Dakota, concluded that SOC at 30–60 and 60–90 cm was 4.3–7.7 Mg C ha^{-1} greater under switchgrass than croplands. Warm-season (C4) grasses can increase SOC, but not STN compared with cool-season (C3) grasses [31].

Nitrogen fertilization can variably influence SOC and STN under perennial grasses. Several researchers [20,32] found that N fertilization did not influence SOC at 0–30 cm under switchgrass after 2–3 yr or under perennial grasses after 5 yr in Alabama and Colorado. In contrast, Rice et al. [33] reported that N fertilization to cool-season grasses increased C sequestration rate at 0–30 cm by 1.6 Mg C ha^{-1} yr^{-1} compared with no N fertilization after 5 yr in Kansas. In Alberta, Canada, Bremer et al. [34] observed that N fertilization to perennial grasses increased C sequestration rate at 0–5 cm by 0.5 Mg C ha^{-1} yr^{-1} compared with no N fertilization after 6–12 yr. In South Dakota, Lee et al. [35] noted C sequestration rate of 2.4 Mg C ha^{-1} yr^{-1} at 0–90 cm under switchgrass after 4 yr.

Soil total C (STC) is composed of SOC and soil inorganic C (SIC). As SOC decreases with increased soil depth, SIC increases and becomes a large proportion of STC, especially in subsoil layers under dryland cropping systems in arid and semiarid regions [36,37]. Drylands occupy 47% of the earth's total surface [38] where potential for C sequestration is substantial [39]. As management practices can influence STC, SOC, and SIC [36,36,40], using STC for measurement of total soil C sequestration instead of SOC due to management practices can substantially reduce the cost of analyzing soil samples, especially in arid and semiarid regions, because it eliminates the analysis of SIC [37]. Bronson et al. [18] found that STC and STN were 11.0 Mg C ha^{-1} and 0.6 Mg N ha^{-1}, respectively, greater in native rangeland than cropland soils in the southern Great Plains. Similarly, Liebig et al. [25] reported that STC at 0–5 and 30–60 cm was greater under switchgrass than croplands at most of the 42 sites in arid and semiarid regions of the northern Great Plains.

Most studies on soil C and N stocks under perennial grasses were compared with those under annual cereal crops. Relatively, few studies exist on the effect of grass species and N fertilization rates on root biomass C and N and STC and STN. We evaluated root biomass C and N and STC and STN stocks under three bioenergy perennial grasses (intermediate wheatgrass, smooth bromegrass, and switchgass), each applied with four N fertilization rates (0, 28, 56, and 84 kg N ha^{-1}) from 2011 to 2013 in eastern Montana. We also compared these parameters under adjacent annual spring wheat applied with recommended N rate in 2013. Our objectives were to: (1) quantify root biomass C and N inputs of perennial grasses with different N rates and their effects on STC and STN after 2–4 yr of grass establishment, (2) compare root biomass C and N, STC, and STN under perennial grasses and annual spring wheat, and (3) determine grass species and N rates that enhance soil C and N sequestration. We hypothesized that perennial grass species and N fertilization rates will variably affect STC and STN stocks due to differences in root C and N inputs and that STC and STN will be greater under perennial grasses than under annual spring wheat.

2. Materials and methods

2.1. Experimental site, treatments, and management

The experiment was conducted on 5% sloping land from 2009 to 2013 at the USDA Conservation District Farm, 11 km north of Culbertson, Montana. At the initiation of the experiment in April 2009, the soil, a Williams loam (fine-loamy, mixed, superactive, frigid, Typic Argiustoll), had 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} bulk density at the 0–15 cm depth. At the experimental site, mean (115-yr average) monthly air temperature ranges from -8 °C in January to 23 °C in July and August. Mean annual precipitation is 341 mm, 80% of which occurs during the growing season (April to October). The cropping history (10 yr) at the site before the experiment initiation was continuous spring wheat under conventional tillage.

Treatments included two cool-season grasses (intermediate wheatgrass and smooth bromegrass) and one warm-season grass (switchgrass), each applied with four N fertilization rates (0, 28, 56, and 84 kg N ha^{-1}). Grasses were established in April 2009 when monoammonium phosphate (11% N, 23% P) was broadcast at 280 kg ha^{1} which supplied N at 31 kg N ha^{-1} and P at 64 kg P ha^{-1}. Nitrogen fertilizer as urea (46% N) was broadcast at the soil surface at 0–84 kg N ha^{-1} in April 2011–2013. Perennial grass as the main plot treatment and N fertilization rate as the split-plot treatment were arranged in a randomized complete block design with four replications. For this study, only three replications were chosen. The size of the main plot was 12.3 m × 30.5 m and the split plot was 3.1 m × 30.5 m.

