




RESEARCH ARTICLE

Switchgrass cropping systems affect soil carbon and nitrogen and microbial diversity and activity on marginal lands

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Abstract

Switchgrass (*Panicum virgatum* L.), as a dedicated bioenergy crop, can provide cellulosic feedstock for biofuel production while improving or maintaining soil quality. However, comprehensive evaluations of how switchgrass cultivation and nitrogen (N) management impact soil and plant parameters remain incomplete. We conducted field trials in three years (2016–2018) at six locations in the North Central Great Lakes Region to evaluate the effects of cropping systems (switchgrass, restored prairie, undisturbed control) and N rates (0, 56 kg N ha⁻¹ year⁻¹) on biomass yield and soil physicochemical, microbial, and enzymatic parameters. Switchgrass cropping system yielded an aboveground biomass 2.9–3.3 times higher than the other two systems (Jayawardena et al., unpublished data) but our study found that this biomass accumulation did not reduce soil dissolved organic C, total dissolved N (TDN), or bacterial diversity. The annual aboveground biomass removal for bioenergy feedstock, however, reduced soil microbial biomass C (MBC) and microbial biomass N (MBN) and bacterial richness in the second and third years; despite this, continuous monocropping of switchgrass improved soil TDN, inorganic N, bacterial diversity, and shoot biomass in the second and/or third years compared with the first year. N fertilization increased aboveground biomass yield by 1.2 times and significantly increased soil TDN, MBN, and the shoot biomass of switchgrass compared with the unfertilized control. Locations with higher C and N contents and lower C:N ratio had higher aboveground biomass, MBC, MBN, and the activity of BG, CBH, and UREA enzymes; by contrast, locations with higher pH had higher soil TDN and activity of NAG and LAP enzymes. Our research demonstrates that switchgrass cultivation could improve or maintain soil N content and N fertilization can increase plant biomass yield. The comprehensive data also can inform future biogeochemical models to successfully implement switchgrass for bioenergy production.

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KEYWORDS

bioenergy, enzyme activity, microbial richness and diversity, N fertilization, soil fertility, switchgrass

1 | INTRODUCTION

Global energy consumption is projected to rise nearly 50% by 2050 compared with 2018, according to the latest International Energy Outlook (US EIA, 2020). Renewable energy, the fastest-growing energy source, is expected to increase by 3.1% per year between 2018 and 2050 (US EIA, 2020). To meet this ever-increasing demand while minimizing environmental harm, biofuels are an important component. However, more information is needed to characterize the productivity of bioenergy crops in relation to soil, climate, cropping system, and management practices. One important crop is switchgrass (*Panicum virgatum* L.), which was chosen by the US Department of Energy (DOE) from >30 herbaceous species as a model bioenergy feedstock crop in 1991 (Vogel, 1996). Switchgrass is a perennial warm-season (C_4) grass that requires relatively low inputs (Fike et al., 2017), is distributed widely in North America (Lewandowski et al., 2003), adapts to diverse conditions (Sanderson et al., 2006; Wright & Turhollow, 2010), grows on marginal land (Sanderson et al., 2006; Wright & Turhollow, 2010), and tolerates biotic and abiotic stresses (Sun et al., 2012). Furthermore, it can establish mutualistic associations with N-fixers (Bahulikar et al., 2021; Roley et al., 2018, 2019), increase soil organic carbon (Lemus & Lal, 2005; Robertson et al., 2011), reduce soil erosion and greenhouse gas emissions (McLaughlin et al., 2002; Williams et al., 2009), and be harvested with conventional hay-making equipment (Mitchell & Schmer, 2012).

