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Nitrogen fertilizer and landscape position affect soil aggregate size distribution, and intra-aggregate carbon and nitrogen under switchgrass in a marginal cropland

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Abstract

Dedicated bioenergy crops such as switchgrass (*Panicum virgatum* L.) can be grown on marginally productive lands and positively influence soil properties. However, nitrogen management, and landscape can alter soil structural attributes under bioenergy crop production. This study investigated the impacts of long-term nitrogen fertilization (0-N, 0 kg N/ha; 56-N, 56 kg N/ha and 112-N, 112 kg N/ha) and landscape positions (shoulder and footslope) on soil organic carbon (SOC) and structural attributes under switchgrass production. The 112-N rate enhanced the proportion of 2–4 mm water-stable aggregates by 49%, aggregate associated carbon in 2–4 mm and >4 mm aggregates by 16 and 24%, respectively, aggregate associated nitrogen in >4 mm aggregates by 33% and reduced soil bulk density by 19% compared to the 0-N rate. Footslope position increased the proportion of 2–4 mm water-stable aggregates by 26% and lowered bulk density by 8% compared to the shoulder position. Results showed a significant N-rate × landscape position interaction on SOC and glomalin related soil protein content in bulk soil. Overall, this study showed that nitrogen application to switchgrass planted at footslope on a marginally yielding cropland improved soil structure and physical conditions.

Introduction

Soil degradation implies a decline in soil quality with a consequent reduction in ecosystem functions and services (Lal, 2009), and is a major threat to the sustainable development (Nearing *et al.*, 2000). Soil physical degradation leads to decline in structural properties such as soil aggregate stability, pore geometry, thereby increasing a soil's susceptibility to crusting, compaction, reduced water infiltration, increased surface runoff, wind and water erosion and ultimately rendering it vulnerable to desertification (Lal, 2015). Restoration of degraded lands can be achieved by planting perennial crops as these can enhance soil carbon sequestration and play a key role in controlling soil and water erosion (Deng *et al.*, 2014). Switchgrass (*Panicum virgatum* L.) is a warm-season perennial C4 grass which is grown as a dedicated bioenergy feedstock on a broad range of soil types (Vogel and Mitchell, 2008). Due to its deep-root system, rapid growth, greater drought tolerance, adaptability and low-maintenance compared with other common grass species, switchgrass can thrive under diverse climatic and environmental conditions (Blanco-Canqui, 2010). Previous research has shown that it has the potential to store a significant quantity of soil carbon (C) in the Northern Great Plains (Frank *et al.*, 2004). It can positively influence soil physical conditions by providing aboveground plant residue inputs and belowground root biomass and improving soil organic carbon (SOC) sequestration rates, increasing soil aggregation and reducing soil erosion (Blanco-Canqui, 2010). Soil aggregation and its stability also influences soil structure-related processes such as pore development and enhancement, water movement and erosion and runoff (Six *et al.*, 2000; Nael *et al.*, 2004). Thus, soil aggregate stability is a well-recognized indicator of formation, degradation and stabilization of soil structure (Six *et al.*, 2004). Previous research on switchgrass-induced alterations in soil aggregation was focused on the comparison between switchgrass and row crops (Marquez *et al.*, 2004; Blanco-Canqui *et al.*, 2005). Stewart *et al.*, (2015) observed that switchgrass soils had significantly greater aggregate stability in the 0–5, 5–10 and 0–30 cm depths compared to no till corn. Zaibon *et al.*, (2017) postulated that the switchgrass production systems enhanced water infiltration into the soil and reduced the runoff compared with row crop management, and recommended switchgrass to be planted on degraded soils for improved water use. Wang *et al*., (2020) indicated that the average water erosion could be reduced by 50% or more when row crops are replaced by switchgrass on slopes with gradients ≥10%, however the effects may vary according to precipitation and site-specific conditions. In contrast, Márquez *et al*., (2017) did not find any apparent difference between the formation of new stable large and small macroaggregates in the soils converted from row crop (corn-soybean) to switchgrass production. These contradictory findings indicate that moderating factors, such as soil type, crop and soil management (e.g. fertilization), climate and topography, among others, may cause variation in the response of structural attributes of soil under switchgrass plantations.

Nitrogen fertilization rate (N rate), a major input factor, can greatly influence both soil physical properties and switchgrass production. Responses of SOC and physical properties to N- fertilization can vary depending upon soils and environmental conditions (Kering *et al*., 2012). Previous studies have reported mixed effects of N-fertilization on soil properties under switchgrass. For instance, no effect of inorganic fertilization was observed on SOC concentration, SOC pools and aggregate stability in some studies (e.g. Stewart *et al*., 2015; Kibet *et al*., 2016; Lai *et al*., 2018). However, positive responses of the SOC, bulk density (ρ_b), porosity and water movement to N-fertilization under switchgrass plantation were observed by other studies (Jung and Lal, 2011; Kumar *et al*., 2019; Singh *et al*., 2019). Recent research on the impact of N-fertilization on soil aggregation under switchgrass showed mixed results. For instance, Valdez *et al*., (2017) reported that N-fertilization reduced SOC in switchgrass plots after 4 years of plantation in Michigan. Higher N-fertilization rates did not improve active carbon and water stable aggregates (WSA) in a 3-year switchgrass field study conducted by Saini *et al*., (2021) in Tennessee. Besides the N rate, landscape position can also influence the growth of switchgrass, and consequently, soil physical properties and nutrient status. Topographical factors e.g. landscape position, gradient and slope can have a major influence on the redistribution of water and minerals, which in turn affect soil structural properties (Shi *et al*., 2019). Guzman and Al-Kaisi (2011) observed lower SOC, WSA and root biomass but higher ρ_b at the midslope compared to the summit and toeslope positions. Studies have reported that SOC and other soil properties at the footslope position were superior to those at other topographic positions (Lai *et al*., 2018; Alagele *et al*., 2019; Singh *et al*., 2019).