After applying fertilizers, plots were tilled using conventional tillage with a field cultivator to a depth of 7–8 cm for seedbed preparation and weed control in April 2009. Following tillage, intermediate wheatgrass, smooth bromegrass, and switchgrass were planted at 17, 24, and 17 kg ha^{-1}, respectively, at 20 cm spacing using a no-till drill. No K fertilizer was applied because the soil test showed high K content. Similarly, no irrigation was applied. In July and October 2009–2013, aboveground biomass was harvested by hand at 5 cm above the ground from two 0.5 m^2 areas randomly within each plot and composited. Biomass yield was determined at oven-dried basis by weighing the biomass and oven drying a subsample at 65 °C for 3 d. Total biomass yield in a year was determined by adding yields from individual cuttings.

For comparing root and soil parameters under perennial grasses and annual cereal crop, spring wheat was planted in a nearby area immediately outside grass plots in April 2013. Under no-tillage, wheat was planted at 71 kg ha^{-1} with a no-till drill as above in three plots (plot size, 3.1 m × 30.5 m) as three replications. Nitrogen fertilizer as urea and monoammonium phosphate at 100 kg N ha^{-1}, P fertilizer as monoammonium phosphate at 29 kg P ha^{-1}, and K fertilizer as muriate of potash (52% K) at 47 kg K ha^{-1} were banded 5 cm to the side and 5 cm below the seed at planting. Herbicides and pesticides were applied as needed before and during wheat growth. In August 2013, spring wheat was harvested from two 0.5 m^2 areas by hand randomly within each plot as above, separated into grain and vegetative biomass (stems and leaves), and oven dried at 60 °C for 3 d. From these, grain and biomass yields were determined on oven-dried basis. After harvesting grain from the rest of the plot using a combine harvester, wheat residue (stems and leaves) was returned to the soil.

2.2. Root and soil sampling and analysis

In October 2011–2013, soil samples containing roots were collected from the 0–120 cm depth using a truck-mounted hydraulic probe (5 cm inside diameter) after final grass biomass harvest [41]. From four random locations within each plot, soil samples were collected from two places between grass rows and two in the row where one sample was collected between grasses and the other above the root crown. These were separated into 0–15, 15–30, 30–60, 60–90, and 90–120 cm depth increments, placed in plastic bags, and stored at 4 °C until roots were separated from the soil. At the same time, a separate undisturbed soil core from 0 to 120 cm was collected using the hydraulic probe as above for determining the soil bulk density. The soil core was separated into 0–15, 15–30, 30–60, 60–90, and 90–120 cm depth increments, oven dried at 110 °C for 24 h, and weighed, from which the bulk density was calculated by dividing the weight of the oven-dried soil by the volume of the core.

In the laboratory, about 50 g of root-free soil sample from each depth was collected before storage for determination of STC and STN concentrations, composited by depth, air dried, and sieved to 2 mm. The STC and STN concentrations (g C or N kg^{-1}) in soil samples were determined by further grinding a subsample to 0.5 mm and igniting it in a high induction furnace C and N analyzer (Elementar Americas Inc., Mt. Laurel, NJ). The process was repeated for soil samples collected in October 2013 under spring wheat. The STC and STN stocks (Mg C or N ha^{-1}) were determined by multiplying STC and STN concentrations by the bulk density and the thickness of the soil layer. As soil was not disturbed during the experimental period, except for tilling the plots for grass planting in 2009, STC and STN stocks were not corrected for the equivalent soil mass. Because bulk density did not differ among treatments and years, average bulk density values of 1.29, 1.36, 1.44, 1.47, and 1.51 Mg m^{-3}, averaged across treatments and years, at 0–15, 15–30, 30–60, 60–90, and 90–120 cm, respectively, were used for calculations of STC and STN stocks. The STC and STN stocks at 0–120 cm were calculated by adding stocks from individual depths.

For root separation, soil samples were washed thoroughly with water using a hydropneumatic elutriator containing a 0.5-mm screen for several hours until all silt and clay particles were removed [42]. After transferring roots and sand particles from the screen to a container, coarse and fine live roots were hand-picked using a forceps. Roots that could not be picked by hand were separated by immersing the sand and root particles in a 2.2 mol L^{-1} NaCl solution where floated roots were picked using a forceps. Roots from four locations within each plot were composited by depth, oven-dried at 65 °C for 7 d, and weighed to determine root biomass yield. Roots were ground to 1 mm and C and N concentrations (g C or N kg^{-1}) in root samples were determined by using the C and N analyzer as above. Root biomass C and N contents (Mg C or N ha^{-1}) were calculated by multiplying root biomass yield by C and N concentrations. Root biomass C and N contents at 0–120 cm were determined by adding C and N contents from individual depths.