Emerging efforts have been devoted to evaluating switchgrass productivity across various locations (Daly et al., 2017; Hong et al., 2014; Wulschleger et al., 2010), identifying the best nitrogen (N) application rates for switchgrass (Hong et al., 2014; Owens et al., 2013; Vogel et al., 2002), and comparing soil carbon (C) sequestration potential between the switchgrass cropping system and conventional cropping systems (Geisseler & Scow, 2014; Jung & Lal, 2011; Kibet et al., 2016; Lai et al., 2018). Compared with the conventional crop fields, marginal lands are not suitable for food production but could be promising for bioenergy feedstock production (Gelfand et al., 2013; Robertson et al., 2017). It is estimated that a total of 385–580 Mha of degraded lands could potentially be available for bioenergy production globally based on abandoned agricultural land (Campbell et al., 2008; Hall et al., 1993; Hoogwijk et al., 2003; Houghton et al., 1993). In the United States, there is 70–100 Mha land that is

currently available as marginal land-based on satellite and county land-use history maps (Campbell et al., 2013; Daly et al., 2017; Robertson et al., 2017), which provides considerable room for bioenergy production in the future. However, a comprehensive assessment of soil fertility, microbial diversity and activity, and plant productivity under switchgrass cropping systems compared with the undisturbed natural ecosystem and the restored prairie system at various locations is relatively unexplored. This information is of significance since it informs the long-term sustainability of switchgrass, which may become a new market opportunity for producers (Soldato et al., 2010). In addition, this information can add to the toolbox of decision-makers, researchers, county agents, and producers to make improved decisions when considering introducing switchgrass.

Thus, the objective of this study was to (1) evaluate the effects of cropping systems (switchgrass system, restored prairie system, and undisturbed control system) and N fertilization rates (0 and $56 \text{ kg N ha}^{-1} \text{ year}^{-1}$) on soil fertility, microbial biomass C (MBC) and N contents, microbial richness and diversity, the activities of C and N cycling-related enzymes, plant traits, and biomass yield and (2) validate how general the effects of cropping system and N fertilization are at six locations spanning Michigan and Wisconsin (Lux Arbor, Lake City, Escanaba, Oregon, Hancock, and Rhinelander) in three continuous years (2016, 2017, and 2018). We are interested in answering four research questions: (1) Compared with the restored prairie and the undisturbed systems with diverse plant species, would the monoculture of switchgrass reduce soil C and N contents, MBC and N, and microbial diversity and activity? (2) As a perennial grass, would the successive cropping of switchgrass in 3 years increase the belowground biomass of switchgrass, enhance soil organic C and total N pools, and stimulate the activity of C and N cycling-related enzymes? (3) As a bioenergy feedstock, would the annual removal of aboveground biomass remove considerable C and N from the soil and lead to decreases in soil dissolved organic carbon (DOC) and TDN contents in the second and/or third years compared with the first year? (4) Would the application of inorganic N fertilizer increase soil inorganic N availability, enzyme activity, and switchgrass biomass yield but negatively impact soil pH and microbial diversity? (5) Do the responses of soil and plant parameters to cropping systems and N fertilization rates vary across locations with different soil types and properties?

2 | MATERIALS AND METHODS

2.1 | Field description and sampling

The marginal land field trials were conducted at DOE Great Lakes Bioenergy Research Center (GLBRC, <https://data.sustainability.glbrc.org/pages/1.html>) at six locations spanning Michigan and Wisconsin [Lux Arbor (LUX), Lake City (LC), Escanaba (ESC), Oregon (ORG), Hancock (HAN), and Rhinelander (RHN)]; the overall trials are described elsewhere (Jayawardena et al., unpublished data), sampling for this study was conducted in three continuous years (2016, 2017, and 2018). The average annual precipitation, the average annual soil temperature, and background soil properties at each location are shown in Table 1. At each location, the experiment was conducted in a split-plot design comprised of three cropping systems (switchgrass system [G5], restored prairie system [G10], and undisturbed control system [G11]) as whole plots and two N fertilization rates (0 and 56 kg N ha⁻¹ year⁻¹) as subplots with four replications. The field trials were established in 2013, and switchgrass (*Panicum virgatum* L., variety Cave-in-Rock) was planted at a seeding rate of 8.07 kg ha⁻¹ in June 2013. The restored prairie system is a mix of 18 species of native grass, legumes, and forbs, seeded in 2013 at a seeding rate of 7.96 kg ha⁻¹, including graminoids (switchgrass, Canada wildrye, big bluestem, little bluestem, Indiangrass, prairie Junegrass), legumes (showy tick-trefoil, roundhead lespedeza, white false indigo), early forbs (black-eyed Susan, Canadian anemone, butterfly milkweed), mid forbs (cup plant, wild bergamot, pinnate prairie coneflower), and late forbs (rigid goldenrod, showy goldenrod, New England aster). The undisturbed control system was not seeded since the establishment of these sites in 2013, and plants in this system are volunteers.