So far, the studies on soil aggregation and aggregate associated C and N have focused either on the effects of biofuel cropping systems *v*. row crops (Marquez *et al*., 2004; Blanco-Canqui *et al*., 2005), or on the effects of topography (Zilverberg *et al*., 2018; Alagele *et al*., 2019), or on the effects of N-fertilization (Valdez *et al*., 2017; Saini *et al*., 2021) separately.

The current study focuses on how do the agricultural management practices (e.g. fertilizer application) interact with topography and impact soil aggregation status under the biofuel crop production systems. This knowledge gap is important to address for maximizing the benefits to soil from biofuel plantations on marginal lands. We hypothesized that the soil structural attributes under switchgrass plantation can improve with increasing rates of N application but may vary at different landscape positions due to aggrading soils on marginal lands. The objective of this study was to evaluate the responses of SOC, total N, water-stable aggregates and aggregate-associated carbon and nitrogen to different N rates and landscape positions under switchgrass planted to a marginal land in eastern South Dakota.

Materials and methods

Study site, experimental design and soil sampling

The current study was conducted near the city of Bristol (45°16′24.55″N, 97°50′13.34″W; altitude: 524.3 m above sea level) located in South Dakota, USA, which falls within the humid continental climate zone, Dfb, according to Köppen climate classification. Soils at the study location are dominated by loamy soils with 2–20% slope; Forman series (Fine-loamy, mixed, frigid Udic Argiborolls) (Mbonimpa *et al*., 2015). Mean daily temperature and mean annual precipitation for 30 years (1986–2015) at the study site were 6.42°C and 619 mm, respectively. The particle size distribution was 39.8% sand, 22.5% clay and 37.7% silt. Study treatments included three nitrogen (N) rates (0-N, 0 kg N/ha; 56-N, 56 kg N/ha and 112-N, 112 kg N/ha) and two landscape positions (shoulder and footslope) laid out in split-plot design with four replications in a complete block structure. Individual plot size was 21.3 m × 365.8 m. The N rate recommendation for switchgrass production as a biofuel crop in the study region under marginal lands (where row crops do not perform for their maximum yield potential due to erosion, lower soil carbon and drainage issues) ranges from 100–150 and sometimes 200 kg N/ha (Reynolds *et al*., 2000; Lai *et al*., 2018; McGowan *et al*., 2018). Switchgrass, a deep-rooted perennial crop is well-suited for these types of marginal lands with N rate ranged from 100 to 150 kg/ha. This study site was established in 2008. Before the initiation of this experiment, the site was under corn (*Zea mays* L.)-soybean (*Glycine max* (L.) Merr.) rotation. On May 17, 2008, Sunburst cultivar of switchgrass (*P. virgatum* L.) was planted with a seeding rate of 10 kg pure live seed/ha. Urea was applied annually in late May or early June as a source of N fertilizer each year since 2008. Thus, three N rate treatments (0-N, 56-N and 112-N) were applied since 2008. Switchgrass was harvested once annually around a killing frost. Additional information of this site can be found in Singh *et al*. (2019).

Soil sampling was carried out in July 2019 as a single sampling timepoint. For aggregate analysis, soil samples were collected from 0–10 cm depth at three randomly selected spots per plot with a hand shovel and composited. Samples were then gently passed through 8 mm sieve to remove any undesirable plant material. These samples were then air dried and used for estimating aggregate size distribution (Guzman and Al-Kaisi, 2011). For bulk soil analysis, three soil samples per plot were randomly collected from 0–10 cm depth using soil auger and composited to measure SOC and total nitrogen (TN) concentrations and glomalin related soil protein (GRSP). Also, three intact soil cores (5 cm long and 5 cm inner diameter) per plot were collected from 0–10 cm depth to determine the ρ_b.

Water stable aggregates, aggregate associated carbon and nitrogen and bulk density

For the determination of WSA and their size distribution, 100 g of air-dried 8 mm sieved soil samples were placed on top of a stack of sieves with 0.053, 0.25, 0.5, 1, 2 and 4 mm diameter openings for wet sieving in deionized water for 5 min at room temperature. Wet sieving was performed using a custom-made sieving machine, by lowering and then raising the sieves with a stroke

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length of 13 mm and a frequency of 90 strokes/min. Prior to wet sieving, each soil aggregate sample was first misted and then submerged in water in the top sieve for at least 5 min to slake off air-dried soil. Following wet sieving, aggregates retained on each sieve were transferred to pre-weighed beakers, dried at 40°C and weighed. The mass of <0.053 mm soil fractions was obtained by the difference between initial sample weight and the sum of sample weights collected on the 0.053, 0.25, 0.5, 1, 2 and 4 mm sieve nest. The data were analysed to compute WSA. The aggregate fractions obtained from each sieve after drying at 40°C were ground using pestle and mortar after removal of coarse organic particles to determine WSA- associated total carbon (TC) and TN via a dry combustion method that used a TruSpec carbon–hydrogen–nitrogen (CHN) analyser (LECO Corporation, St. Joseph, MI). Inorganic C in the samples was below the detection limits; thus, total C was considered as SOC in this study. The ρ_b was determined from the intact soils cores by oven-drying the samples at 105°C until a constant weight was observed.