2.3. Data analysis

Data for root biomass C and N contents and STC and STN stocks at a depth were analyzed using the MIXED procedure of SAS after testing for homogeneity of variance [43]. Grass, N fertilization rate, year, and their interactions were considered as fixed effects, and replication and replication × grass as random effects. Means were separated by using the least square means test when treatments and their interactions were significant [43]. Linear regression analysis was used to determine changes in root biomass C and N as well as STC and STN stocks for grass species with N rates and years. Statistical significance was evaluated at $P \leq 0.05$, unless otherwise stated. Because of non-randomization of spring wheat plots with grass plots and incomplete year data (collected only in 2013), data for spring wheat could not be used for statistical analysis, but were shown only for comparison with perennial grasses.

3. Results

3.1. Root biomass carbon

Data on root biomass yield have been presented in a separate manuscript. Therefore, only results on root biomass C and N are presented for this study. Root biomass C varied among grass species and years at 0–15, 15–30, 30–60, and 0–120 cm (Table 1). Interactions were significant for grass × year and N rate × year at 0–15 and 0–120 cm.

Root biomass C at 0–15 cm, averaged across N fertilization rates, was greater in intermediate wheatgrass and switchgrass than smooth bromegrass in 2011, but was greater in smooth bromegrass than intermediate wheatgrass in 2012 (Table 2). Root biomass C was greater in 2011 than 2012 in intermediate wheatgrass and switchgrass, but the trend reversed in smooth bromegrass. At 0–120 cm, root biomass C was similarly greater in intermediate wheatgrass and switchgrass than smooth bromegrass in 2011. Root biomass C was greater in 2011 than 2012 in intermediate wheatgrass, but was greater in 2013 than 2011 in smooth bromegrass. Averaged across grass species, root biomass C at 0–15 cm was greater with 56 kg N ha^{-1} than other N rates in 2011. At 0–120 cm, root biomass C was greater with 0 than 84 kg N ha^{-1} in 2011, but the trend reversed in 2013. Averaged across N rates and years, root biomass C was greater in intermediate wheatgrass than smooth bromegrass at 0–15, 15–30, and 0–120 cm, but was greater in smooth bromegrass than switchgrass at 30–60 cm (Table 1). Averaged across treatments, root biomass C was greater in 2011 than 2012 at 0–15 cm, but was greater in 2013 than 2011 or 2012 at 15–30, 30–60, and 0–120 cm.

3.2. Root biomass nitrogen

Root biomass N varied with grass species at 15–30 and 30–60 cm and with year at 0–15, 15–30, 30–60, and 0–120 cm (Table 3). The grass × year interaction was significant at 0–15 and 0–120 cm. Averaged across N fertilization rates, root biomass N at 0–15 and 0–120 cm was greater in intermediate wheatgrass than other grasses in 2011 (Table 2). At both depths, root biomass N declined from 2011 to 2013 in intermediate wheatgrass and switchgrass. Averaged across N rates and years, root biomass N was greater in intermediate wheatgrass than other grasses at 15–30 cm, but was greater in smooth bromegrass than switchgrass at 30–60 cm (Table 3). Averaged across treatments, root

Table 1
Perennial grass root biomass C at the 0–120 cm depth averaged across N fertilization rates as affected by grass species and year.

Grass species[a]	Year	Root biomass C (Mg C ha^{-1})					
		0-15 cm	15-30 cm	30-60 cm	60-90 cm	90-120 cm	0-120 cm
IW		3.28a[b]	0.67a	0.87ab	0.57	0.34	5.73a
SB		2.42b	0.54b	1.08a	0.52	0.19	4.75b
SG		3.45a	0.52b	0.68b	0.49	0.22	5.36ab
SW[c]		0.21	0.04	0.09	0.08	0.02	0.44
	2011	3.44a	0.44b	0.71b	0.50	0.28	5.37ab
	2012	2.76b	0.62a	0.75b	0.47	0.25	4.85b
	2013	2.93ab	0.66a	1.17a	0.59	0.21	5.56a
Significance							
Grass (G)		*	*	*	NS	NS	*
N fertilization rate (N)		NS	NS	NS	NS	NS	NS
G × N		NS	NS	NS	NS	NS	NS
Year (Y)		*	***	**	NS	NS	*
G × Y		*	NS	NS	NS	NS	**
N × Y		*	NS	NS	NS	NS	*
G × N × Y		NS	NS	NS	NS	NS	NS

*Significant at $P = 0.05$.
**Significant at $P = 0.01$.
***Significant at $P = 0.001$; NS, not significant.
[a] Perennial grasses are IW, intermediate wheatgrass; SB, smooth bromegrass; and SG, switchgrass.
[b] Numbers followed by different letters within a column in a set are significantly different at $P = 0.05$ by the least square means test.
[c] SW is annual spring wheat. Root biomass C for spring wheat was determined in 2013 and is used for comparison only with perennial grasses, but not for data analysis.

biomass N was greater in 2011 than 2012 and 2013 at 0–15 and 0–120 cm, but was greater in 2012 than 2011 and 2013 at 15–30 cm, and greater in 2013 than 2011 at 30–60 cm. Overall root biomass N at various soil depths was 7–22 times greater in perennial grasses than annual spring wheat.