Each plot is either 20 m × 20 m (LC, ESC, ORG, HAN, and RHN) or 20 m × 12 m (LUX), depending on the site. To replace the soil N lost through the annual removal of aboveground biomass, urea (44–0–0) was applied at 56 kg N ha⁻¹ to the fertilized subplots in May each year by a Ford 1510 tractor (Ford Motor Company) and a Grandy 10-ft drop spreader (Gandy Company). No phosphorus (P) and potassium (K) fertilizer were applied in these plots. The crops were managed under conventional production practices consistent with best management practices as recommended by Wisconsin (UW) and Michigan State University (MSU) extension agronomists. During mid-October and mid-November each year, switchgrass in the switchgrass cropping system and grasses in the restored prairie system (18-species mix including forbs, grasses, and legumes) were chopped at 15 cm height with Kemper mounted forage harvester C2200 (Maschinenfabrik

Kemper GmbH & Co. KG, Stadtlon), AGCO RT120A tractor (AGCO Corporation), and Meyer 4110 Forage wagon (Meyer Manufacturing Corporation). For the undisturbed control system, 1 m × 2 m of volunteer plants were sampled from each split-plot as a reference. The fertilization, sampling, and harvesting dates at all sites during 2016–2018 are shown in Table S1.

Rhizosphere soils were collected in July each year from the six locations for the determination of soil physicochemical, microbial, and enzymatic parameters. A total of 432 rhizosphere soil samples (6 locations × 3 cropping systems × 2 N rates × 4 replicate plots × 3 pseudo cores) were collected each year. Meanwhile, three switchgrass plants were sampled at the same spots as the soil cores in each split-plot for determinations of plant aboveground height and shoot dry biomass. Switchgrass root samples in the ingrowth cores (stiff plastic mesh pots with a 5 cm diameter, a 13 cm height, and a hole size of 2 mm) that were randomly placed in each split-plot were also collected for determination of root growth in that year, including root dry biomass, root length, and root width. The ingrowth cores were stapled in the field in winter each year to form a cylinder with a plastic cap at the bottom. A mix of field soil, removed from the installation site, and perlite was added to the cores in a 1:1 ratio. At sampling, the rhizosphere soil and plant samples were put in an ice box immediately. After being transferred into the laboratory on the same day, the soil was divided into three portions: one was used for the determination of soil moisture, one was air-dried, ground, and passed 2-mm sieve for physicochemical analysis, and the other one was passed 2-mm sieve and stored at –80°C for enzymatic and genomics analyses. The plant roots were rinsed with water and Tween® 20 (Sigma Aldrich Co LLC) over a 1-mm sieve in the lab to remove soil particles that could interfere with scanning, separated with shoots for determining the fresh weight of each part, scanned for measuring root length and width, and put into the oven for drying. At field harvest during mid-October and mid-November, switchgrass in each plot was chopped at 15 cm height and weighed on the same day as the fresh biomass. A known weight of plant subsample from each plot was dried at 60°C for at least 48 h until constant weight for determining plant moisture and calculating dry biomass.