Soil organic carbon, nitrogen and glomalin related soil protein from bulk soil

Bulk soil samples were air-dried and ground to a fine powder and analysed for TC and TN concentration via a dry combustion method using a CHN analyser. GRSP concentration was determined following the procedure outlined by Wright and Upadhyaya (1998). Briefly, 3 g of air-dried soil sample was placed in a tube and 24 ml of extractant buffer (20 mM sodium citrate, pH 7.0) was added to it and mixed. The tubes were autoclaved at 121°C (15 psi) for 30 min, then cooled and centrifuged at 10 000 *g*. Soil protein concentration in solution was determined with a pierce bovine serum albumin (BSA) protein assay kit (Thermo Scientific, IL, USA). Following the colour reaction, the absorbance of the samples and the blank was measured at 562 nm using a spectrophotometer. The concentration of GRSP was determined from the absorbance values of samples via a standard curve of 0–2000 u g/ml of BSA and GRSP expressed as mg/g of dry soil.

Statistical analysis

Statistical analysis of data was conducted using the pairwise differences method to compare least-squares means estimated by a mixed model using the GLIMMIX procedure in SAS 9.4 (SAS 2013). Nitrogen rate, landscape position and N rate × landscape position were considered as fixed effects and replication and replication × N rate as random effects. Analysis of variance was performed to test the fixed effects of the N rate and landscape position on the soil properties on the basis of mixed model and P values were adjusted by Tukey method in the SAS 9.4. The Kolmogorov–Smirnov test was applied to check for normality assumption and data were transformed (e.g. <0.053 mm aggregates) via log transformation when necessary (Box and Cox, 1981). Statistical differences were considered significant at $\alpha = 0.05$ level. Simple linear regressions were used to determine relationships between measured soil parameters using SAS REG procedure.

Results

Aggregate size distribution and aggregate associated SOC and TN concentration

Interaction between N application rate and landscape position was observed for the proportions of 1–2 mm and >4 mm aggregates (Table 1). At footslope, 112-N enhanced the proportion of 1–2 mm aggregates by 75 and 62% compared to the 0-N ($P < 0.01$) and 56-N treatments ($P = 0.014$). Under 112-N, the proportion of 1–2 mm aggregates was 60% more at footslope than that at the shoulder position ($P = 0.014$). At shoulder, 112-N increased the proportion of >4 mm aggregates by 62% compared to the 56-N ($P = 0.03$). Under 56-N, the proportion

Table 1. Soil aggregate size distribution as influenced by nitrogen rates (0-N, 0 kg N/ha; 56-N, 56 kg N/ha and 112-N, 112 kg N/ha) under switchgrass planted at different landscape positions (shoulder and footslope)

Position	N Rate	Aggregate size (mm)						
		<0.053	0.053–0.25	0.25–0.50	0.50–1	1–2	2–4	>4
		%						
Shoulder	0-N	14.5 ± 4.18	3.3 ± 0.67	6.9 ± 1.35	17.7 ± 3.37	15.5 ± 3.13^{b†}	20.0 ± 1.60	22.1 ± 2.46^{bc}
	56-N	15.9 ± 3.41	4.3 ± 1.57	6.5 ± 1.00	13.7 ± 1.91	17.1 ± 0.83^{b}	23.5 ± 1.18	19.0 ± 3.04^{c}
	112-N	15.0 ± 1.76	0.9 ± 0.11	3.0 ± 0.62	9.0 ± 1.23	16.1 ± 1.44^{b}	25.2 ± 2.14	30.8 ± 0.89^{ab}
Footslope	0-N	16.0 ± 6.80	3.3 ± 1.49	5.7 ± 1.31	10.9 ± 1.97	14.7 ± 2.01^{b}	21.4 ± 1.49	28.0 ± 2.11^{abc}
	56-N	11.3 ± 3.30	2.3 ± 1.32	3.3 ± 2.08	6.3 ± 2.26	15.9 ± 0.80^{b}	28.2 ± 4.99	32.7 ± 3.48^{a}
	112-N	3.7 ± 1.35	0.9 ± 0.15	2.7 ± 0.81	8.8 ± 2.14	25.7 ± 1.23^{a}	36.6 ± 1.94	21.5 ± 0.14^{bc}
Average	0-N	15.3 ± 3.71	3.3 ± 0.75^{A‡}	6.3 ± 0.90	14.3 ± 2.22	15.1 ± 1.73	20.7 ± 1.04^{B}	25.0 ± 1.86
	56-N	13.6 ± 2.36	3.3 ± 1.02^{AB}	4.9 ± 1.23	10.0 ± 1.95	16.5 ± 0.58	25.9 ± 2.53^{AB}	25.9 ± 3.36
	112-N	9.3 ± 2.38	0.9 ± 0.08^{B}	2.8 ± 0.47	8.9 ± 1.14	20.9 ± 2.02	30.9 ± 2.54^{A}	26.2 ± 1.81
	Shoulder	15.1 ± 1.72^{A§}	2.8 ± 0.67	5.4 ± 0.76	13.4 ± 1.63^{A}	16.2 ± 1.09	22.9 ± 1.09^{B}	24.0 ± 1.93
	Footslope	10.3 ± 2.78^{B}	2.2 ± 0.67	3.9 ± 0.87	8.7 ± 1.24^{B}	18.8 ± 1.67	28.8 ± 2.51^{A}	27.4 ± 1.85