3.3. Soil total carbon

Differences in root biomass C inputs resulted in variations in STC among grass species at 90–120 cm and years at 0–15, 30–60, and 90–120 cm (Table 4). Significant interactions occurred for grass × N rate at 30–60 and 60–90 and grass × year at 0–15, 30–60, and 0–120 cm.

Averaged across years, STC at 30–60 cm was greater under intermediate wheatgrass and switchgrass than smooth bromegrass with 28 kg N ha^{-1} and greater under intermediate wheatgrass than smooth bromegrass and switchgrass with 84 kg N ha^{-1} (Fig. 1). There was significant ($P \leq 0.10$) linear response of STC with N fertilization rate for smooth bromegrass where STC at 30–60 cm increased by 0.24 Mg C ha^{-1} with an increase in N rate by 1 kg N ha^{-1}. At 60–90 cm, STC was greater under smooth bromegrass than switchgrass with 0 kg N ha^{-1}, greater under intermediate wheatgrass than switchgrass with 28 kg N ha^{-1}, and greater under switchgrass than intermediate wheatgrass with 84 kg N ha^{-1}. Significant linear

Table 2
Interaction between grass species, N fertilization rate, and year on perennial grass root biomass C and N at 0–15 and 0–120 cm depths.

Grass species[a]	N fertilization rate (kg N ha^{-1})	Root biomass C (Mg C ha^{-1})					
		0-15 cm			0-120 cm		
		2011	2012	2013	2011	2012	2013
IW		4.21a[b]A[c]	2.67bB	2.96B	6.27aA	4.89B	5.41AB
SB		1.92bB	3.71aA	2.64AB	3.44bB	4.81AB	5.37A
SG		4.17aA	2.92abB	3.27AB	5.74a	4.65	4.88
		0-15 cm			0-120 cm		
	0	3.21b	2.57	2.86	6.45aA	4.62B	4.05bB
	28	2.72b	3.24	3.24	5.44ab	4.80	5.39ab
	56	4.54aA	2.55B	2.51B	5.79ab	4.37	5.38ab
	84	3.19b	2.69	3.22	4.41b	4.36	5.53a
		Root biomass N (kg N ha^{-1})					
		0-15 cm			0-120 cm		
IW		151.8aA	95.5B	72.9B	198.2aA	149.4B	117.9C
SB		76.6c	92.3	79.6	112.1b	142.9	135.8
SG		117.8bA	86.3AB	70.7B	147.8bA	126.6AB	98.8B

[a] Perennial grasses are IW, intermediate wheatgrass; SB, smooth bromegrass; and SG, switchgrass.
[b] Numbers followed by different lowercase letters within a column (year) between grasses in a depth are significantly different at $P = 0.05$ by the least square means test.
[c] Numbers followed by different uppercase letters within a row (grass) between years in a depth are significantly different at $P = 0.05$ by the least square means test.

Table 3
Perennial grass root biomass N at the 0–120 cm depth averaged across N fertilization rates as affected by grass species and year.

Grass species[a]	Year	Root biomass N (kg N ha^{-1})					
		0-15 cm	15-30 cm	30-60 cm	60-90 cm	90-120 cm	0-120 cm
IW		106.7	14.0a[b]	18.5ab	12.7	8.5	160.4
SB		82.5	10.6b	24.6a	12.4	5.9	136.0
SG		91.6	10.6b	13.1b	9.2	5.0	129.5
SW[c]		4.2	0.9	1.8	1.6	0.4	8.9
	2011	115.1a	9.2c	15.4b	11.5	7.2	158.4a
	2012	91.3b	14.8a	17.6ab	11.3	6.5	141.5b
	2013	74.4b	11.3b	23.3a	11.4	3.4	123.8c
Significance							
Grass (G)		NS	*	*	NS	NS	NS
N fertilization rate (N)		NS	NS	NS	NS	NS	NS
G × N		NS	NS	NS	NS	NS	NS
Year (Y)		***	***	*	NS	NS	*
G × Y		*	NS	NS	NS	NS	*
N × Y		NS	NS	NS	NS	NS	NS
G × N × Y		NS	NS	NS	NS	NS	NS

*Significant at $P = 0.05$.
***Significant at $P = 0.001$; NS, not significant.
[a] Perennial grasses are IW, intermediate wheatgrass; SB, smooth bromegrass; and SG, switchgrass.
[b] Numbers followed by different letters within a column in a set are significantly different at $P = 0.05$ by the least square means test.
[c] SW is annual spring wheat. Root biomass N for spring wheat was determined in 2013 and is used for comparison only with perennial grasses, but not for data analysis.

responses of STC with N rate occurred for smooth bromegrass and switchgrass. An increase in N rate by 1 kg N ha^{-1} increased STC at 60–90 cm by 0.37 Mg C ha^{-1} for switchgrass, but decreased by 0.02 Mg C ha^{-1} for smooth bromegrass.