2.2 | Soil physicochemical properties determination

DOC is a component of the soil active organic carbon (Matlou & Haynes, 2006), which is an organic carbon source for microorganisms in the soil. In this study, soil carbon (C) and nitrogen (N) in the labile organic pools

TABLE 1 Site description and background soil properties

Location	Lux Arbor (LUX) MI south	Lake City (LC) MI central	Escanaba (ESC) MI north	Oregon (ORG) WI south	Hancock (HAN) WI central	Rhineland (RHN) WI north
Coordinates (latitude, longitude)	42.4764, -85.4519	44.2961, -85.1996	45.7627, -87.1877	42.9661, -89.3561	44.1194, -89.5338	45.6656, -89.2180
Annual soil temperature (°C)	10.1	6.3	5.6	8.3	6.7	5
Annual precipitation (mm)	1005	823	724	940	1134	800
Soil series	Kalamazoo/Oshtremo	Croswell	Onaway	Dodge	Plainfield	Vilas
Taxonomic class	Typic Hapludalf (Alfisol)	Oxyaquic Haplorthod (Spodosol)	Inceptic Hapludalf (Alfisol)	Typic Hapludalf (Alfisol)	Typic Udipsamment (Entisol)	Entic Haplorthod (Spodosol)
Surface (0–20 cm) soil texture class	Loam	Loamy sand	Sandy loam	Silt loam	Loamy sand	Sandy loam
Sand (%)	51.1	84.7	57.1	9.1	87.6	59.1
Silt (%)	31.7	7.8	27.7	74.8	5.9	25.8
Clay (%)	17.2	7.5	15.2	16.1	6.5	15.1
Bulk density (g cm ⁻³)	1.75 ± 0.09	1.51 ± 0.23	1.26 ± 0.16	1.11 ± 0.37	1.44 ± 0.03	1.35 ± 0.20
pH	5.8	7.3	7	6.9	6.3	5.7
CEC (cmol kg ⁻¹)	8.8	5.6	10.4	13	3.4	7.6
Organic C (%)	0.77	0.92	1.73	1.58	0.69	1.09
Total N (%)	0.06	0.06	0.15	0.16	0.05	0.07
C:N ratio	12.6	14.4	11.4	10.2	12.7	15.9
Inorganic C (%)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Inorganic P (ppm)	12	24	14	9	128	193
K (ppm)	52	45	43	81	84	105
Ca (ppm)	674	760	1305	1451	413	409
Mg (ppm)	70	101	151	392	74	53
Na (ppm)	6	229	16	7	42	2

Abbreviations: MI, Michigan; WI, Wisconsin.

Note. Soil pH was determined using 1:1 dry soil:water suspension. Inorganic P was determined using Bray-Kurtz P1 extraction. More information of the marginal land experimental site and background soil properties can be found at Great Lakes Bioenergy Research Center website (<https://data.sustainability.glbc.org/pages/1.html>) and Kasmerchak and Schaezel (2018).

(DOC, total dissolved N [TDN]), inorganic pools (NH_4^+ , NO_3^- , plant-available N), microbial pools (MBC, microbial biomass N [MBN]), as well as soil pH and soil moisture were determined.

Six grams of soil were extracted by 30 ml of 0.5 M K_2SO_4 and filtered with Whatman® filter paper (Grade 202) as described in Smercina et al. (2021). DOC and TDN were determined using a vario TOC cube (Elementar Americas Inc.) following the manufacturer's instruction. Soil inorganic N (NH_4^+ , NO_3^-) in the extractant was determined using 96-well high-throughput colorimetric methods as described by Smercina et al. (2021). Soil MBC and N (MBN) were determined by the chloroform fumigation direct extraction method as described by Anderson and Domsch (1978) and Gregorich et al. (1990) and a vario TOC cube (Elementar Americas Inc.) following the manufacturer's manual. MBC and MBN were calculated using DOC and TDN contents according to the equations reported by Beck et al. (1997) and Brookes et al. (1985). Soil pH was determined in a 1:2 (w/v) soil: deionized water extractant with a VWR Symphony B20PI Benchtop pH meter (VWR International, LLC) (Schofield & Taylor, 1955). Soil moisture was measured following the gravimetric method described by Reynolds (1970).