†Means with different lowercase letters within a column for the nitrogen rate and landscape position are significantly different at $P < 0.05$. No lowercase letters are shown if the nitrogen rate × landscape position interaction was not significant. Values are means with standard errors.
‡Nitrogen rate means (averaged across landscape positions) followed by different uppercase letters are significantly different at $P < 0.05$.
§Landscape position means (averaged across nitrogen rates) followed by different uppercase letters are significantly different at $P < 0.05$.

of >4 mm aggregates were 72% more at footslope than that at the shoulder position ($P < 0.01$). Thus, the effect of N application on 1–2 mm and >4 mm aggregate fractions depended on the landscape position. No interaction between N application rate and landscape position was observed for rest of the aggregate sizes. Averaged across landscape positions, the 112-N treatment increased the proportion of 2–4 mm aggregates compared with the 0-N by 49% ($P = 0.02$) and results for the 56-N were similar to those of 0-N and 112-N treatments (Table 1). The proportion of 0.053–0.25 mm aggregates was lower ($P = 0.04$) under the 112-N compared to the 0-N (Table 1), which may likely be due to the binding of small aggregates leading to the formation of large aggregates. Averaged across N application rates, the proportion of 2–4 mm aggregates was 26% higher at the footslope position than that at the shoulder position ($P = 0.02$; Table 1). However, the proportions of 0.50–1 mm and <0.053 mm soil fractions were lower at the footslope than at the shoulder position.

For aggregate associated SOC concentration, no interaction between N application rate and landscape position was observed (Table 2). Averaged across landscape positions, the 112-N treatment increased SOC concentration in >4 mm aggregates compared to that of the 56-N and 0-N treatments ($P = 0.04$; Table 2). Similarly, SOC in 2–4 mm aggregates was 16% higher under 112-N than the 0-N treatment. Nitrogen application did not affect SOC in the remaining aggregate size fractions, though followed the trend 0-N < 56-N < 112-N. Averaged across N application rates, the landscape positions did not affect aggregate associated SOC concentration, however, in general, the footslope position had numerically higher ($P > 0.05$) aggregated associated SOC than the shoulder position.

For aggregate associated TN concentration, interaction between N application rate and landscape position was observed for 0.25–0.50 mm and 2–4 mm aggregate sizes (Table 3). At shoulder, 112-N increased the TN concentration in 0.25–0.50 mm aggregates by 54% compared to the 56-N ($P = 0.04$). The TN concentration in 2–4 mm aggregates was 58% greater in 112-N treatment at footslope compared to 0-N treatment at shoulder position. No interaction between N application rate and landscape position was observed for TN concentration in the rest of the aggregate sizes. Averaged across the landscape positions, 112-N increased the concentration of TN in >4 mm aggregates compared to the 0-N treatment ($P = 0.04$; Table 3). Nitrogen application did not influence TN in the other aggregate fractions ($P > 0.05$). Averaged across N application rate, the aggregate associated TN was similar at both the landscape positions.

Soil organic carbon, bulk density, total nitrogen and glomalin concentration in bulk soil

For SOC concentration in bulk soil, interaction between N application rate and landscape position was observed (Table 4). At shoulder position, 56-N and 112-N treatments increased the SOC concentration in bulk soil by 13% each compared to the 0-N treatment ($P < 0.01$). Under 0-N, the SOC concentration in bulk soil was 11% more at footslope than that at the shoulder position ($P < 0.01$; Table 4). No interaction between N application rate and landscape position was observed for the soil ρ_b (Table 4). Averaged across landscape positions, ρ_b was reduced by 19% with 112-N compared to the 0-N treatment ($P = 0.04$; Table 4). Averaged across N application rates, ρ_b was higher (1.27 Mg/m^3) at shoulder position than that at footslope position (1.17 Mg m^{-3}). TN concentration in bulk soil was not influenced by N rate and landscape position. For GRSP concentration in bulk soil, interaction between N application rate and landscape position was observed. At footslope position, 56-N treatment increased the GRSP concentration in bulk soil by 79% and 46% compared to the 0-N and 112-N treatments, respectively ($P < 0.01$; Table 4). Under 56-N and 112-N, the GRSP concentration in bulk soil was higher at footslope (1.18 and 0.81 mg/g, respectively) than that at the shoulder position (0.59 and 0.48 mg/g, respectively) ($P < 0.01$).