Averaged across N rates, STC at 0–15 cm was greater under switchgrass than smooth bromegrass in 2011, but was greater under intermediate wheatgrass than switchgrass in 2012, and greater under smooth bromegrass than intermediate wheatgrass in 2013 (Table 5). At 30–60 cm, STC was greater under intermediate wheatgrass than other grasses in 2012, but was greater under switchgrass than smooth bromegrass in 2013. At 0–120 cm, STC was greater under intermediate wheatgrass than switchgrass in 2012. Changes in STC at 0–15, 30–60, and 0–120 cm with years varied among grasses. Considering STC under nearby annual spring as the baseline value, significant ($P < 0.10$) linear trend in STC at 0–15 cm with year was found only for smooth bromegrass where STC increased at 0.80 Mg C ha^{-1} yr^{-1}. Averaged across N rates and years, STC at 90–120 cm was greater under smooth bromegrass than other grasses (Table 4). Overall STC at various depths were 3–31% greater under perennial grasses than annual spring wheat.

3.4. Soil total nitrogen

Differences in root biomass N among treatments resulted in significant variations in STN among perennial grasses at 15–30 cm and among years at 15–30, 30–60, 90–120, and 0–120 cm (Table 6). Averaged across N fertilization rates and years, STN at 15–30 cm was greater under intermediate wheatgrass than smooth bromegrass and switchgrass. Averaged across treatments, STN at 15–30 and 30–60 cm was greater in 2012 than 2011 and 2013. At 90–120 and 0–120 cm, STN was greater in 2012 than 2013. There was no significant trend of STN with year at all depths. Overall STN at various depths was 4–19% greater under perennial grasses than annual spring wheat.

Table 4
Soil total C (STC) at the 0–120 cm depth averaged across N fertilization rates as affected by perennial grass species and year.

Grass species[a]	Year	STC (Mg C ha^{-1})					
		0-15 cm	15-30 cm	30-60 cm	60-90 cm	90-120 cm	0-120 cm
IW		19.4	14.6	77.8	113.1	102.0b[b]	326.9
SB		19.2	13.0	65.3	118.3	110.5a	326.3
SG		19.0	13.2	77.1	109.6	98.0b	316.9
SW[c]		16.6	10.4	63.3	111.4	97.6	299.3
	2011	19.8a	13.6	76.9a	113.8	98.7b	322.8
	2012	18.6b	14.0	67.3b	114.1	104.9ab	318.9
	2013	19.2ab	13.2	72.1ab	113.1	107.0a	324.6
Significance							
Grass (G)		NS	NS	NS	NS	*	NS
N fertilization rate (N)		NS	NS	NS	NS	NS	NS
G × N		NS	NS	*	*	NS	NS
Year (Y)		*	NS	*	NS	*	NS
G × Y		*	NS	*	NS	NS	*
N × Y		NS	NS	NS	NS	NS	NS
G × N × Y		NS	NS	NS	NS	NS	NS

*Significant at $P = 0.05$; NS, not significant.
[a] Perennial grasses are IW, intermediate wheatgrass; SB, smooth bromegrass; and SG, switchgrass.
[b] Numbers followed by different letters within a column in a set are significantly different at $P = 0.05$ by the least square means test.
[c] SW is annual spring wheat. Soil total C for spring wheat was determined in 2013 and is used for comparison with for perennial grasses, but not for data analysis.