2.3 | Soil microbial richness and diversity assessment

Soil microorganisms play a critical role in organic matter decomposition, nutrient cycling, and soil productivity (Fierer et al., 2021; Ramírez et al., 2020). Soil DNA for 2016 and 2017 was extracted from 0.5 g soil using a PowerSoil® DNA Isolation Kit (MO BIO Laboratories Inc.) following the manufacturer's protocol. Soil DNA for 2018 was extracted in 96-well plates using the KingFisher Flex Purification System (Thermo Fisher Scientific) with the PowerSoil® kit. The extracted soil DNA was electrophoresed on 1% agarose gels, and the quality and quantity of DNA were evaluated using a NanoDrop-1000 spectrophotometer (Thermo Fisher Scientific). For bacteria, the V4 hypervariable region of 16S rRNA genes was amplified using 515F/806R primers (Caporaso et al., 2011), and the Illumina compatible libraries were prepared using primers containing both the target sequences and the dual indexed Illumina compatible adapters (Kozich et al., 2013) by Michigan State University (MSU) Research Technology Support Facility (RTSF) Genomics core. For fungi, ITS1 region was amplified using ITS1-F/ITS2 primers (White et al., 1990), and libraries were multiplexed using a three-step PCR sequence as described by Chen

et al. (2018). The completed libraries were normalized using Invitrogen SequelPrep DNA Normalization plates and pooled and cleaned up using AmpureXP magnetic beads. Libraries were then paired-end sequenced by MSU RTSF Genomics core on a MiSeq platform (Illumina Inc.) using the v2 kit for bacterial libraries and the v3 kit for fungal libraries. Bioinformatics and sequence processing was conducted using Quantitative Insights Into Microbial Ecology (QIIME) 2 (Bolyen et al., 2019) and USEARCH (Edgar, 2010), and details can be found in the Supporting information. A total of 21,450 bacterial OTUs and 2824 fungal OTUs were rarefied to 10,000 reads to evaluate the two richness indices, Chao1 (Chao, 1984) and Abundance-based Coverage Estimator (ACE) (Chao & Lee, 1992), and two diversity indices, Shannon-Weiner (Shannon, 1948) and reverse Simpson (Invsimpson) (Simpson, 1949). All diversity metrics were calculated using the vegan package in R (Oksanen et al., 2014).

2.4 | Soil carbon (C) and nitrogen (N) cycling-related enzymes activity assay

Soil enzyme activities are the indicators of microbial community and functions. Soil extracellular enzymes decompose substrates of varying composition and complexity (Jian et al., 2016; Sinsabaugh, 2010) and play an important role in C sequestration and N cycling (Bowles et al., 2014; Keane et al., 2020). In this study, five C and N cycling-related enzymes, cellobiohydrolase (CBH, also called β -d-cellobiosidase) and β -glucosidase (BG), urease (UREA), N-acetyl- β -glucosaminidase (NAG), and leucine aminopeptidase (LAP), were determined to evaluate how switchgrass cultivation and N rate affect soil C sequestration and N availability.

Soil BG (EC 3.2.1.21) and CBH (EC 3.2.1.91) are commonly studied extracellular glycosidases to reveal the potential microbial activities associated with fast-turnover organic C (Klose & Tabatabai, 2002; Sinsabaugh et al., 2008). Soil BG catalyzes the hydrolysis of β -D-glucopyranoside and is involved in the saccharification of cellulose (Bandick & Dick, 1999; Deng & Tabatabai, 1994; Tabatabai, 1994; Turner et al., 2002); CBH hydrolyzes the end of the cellulose chain and produces glucose or cellobiose as the end product (Lynd et al., 2002). Of the three N-cycling enzymes, UREA (EC 3.5.1.5) regulates the soil N transformation and is in charge of the hydrolysis of urea into ammonia and CO_2 (Kong et al., 2008); soil NAG (EC 3.2. 1.30) and LAP (EC 3.4.11.1) regulate the release of plant-available N from organic compounds (Sinsabaugh et al., 2008). Activities of NAG, BG, CBH, and LAP were measured