Table 2. Aggregate associated soil organic carbon (SOC) concentration (g/kg) in different sizes of water stable aggregates from soils managed with varying nitrogen rates (0-N, 0 kg N/ha; 56-N, 56 kg N/ha and 112-N, 112 kg N/ha) under switchgrass planted at different landscape positions (shoulder and footslope)

Position	N Rate	Aggregate size (mm)					
		0.053–0.25	0.25–0.50	0.50–1	1–2	2–4	>4
		g/kg					
Shoulder	0-N	23.3 ± 1.18[†]	23.6 ± 1.66	24.2 ± 0.91	25.8 ± 0.62	22.0 ± 1.03	24.5 ± 1.06
	56-N	24.3 ± 1.59	23.8 ± 1.73	24.8 ± 0.91	24.7 ± 0.66	25.6 ± 0.58	25.7 ± 1.18
	112-N	27.2 ± 0.89	28.6 ± 2.52	27.5 ± 1.49	27.7 ± 1.47	27.3 ± 1.21	30.7 ± 0.93
Footslope	0-N	25.4 ± 1.27	26.6 ± 1.35	25.5 ± 1.42	25.0± 1.70	26.6 ± 1.22	25.4 ± 1.49
	56-N	24.9 ± 2.25	28.8 ± 2.12	28.4 ± 2.10	28.7 ± 1.90	24.7 ± 1.19	26.2 ± 1.17
	112-N	28.1 ± 1.01	24.9 ± 0.42	28.9 ± 0.37	28.0 ± 0.46	29.2 ± 1.97	31.0 ± 0.80
Average	0-N	24.3 ± 0.90	25.1 ± 1.14	24.9 ± 0.82	25.4 ± 0.86	24.3 ± 1.14[B‡]	24.9 ± 0.87[B]
	56-N	24.6 ± 1.28	26.3 ± 1.58	26.6 ± 1.27	26.7 ± 1.20	25.2 ± 0.63[AB]	26.0 ± 0.77[B]
	112-N	27.7 ± 0.64	26.7 ± 1.38	28.2 ± 0.75	27.9 ± 0.72	28.2 ± 1.13[A]	30.8 ± 0.57[A]
	Shoulder	24.9 ± 0.83[§]	25.3 ± 1.26	25.5 ± 0.74	26.1 ± 0.64	25.0 ± 0.84	26.9 ± 0.98
	Footslope	26.1 ± 0.94	26.7 ± 0.91	27.6 ± 0.89	27.2 ± 0.92	26.8 ± 0.96	27.5 ± 0.97

[†]No lowercase letters are shown if the nitrogen rate × landscape position interaction was not significant. Values are means with standard errors.
‡Nitrogen rate means (averaged across landscape positions) followed by different uppercase letters are significantly different at $P < 0.05$.
§No uppercase letters are shown if landscape position means (averaged across nitrogen rates) were not significantly different at $P < 0.05$.

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Table 3. Aggregate associated total nitrogen (TN) concentration (g/kg) in different sizes of water stable aggregates from soils managed with varying nitrogen rates (0-N, 0 kg N/ha; 56-N, 56 kg N/ha and 112-N, 112 kg N/ha) under switchgrass planted at different landscape positions (shoulder and footslope)

Position	N Rate	Aggregate size (mm)					
		0.053–0.25	0.25–0.50	0.50–1	1–2	2–4	>4
		g/kg					
Shoulder	0-N	1.42 ± 0.04	1.42 ± 0.08^{b†}	1.57 ± 0.10	1.63 ± 0.13	1.28 ± 0.08^{b}	1.51 ± 0.21
	56-N	1.69 ± 0.11	1.55 ± 0.09^{ab}	1.65 ± 0.07	1.77 ± 0.11	1.95 ± 0.11^{ab}	2.07 ± 0.21
	112-N	1.79 ± 0.13	2.19 ± 0.36^{a}	1.94 ± 0.20	1.92 ± 0.17	1.84 ± 0.18^{ab}	2.29 ± 0.08
Footslope	0-N	1.64 ± 0.13	1.97 ± 0.16^{ab}	1.89 ± 0.16	1.74 ± 0.18	1.94 ± 0.17^{ab}	1.96 ± 0.22
	56-N	1.49 ± 0.16	1.89 ± 0.15^{ab}	1.83 ± 0.10	1.88 ± 0.21	1.35 ± 0.16^{ab}	1.77 ± 0.27
	112-N	1.76 ± 0.20	1.53 ± 0.03^{ab}	1.65 ± 0.09	1.72 ± 0.05	2.02 ± 0.20^{a}	2.34 ± 0.12
Average	0-N	1.53 ± 0.08	1.69 ± 0.13	1.73 ± 0.11	1.68 ± 0.11	1.61 ± 0.15	1.74 ± 0.17^{B‡}
	56-N	1.59 ± 0.10	1.72 ± 0.10	1.74 ± 0.06	1.82 ± 0.11	1.65 ± 0.15	1.92 ± 0.17^{AB}
	112-N	1.78 ± 0.11	1.86 ± 0.21	1.80 ± 0.12	1.82 ± 0.09	1.93 ± 0.13	2.32 ± 0.07^{A}
	Shoulder	1.64 ± 0.07§	1.72 ± 0.15	1.72 ± 0.09	1.77 ± 0.08	1.69 ± 0.11	1.96 ± 0.14
	Footslope	1.63 ± 0.09	1.80 ± 0.09	1.79 ± 0.07	1.78 ± 0.09	1.77 ± 0.13	2.02 ± 0.13