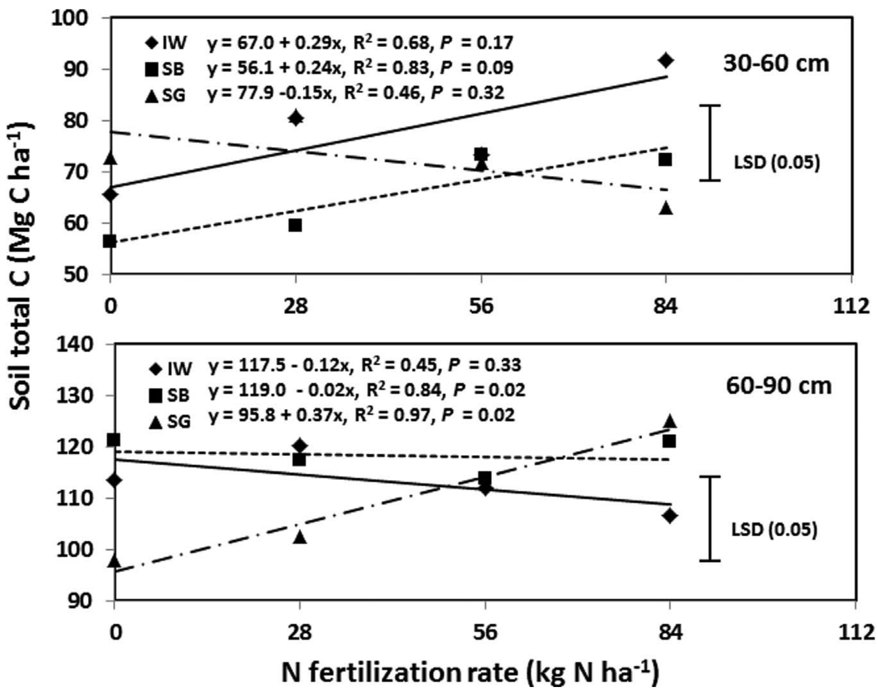

Fig. 1. Response of soil total C (STC) at 30–60 and 60–90 cm depths with N fertilization rate for various perennial grasses. Perennial grasses are IW, intermediate wheatgrass; SB, smooth bromegrass; and SG, switchgrass. LSD (0.05) is the least significant difference in STC between perennial grasses at a N fertilization rate at $P = 0.05$.

Table 5
Interaction of perennial grass species and year on soil total C (STC) at 0–15, 30–60, and 0–120 cm depths averaged across N fertilization rates.

Soil depth	Grass species[a]	STC (Mg C ha^{-1})			Change in STC with year (ΔSTC)		
		2011	2012	2013	ΔSTC (Mg C ha^{-1} yr^{-1})	R^2	P
0-15 cm	IW	19.6ab[b]	19.7a	19.0b	0.66	0.60	0.22
	SB	18.8bB[c]	18.3abAB	20.2aA	0.80	0.85	0.07
	SG	20.7aA	17.8bB	18.3abB	0.33	0.10	0.68
30-60 cm	IW	81.0	81.4a	70.9ab	2.56	0.25	0.50
	SB	73.8A	58.4bB	63.8bAB	−0.62	0.03	0.84
	SG	72.8B	61.9bAB	81.6aA	3.27	0.37	0.39
0-120 cm	IW	330.3	335.3a	315.3	5.40	0.32	0.43
	SB	330.5	324.1ab	325.8	6.51	0.63	0.20
	SG	305.3AB	299.9bB	333.4A	6.73	0.50	0.29

[a] Perennial grasses are IW, intermediate wheatgrass; SB, smooth bromegrass; and SG, switchgrass.
[b] Numbers followed by different lowercase letters within a column (year) between grasses in a depth are significantly different at $P = 0.05$ by the least square means test.
[c] Numbers followed by different uppercase letters within a row (grass) between years in a depth are significantly different at $P = 0.05$ by the least square means test.

4. Discussion

Increased root growth due to above-average growing season (April–October) and annual precipitation enhanced root biomass and therefore root C content at 0–15 and 0–120 cm in intermediate wheatgrass and switchgrass in 2011. Growing-season and annual precipitation were 81 and 56 mm, respectively, greater in 2011 than the 115-yr average (Table 7). Smooth bromegrass, however, did not grow well in initial years of establishment, resulting in poor root growth and root biomass C in 2011. In eastern Canada, smooth bromegrass yielded lower root biomass at 0–15 cm than most other grasses in the year of establishment [44]. Smooth bromegrass yielded better than other grasses in successive years, resulting in greater root biomass C than intermediate wheatgrass in 2012 when growing season and annual precipitation were lower than the 115-yr average (Table 7). Bollinder et al. [44] found that perennial grasses usually have lower root biomass at the surface layer in the year of establishment and reach maximum at 2–4 yr. Our results, however, showed declining root biomass C in successive years in intermediate wheatgrass and switchgrass due to reduced root growth, with lower biomass C during below-average precipitation in 2012.

Increased N availability due to N fertilization likely increased root growth and therefore root biomass C at 0–15 cm with 56 kg N ha^{-1} in 2011 and at 0–120 cm with 84 kg N ha^{-1} in 2013 when growing-season and annual precipitation were greater than the 115-yr average. Increased N fertilization rate from 0 to 140 kg N ha^{-1} increased switchgrass root biomass [41]. Similarly, increased N rate from 0 to 290 kg N ha^{-1} increased smooth bromegrass root biomass [45]. We, however, did not observe significant linear or quadratic responses of root biomass C to N fertilization rate as reported by Heggenstaller et al. [41]. The reasons for reduced root biomass C at 0–120 cm with increased N rate in 2011 were not known. Heggenstaller et al. [41] also observed that increased N rate reduced root biomass in eastern gamagrass (*Tripsacum dactyloides* L.) and indianagrass (*Sorghastrum nutans* [L] Nash).

It is not surprising to observe greater root biomass C at 0–15 cm than at other depths for perennial grasses (Table 1), as most of the roots are concentrated in the surface soil layer due to increased availability of soil water and nutrients [46]. About 70–90% of total root biomass in perennial forages to a 1 m depth occurs at the 0–20 cm layer [47]. Increased root growth increased root biomass C at 0–15, 15–30, and 0–120 cm in intermediate wheatgrass compared with other grasses. An exception occurred at 30–60 cm where smooth bromegrass had greater root biomass C than other grasses. Skinner [48] found increased root biomass of some perennial grasses at subsurface layers than the surface layer due to increased soil water availability. Our root biomass C of 5.1 Mg C ha^{-1} at 0–90 cm for perennial grasses was within the reported value of 2.2–6.5 Mg C ha^{-1} at 0–75 cm in Alabama [23] and Quebec, Canada [22]. Overall root biomass C for perennial grasses at various depths was 12–15 times greater than annual spring wheat (Table 1). Greater root biomass and C content in perennial grasses than annual cereal crops have been reported by several researchers [22,23].