by a fluorometric method (DeForest, 2009; German et al., 2012; Kim et al., 2018; Saiya-Cork et al., 2002) and a BioTek microplate reader (BioTek Instruments). Activities of NAG, BG, and CBH were measured at an excitation wavelength of 370 nm and an emission wavelength of 455 nm, and activity of LAP was determined at an excitation wavelength of 350 nm and an emission wavelength of 430 nm. Their activities were expressed as $\text{nmol g}^{-1} \text{ dry soil h}^{-1}$. Soil UREA activity was measured using the method described by Sinsabaugh et al. (2000) and urea (Millipore Sigma, 57–13-6) and determined spectrophotometrically at 610 nm. The activity of UREA was expressed as $\text{nmol NH}_4^+ \text{ g}^{-1} \text{ soil h}^{-1}$.

2.5 | Determination of plant traits and biomass yield

In July each year, the aboveground plant height, shoot biomass, root biomass, root length, and root width of switchgrass in each plot were determined. Switchgrass roots from the core were washed and scanned with an Epson Perfection V600 Photo Scanner (Seiko Epson Corporation), and the 1200-dpi image was compressed to 400-dpi and analyzed with Gia Roots to measure the length and width of the longest roots (Galkovsky et al., 2012). The shoot and root were oven-dried at 60°C for at least 48 h until a constant weight for determining shoot dry biomass and root dry biomass. At field harvest during mid-October and mid-November each year, all plants were weighed on the same day as the fresh biomass. Plant subsamples were collected and dried at 60°C for at least 48 h until constant weight for plant moisture determination and dry biomass calculation.

2.6 | Statistical analysis

Multivariate analysis of variance (MANOVA) was performed in R (<https://www.r-project.org/>) to evaluate the effects of cropping system and N level on soil physicochemical properties, microbial richness and diversity, enzymes activity, plant traits, and biomass yield, as well as to evaluate the variations across the six locations and in the 3 years. A full model was used to see if there are four-way interactions of cropping system \times N rate \times year \times location in this study. Based on the Pillai's trace, no significant four-way interactions was found at 0.05 significance level for all tested dependent variables; thus, the dropped model without the four-way interaction was used as the final model. Mean separation was done using Tukey's HSD post hoc procedure in R at a significance level of 0.05. Pearson's correlation (r) was

calculated between each pair of variables using "rcorr" function and "Hmisc" package.

3 | RESULTS

3.1 | Soil physicochemical properties under switchgrass cropping systems

The six locations have diverse soil types and background properties (Table 1; Kasmerchak & Schaetzl, 2018). Briefly, Escanaba (ESC) and Oregon (ORG) had considerably higher organic C and total N contents but lower C/N ratio than the other four locations; Lake City (LC) and Escanaba (ESC) had the highest background soil pH ($\text{pH} \geq 7$), which was followed by ORG and HAN (pH ranges from 6 to 7) and lowest in LUX and RHN ($\text{pH} \leq 6$).

Soil DOC content was not impacted by N rate but interactively affected by cropping system \times year \times location ($p \leq 0.05$) (Table 2). The switchgrass system and the two reference systems were not significantly different overall in soil DOC content (Figure 1a, Figure S1a–c). At LUX, LC, ESC, and ORG, soil DOC content in the switchgrass system increased by 1–1.4 times in the second and third years compared with the first year; at HAN and RHN, soil DOC content in the switchgrass system decreased by 10%–30% in the second year compared with the first year (Figure S1a–c). Nevertheless, the DOC contents at LUX, LC, ESC, and RHN were significantly higher than those at ORG and HAN, particularly in the second year (Figure 1d). In the switchgrass system, the average DOC content was 95.2–110.1 mg kg^{-1} at LUX, LC, ESC, and RHN but only 50.8–61.2 mg kg^{-1} at ORG and HAN (Figure S1a–c).