†Means with different lowercase letters within a column for the nitrogen rate and landscape position are significantly different at $P < 0.05$. No lowercase letters are shown if the nitrogen rate × landscape position interaction was not significant. Values are means with standard errors.
‡Nitrogen rate means (averaged across landscape positions) followed by different uppercase letters are significantly different at $P < 0.05$.
§No uppercase letters are shown if landscape position means (averaged across nitrogen rates) were not significantly different at $P < 0.05$.

Discussion

Aggregate size distribution and aggregate associated SOC and TN concentration

Organic matter is considered an important binding agent for aggregation of soil (Tisdall and Oades, 1982), and therefore, the increase in the proportion of 2–4 mm aggregates with the increasing N rate in this study can be attributed to the increase in aggregate associated SOC (Table 2). The accumulation of OC and N in macroaggregate fractions has been well documented in different soil management systems (Monreal *et al.*, 1995; Six *et al.*, 1998). Our results are consistent with previous studies that reported an increment in aggregate associated SOC and TN increased stability of aggregates with the fertilizer application (Subbian *et al.*, 2000; Jagadamma *et al.*, 2008; Yu *et al.*, 2012; Ghosh *et al.*, 2018; Yan *et al.*, 2021). Yajun *et al.*, (2017) also reported that the application of N and phosphorus fertilizers significantly enhanced the SOC and N stocks in >2 mm aggregates at 0–20 cm depth.

Higher proportion of 2–4 mm aggregates and lower proportion of 0.50–1 mm and <0.053 mm soil fractions at footslope suggested that a portion of the water stable macroaggregates displaced at shoulder position, transported by surface flow and redeposited

Table 4. Soil organic carbon (SOC), bulk density (ρ_b), total nitrogen (TN) and glomalin related soil protein (GRSP) content monitored from soils managed with varying nitrogen rates (0-N, 0 kg N/ha; 56-N, 56 kg N/ha and 112-N, 112 kg N/ha) under switchgrass planted at different landscape positions (shoulder and footslope)

Position	N Rate	SOC (g/kg)	ρ_b (Mg m^{-3})	TN (g/kg)	GRSP (mg/g)
Shoulder	0-N	22.4 ± 0.19^{b†}	1.45 ± 0.08	1.86 ± 0.05	0.50 ± 0.03^{c}
	56-N	25.3 ± 0.15^{a}	1.21 ± 0.07	2.27 ± 0.09	0.59 ± 0.05^{c}
	112-N	25.4 ± 0.34^{a}	1.15 ± 0.04	2.29 ± 0.04	0.48 ± 0.08^{c}
Footslope	0-N	24.8 ± 0.57^{a}	1.25 ± 0.05	2.14 ± 0.27	0.66 ± 0.02^{bc}
	56-N	24.2 ± 0.38^{a}	1.21 ± 0.06	1.91 ± 0.26	1.18 ± 0.05^{a}
	112-N	25.6 ± 0.32^{a}	1.04 ± 0.03	2.51 ± 0.09	0.81 ± 0.03^{b}
Average	0-N	23.6 ± 0.52	1.35 ± 0.06^{A‡}	2.00 ± 0.14	0.58 ±0.03
	56-N	24.7 ± 0.29	1.21 ± 0.04^{AB}	2.09 ± 0.15	0.89 ± 0.12
	112-N	25.5 ± 0.22	1.09 ± 0.03^{B}	2.40 ± 0.06	0.65 ± 0.07
	Shoulder	24.4 ± 0.43	1.27 ± 0.05^{A§}	2.14 ± 0.07	0.52 ± 0.03
	Footslope	24.8 ± 0.29	1.17 ± 0.04^{B}	2.19 ± 0.14	0.89 ± 0.07

†Means with different lowercase letters within a column for the nitrogen rate and landscape position are significantly different at $P < 0.05$. No lowercase letters are shown if the nitrogen rate × landscape position interaction was not significant. Values are means with standard errors.
‡Nitrogen rate means (averaged across landscape positions) followed by different uppercase letters are significantly different at $P < 0.05$.
§Landscape position means (averaged across nitrogen rates) followed by different uppercase letters are significantly different at $P < 0.05$.

at the footslope, and the depositional materials aided in the development of WSA at footslope position (Tang *et al*., 2010). Guzman and Al-Kaisi (2011) also found that the midslope landscape position had lower index of aggregate stability compared to the toeslope position, which they attributed to lower root biomass and soil organic matter, caused by the soil erosion at the midslope position. Furthermore, roots form and stabilize aggregates either directly by mechanical entanglement with soil particles, or indirectly by providing organic compounds, exudates, rhizodeposits and root turnover and stimulating microbial activity (Jastrow *et al*., 1998; Gale *et al*., 2000; Bronick and Lal, 2005). Thus, soil microbes, fungal hyphae, microbial (bacterial and fungal) and plant mucilages play a crucial role in stabilization of macroaggregates (Roberson *et al*., 1991; Gupta and Germida, 2015). A previous research conducted at the current study site also reported higher bacterial, fungal, actinomycetes, AM fungi, total microbial biomass and microbial biomass carbon at the footslope position (Sekaran *et al*., 2019).