Differences in soil water availability at various depths appeared to influence root biomass C among years. Increased water availability at

Table 6
Soil total N (STN) at the 0–120 cm depth averaged across N fertilization rates as affected by perennial grass species and year.

Grass species[a]	Year	STN (Mg N ha^{-1})					
		0-15 cm	15-30 cm	30-60 cm	60-90 cm	90-120 cm	0-120 cm
IW		2.02	1.66a[b]	3.35	2.38	1.98	11.39
SB		2.10	1.49b	3.36	2.40	2.04	11.39
SG		2.03	1.47b	3.03	3.14	2.01	11.68
SW[c]		1.82	1.30	3.12	2.39	1.93	10.56
	2011	2.04	1.53b	3.11b	3.12	2.01ab	11.81ab
	2012	2.11	1.64a	3.45a	2.55	2.15a	11.88a
	2013	2.01	1.43b	3.18b	2.24	1.88b	10.74b
Significance							
Grass (G)		NS	*	NS	NS	NS	NS
N fertilization rate (N)		NS	NS	NS	NS	NS	NS
G × N		NS	NS	NS	NS	NS	NS
Year (Y)		NS	***	*	NS	*	*
G × Y		NS	NS	NS	NS	NS	NS
N × Y		NS	NS	NS	NS	NS	NS
G × N × Y		NS	NS	NS	NS	NS	NS

*Significant at $P = 0.05$.
***Significant at $P = 0.001$; NS, not significant.
[a] Perennial grasses are IW, intermediate wheatgrass; SB, smooth bromegrass; and SG, switchgrass.
[b] Numbers followed by different letters within a column in a set are significantly different at $P = 0.05$ by the least square means test.
[c] SW is spring wheat. Soil total N for spring wheat was determined in 2013 and is used for comparison with for perennial grasses, but not for data analysis.

Table 7
Monthly total, crop growing season (April–October), and annual precipitation (mm) from 2011 to 2013 at the experimental site.

Month	2011	2012	2013	115-yr average
January	2	0	2	9
February	4	2	1	5
March	7	4	12	14
April	35	27	9	22
May	172	58	121	51
June	71	82	121	71
July	42	26	49	68
August	25	12	73	34
September	17	0	41	29
October	16	46	9	22
November	2	10	6	11
December	4	3	9	10
April–October	378	251	423	297
January–December	397	271	453	341

the soil surface during the year with above-average precipitation enhanced root growth and therefore root biomass C at 0–15 cm in 2011. As water moved down into the soil profile, increased root proliferation may have increased root biomass C at 15–30 and 30–60 cm in 2012 and 2013. Higher precipitation certainly increased total root biomass C at 0–120 cm in 2013 than 2012 (Tables 1 and 7).

As with root biomass C, increased root growth increased root biomass N at 0–15 and 0–120 cm in intermediate wheatgrass than other grasses during above-average precipitation in 2011 (Table 2). Reduction in N concentration with year, however, reduced root biomass N from 2011 to 2013, especially in intermediate wheatgrass and switchgrass. Increased root biomass N at 15–30 cm in intermediate wheatgrass and at 30–60 cm in smooth bromegrass (Table 3) was also a result of increased root biomass yield, a case similar to that observed for root biomass C (Table 1). Similarly, variations in root biomass N among years at different depths were also related to differences in root biomass yield, similar to those observed for root biomass C.

The trends in STC under perennial grasses with N fertilization rates were not related to root C input. The different trends of STC at 30–60 and 60–90 cm with N rates suggest that N fertilization rate had a variable effect on STC at various depths after 2–4 yr of perennial grass establishment. Several researchers [4,20,32] did not find significant effect of N fertilization on SOC at 0–30 cm under perennial grasses after 2–5 yr. Only after 4–12 yr, N fertilization increased SOC at 0–90 cm by 0.5–2.4 Mg C ha^{-1} yr^{-1} compared with no N fertilization under switchgrass in USA and Canada [33–35]. It is likely that longer than 4 yr of the present study is needed to observe the effect of N fertilization on root C input and STC under perennial grasses.