The increase in aggregate associated SOC and TN associated with the N fertilization may be due to the increased input of plant material, above- and below-ground biomass and the transfer of photosynthetically fixed atmospheric C into the soil (Bronick and Lal, 2005). Nitrogen fertilization can help to build soil organic matter by enhancing total crop production. Greater amount of residue returned to the soil due to enhanced crop production, then breaks down and contribute to soil organic matter (Conant *et al*., 2001; Munroe and Van Eerd, 2016). Greater SOC and biological activity with fertilizer application increase the water stable aggregation (Haynes and Naidu, 1998). Nitrogen addition can stimulate soil C storage both by increasing soil C input and by decreasing decomposition rates (Huang *et al*., 2020). Fertilizer application mainly enhanced switchgrass biomass yields, consequently increasing the return amounts of stubble and root exudates at the current study site (Hong *et al*., 2014), which might have resulted in the addition of exogenous C and N into soil via rhizodeposition. Heggenstaller *et al*., (2009) reported that N fertilization had positive effects on both aboveground harvested biomass (shoot biomass) and root biomass of switchgrass. While studying switchgrass contributions to SOC as influenced by N fertilization, Stewart *et al*., (2016) demonstrated that increasing N fertilizer rate increased aboveground biomass, belowground root biomass and biomass C and N, suggesting increased belowground production for additional nutrient acquisition. They further reported that the N treatments that maximized belowground root biomass incorporated more belowground root biomass C into the soil C pool after 9 years (Stewart *et al*., 2016).

The greater concentration of aggregate-associated TN concentration in 2–4 mm aggregates in 112-N treatment at footslope could be attributed to the combined effect of higher N-fertilization and the soil erosion depositional processes at the footslope position. Ayoubi *et al*., (2012) also reported higher SOC and TN concentrations in aggregates at the lower slope gradients compared to the steep slopes. Macroaggregates can physically protect recently added OC and N from microbial decomposition and mineralization (Blanco-Canqui and Lal, 2004) by creating a physical barrier between the substrates and microbes, therefore can promote the accumulation of OC and N in them. With increase in N fertilization, the higher the SOC content (in 2–4 mm and >4 mm fractions), the greater the TN content in >4 mm fractions, when averaged across landscape positions (Tables 2 and 3). Pu *et al*., (2012) observed linear relationships between SOC and TN concentrations while studying SOC and TN dynamics under different terrain positions. The increase in SOC and TN concentration in the macroaggregates with N fertilization contributed to increased C and N sequestration into the soil. The storage of SOC and TN in the macroaggregates is propitious to improve soil fertility, mitigate greenhouse gas fluxes (e.g. CO_2 and N_2O) and to improve agroecosystem sustainability (Lal, 2004).

Soil organic carbon, bulk density, total nitrogen and glomalin concentration in bulk soil

The increase in SOC with increased N rates may be linked to increased C sequestered in crop biomass, and later returned to the soil as crop residue (Aulakh *et al*., 2001). The long-term application of mineral fertilizer led to an increased soil C content due to an enhancement in plant productivity and residue input (Campbell and Zentner, 1993). Previous studies have reported an increase in switchgrass belowground root biomass production along with high level of nutrient allocation to roots with increasing N fertilization compared with unfertilized conditions (Heggenstaller *et al*., 2009; Garten Jr *et al*., 2011).

Reduction in soil ρ_b with increasing N rate could be attributed to increase in SOC concentration in bulk soil which was likely in response to higher litter production (Hong *et al*., 2014; Bhattarai *et al*., 2021) and consequently more retention of residue over soil surface under 112-N treatment. The significantly negative relation between ρ_b and SOC found in this study further corroborate these findings (Fig. 1). Dense and deep root matrix of switchgrass can loosen the soil along with addition of soil organic matter thereby reducing the soil ρ_b (Thomas *et al*., 1996). The increase in SOM, soil structure and root biomass at the footslope can primarily contribute to decreased soil ρ_b (Guzman and Al-Kaisi, 2011). A previous study conducted at the current study site also reported a reduction in ρ_b at the footslope position (Singh *et al*., 2019). The improvement in soil aggregation and overall soil structure can reduce soil ρ_b at the footslope position. Landscape position can influence soil erosion and the distribution of SOM (Guzman and Al-Kaisi, 2011). Soil and its associated nutrients (e.g. nitrogen) generally erode from shoulder or summit positions and are deposited at the footslope positions (Papiernik *et al*.,