Although there was no significant relationship between STC with root C input as influenced by grass species and N rate, STC tended to increase with increased root biomass C returned to the soil (Tables 2 and 5). This suggests that increased root C input probably increased STC. As there was no consistent increase in STC with year among grasses, differences in turnover rates of root C into SOC may have influenced STC levels among grasses from 2009 to 2013. It usually takes 4 yr for complete turnover of grass roots to SOC and turnover rates can be influenced by root properties, such C/N ratio, lignin content, and lignin content/N ratio [4,22]. Omenode and Vyn [26] reported that SOC was greater under switchgrass than mixed grasses of little bluestem (*Schizachyrium scoparium* [Michx] Nash), indiangrass (*Sorghastrum nutans* [L] Nash), and big bluestem (*Andropogon gerardii* Vitman) after 6–8 yr. In contrast, Ma et al. [20] found that SOC was lower under switchgrass than Bermuda grass (*Cynodon dactylon* L.) after 10 yr. Several researchers [4,49] found that SOC was greater under legume than nonlegume grasses. Both cool- and warm-season grasses are equally effective in increasing soil C stock [5,32]. Because of variations in STC among grass species and years at various depths, we also conclude that both cool-season grasses, such as intermediate wheatgrass and smooth bromegrass, and warm-season grasses, such as switchgrass, can be effective in storing C in the soil. As soil C stock depends on age of grass establishment, grass species, and climate [20,25], long-term study may be needed to evaluate the effect of grass species on STC.

The reasons for greater STC at 90–120 cm under smooth bromegrass than other grasses (Table 4) were not known, as root biomass C at this depth, although not significant, was lower in smooth bromegrass (Table 1). Increased STC at all depths under perennial grasses compared with annual spring wheat, however, was due to increased root C input, as root biomass C was 7–15 times greater in perennial grasses (Table 1). This suggests that STC can be greater under perennial grasses where only root biomass was returned to the soil than annual spring wheat where both root and shoot (stems and leaves) biomass were returned to the soil after grain harvest. Increased STC and SOC under perennial grasses compared with annual crops have been known [22,25,26,29]. Increased STC at lower depths under perennial grasses and annual spring wheat was probably a result of presence of large amount of SIC in subsoil layers, as soils under dryland cropping systems in arid and semiarid regions are enriched with SIC, especially in the underlying layers [37,40]. Increased STC or SOC in underlying soil layers is beneficial for soil C sequestration, as SOC in subsoils is mineralized less than in the surface soil due to reduced microbial activity [25,26]. Liebig et al. [25] found that both STC and SOC at 30–60 and 60–90 cm were greater under switchgrass than croplands.

The STC at various depths, averaged across treatments, exhibited various trends with year (Table 4). Considering STC under annual spring wheat as the baseline value, significant linear trends of STC with year were observed only at 90–120 cm (R^2 = 0.84, $P \leq 0.10$), with C sequestration rate of 2.5 Mg C ha^{-1} yr^{-1}, respectively. This suggests that C sequestration can be increased in deep subsoil layers under perennial grasses.

The STN under grasses and spring wheat declined from 0–15 to 15–30 cm, increased slightly at 30–60 cm due to increased thickness of the soil layer, and then declined (Table 6). This was largely reflected by the distribution of root biomass N with depth (Table 3). The greater STN at 15–30 cm under intermediate wheatgrass than other grasses was due to increased root biomass N. Cool-season grasses, such as intermediate wheatgrass, can increase STN compared with warm season grasses, such as switchgrass [31]. Although root biomass N varied among grasses at 30–60 cm, STN at this depth did not differ.

The greater STN at 15–30, 30–60, 90–120, and 0–120 cm in 2012 than other years was likely a result of reduced N mineralization due to lower precipitation. The growing season precipitation was 127–172 mm and annual precipitation 126–182 mm lower in 2012 than 2011 and 2013 (Table 7). Similarly, total annual precipitation was 70 mm lower in 2012 than the 115-yr average. It appears that reduced microbial activity due to decreased soil water availability reduced N mineralization, thereby increasing STN in 2012. The STN can vary with year due to variations in soil temperature and water content [36,37]. As with STC, greater STN under perennial grasses than annual spring wheat was due to increased root biomass N input to the soil (Tables 3 and 6).

5. Conclusions

Differences in grass species and N fertilization rates resulted in variations for root biomass C and N returned to the soil and STC and STN stocks to a depth of 120 cm after two to four years of grass plantation. Root biomass C and N at various depths increased with intermediate wheatgrass and switchgrass, but decreased with smooth bromegrass in 2011 and 2013 when the annual precipitation was above the 115-yr average. In these years, N fertilization rate had a variable effect on root biomass C. The STC at different depths varied with grass species and N rates in various years and did not correspond to trends in root biomass C with N rates and years. Increased root biomass N, however, increased STN at 15–30 cm. The STC increased in deep soil layers possibly due to the presence of large amount of SIC in subsoil layers, but STN decreased. Overall STC and STN at 0–120 cm were greater under perennial grasses than annual spring wheat due to increased root C and N inputs. More than four years of study may be needed to evaluate the effects of grass species and N fertilization rate on STC and STN stocks under perennial grasses in semiarid regions of the northern Great Plains.

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