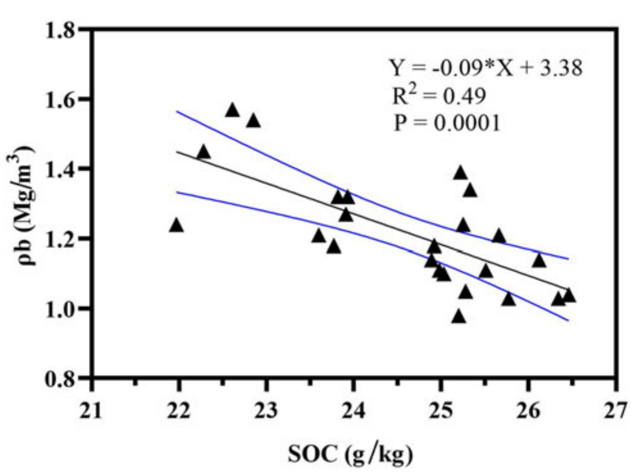

Figure 1. Relationship between soil bulk density (ρ_b) and organic carbon (SOC) content in bulk soil managed with varying nitrogen rates (0-N, 0 kg N/ha; 56-N, 56 kg N/ha and 112-N, 112 kg N/ha) under switchgrass planted at different landscape positions (shoulder and footslope).

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2007). Although soil erosion was not directly measured in the current study, simulation modelling research using historical management practices at this site has reported that tillage erosion, runoff and subsurface flow promoted soil, nutrient and SOM transport from the shoulder position to the footslope position (Mbonimpa *et al.*, 2015).

Application of N may have increased the formation of arbuscular mycorrhizal (AM) structures within plant roots (Treseder *et al.*, 2007) and consequently, the concentration of GRSP in the soil, however the GRSP was highest in the 56-N treatment. Lower GRSP concentration in 112-N treatment compared to the 56-N treatment could be due to the reason that the application of N can enhance the nutrient availability to plants and reduce their allocation of C to their associated microbes (Johnson *et al.*, 2003). Interestingly, a previous research conducted at the current study site found lower AM fungi biomass and total fungi biomass in 112-N (Sekaran *et al.*, 2019). Other studies have also reported a reduction in AM fungi biomass, diversity, root colonization and hyphal growth and GRSP with N addition in switchgrass (Emery *et al.*, 2017) and other natural and agroecosystems (Oates *et al.*, 2016). A meta-analysis reported that the reduction in AM fungi abundance was slightly more pronounced under greater N application rates, and that the N addition effects on AM colonization were positive in only 23% of studies (Treseder, 2004). Other factors such as climate, litter quantity and quality, AM diversity also influence the GRSP concentration in soils (Wilson *et al.*, 2009). These trends in GRSP content may not directly explain the higher aggregation with increasing fertilization in this study since a variety of gluing agents are involved in soil aggregation; and fertilization can significantly increase other organic aggregating agents such as organic matter compounds (Zhang *et al.*, 2016). The aggregate formation in soil is a result of many interactions occurring between a variety of cementing agents; and fertilization can affect different cementing agents in soil differently. Fertilization can increase the content of other gluing agents e.g. carbohydrates, extracellular polysaccharides, phenols, that play a crucial role in soil aggregation. For instance, Guo *et al.*, (2019) reported that the application of mineral fertilizer generally increased all the measured soil carbohydrates viz., acid-soluble, hot-water-soluble and cold-water-soluble carbohydrates, that aided in improving soil fertility, soil structure and increasing C sequestration. In another study, Sher *et al.*, (2020) found that soils with added N and P fertilizers had the highest switchgrass root biomass, extracellular polysaccharides and percentage of water-stable soil aggregates. They demonstrated that switchgrass cultivation can promote microbial production of extracellular polysaccharides, providing a mechanism to enhance aggregation in marginal soils.

The increased AM fungi and the microbial community at footslope position might be responsible for higher GRSP content at this landscape position. A prior research conducted at the current study site found significantly higher bacterial, fungal, actinomycetes, AM fungi, total microbial biomass and microbial biomass carbon at footslope compared to shoulder position (Sekaran *et al.*, 2019). Furthermore, the deep root system of switchgrass can form abundant and dense network with AM fungi (Emery *et al.*, 2017), and this interdependence between plant roots and fungi is highly effective in warm-season native grasses such as switchgrass (Brejda *et al.*, 1998). The AM fungi can stabilize soil aggregates directly by forming a hyphal network around soil particles, and indirectly by the hyphal exudation of GRSP (Rillig, 2004). Glomalin acts as a biological glue that prevents aggregates from rupturing by forming a waxy coating over the aggregates and thus has a cementing capacity to bind soil particles together (Wright and Upadhyaya, 1998). It has been reported that half-life of glomalin in soil ranges between 6–42 years and its relatively slow turnover in soil contributes to lasting effects on soil aggregation (Rillig, 2004; Welemariam *et al.*, 2018).

Conclusions

This study showed that nitrogen application to switchgrass stand planted at footslope position can improve soil quality in marginally yielding cropland. The results suggest that switchgrass crop management using N application rates in the recommended range (112 kg N/ha) is a beneficial way to return marginal land to production in eastern South Dakota. The main benefits expected from due fertilization are better physical structure of the soil, sequestration of SOC and nitrogen especially in the large soil aggregates, and improvement of footslope *vs.* shoulder in terms of proportion of 2–4 mm aggregates, and GRSP. The responses of soil aggregate stability indicators to the N fertilization can be mediated by the landscape position. Farmers' decisions are based both on economic and environmental considerations and further long-term research is needed to also investigate the economic impacts of switchgrass production managed with different nitrogen fertilization rates under diverse soil and environmental conditions.

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