Soil TDN was significantly affected by the interaction of year \times location ($p \leq 0.01$) and the main effect of N rate ($p \leq 0.001$) (Table 2). The average TDN was 1.3–1.4 times higher in the second and third years than in the first year, significantly higher at ESC (18.5 mg kg^{-1}) and LC (17.4 mg kg^{-1}) than at the other four locations (12.3–14.7 mg kg^{-1}), and significantly higher in the fertilized soils (16.2 mg kg^{-1}) than in the unfertilized soils (14.4 mg kg^{-1}) (Figure 1f–h). Of soil TDN, NH_4^+ was significantly affected by the interactions of cropping system \times N rate ($p \leq 0.05$) and year \times location ($p \leq 0.001$), whereas NO_3^- was only significantly affected by N rate ($p \leq 0.05$) (Table 2). Switchgrass cropping system did not decrease soil TDN, NH_4^+ , or NO_3^- content compared with the undisturbed control system and the restored prairie system (Figures 1e and 2a,c).

Soil MBC and N (MBN) were strongly correlated with each other ($r = 0.821$, $p \leq 0.001$) and both were affected by cropping system, year, and location (Table 3; Figure 3a–h, Figure S2a–f). Soil MBC was significantly affected by the

interaction of year \times location ($p \leq 0.05$) and the main effect of cropping system ($p \leq 0.01$), whereas MBN was significantly affected by the interaction of cropping system \times year ($p \leq 0.05$) and the main effect of location ($p \leq 0.0001$) (Table 2). Both MBC and MBN contents were lowest in the switchgrass system, followed by the restored prairie system, and highest in the undisturbed control system (Figure 3a,e); both were lower in the second and third years compared with the first year (Figure 3c,g). Of the six locations, both MBC and MBN was highest at ORG and ESC, followed by LC and LUX, and lowest at RHN and HAN (Figure 3d,h). Both MBC and MBN were significantly correlated with soil moisture (r values = 0.268–0.135, p values ≤ 0.01) and NH_4^+ content (r values = 0.116–0.136, p values ≤ 0.05) (Table 3).

Soil pH was significantly affected by the main effects of cropping system ($p \leq 0.01$) and location ($p \leq 0.0001$) (Table 2; Figure 2d). Of the three cropping systems, the average pH was lowest in the switchgrass system (pH 6.1), followed by the restored prairie system (pH 6.3), and highest in the undisturbed control system (pH 6.4). Of the six locations, soil pH was highest at LC and ESC (pH 6.6–6.8), followed by LUX and HAN (pH 6.2–6.3), and lowest at RHN (pH 5.3). Compared with background soils pH in 2013, soil pH decreased at LC, ESC, and RHN, did not change at HAN, but increased at LUX (Table 1; Figure 2d). Soil pH was significantly correlated with most microbial richness and diversity indices and enzyme activities (Table 3). Soil moisture (%) was significantly affected by the interactions of cropping system \times N rate ($p \leq 0.05$) and year \times location ($p \leq 0.001$) (Tables 2; Figure 2f). Soil moisture was greatest at 56 kg N ha^{-1} in the restored prairie system and lowest at 0 kg N ha^{-1} in the undisturbed control system across the three systems; soil moisture was greatest at ORG and lowest at HAN across the six locations. Soil moisture was significantly correlated with soil microbial richness and diversity, enzyme activities, and plant biomass (Table 3).

3.2 | Soil microbial richness and diversity under switchgrass cropping systems

For the soil bacterial community, the number of 16S rRNA OTUs and both richness indices (Chao1 and Abundance-based Coverage Estimator) were not impacted by N rate but interactively affected by cropping system \times year \times location (p values ≤ 0.01); by contrast, neither diversity indices (Shannon and Invsimpson) were impacted by cropping system or N rate, but both indices were significantly affected by the interaction of year \times location (p values ≤ 0.001) (Table 2). At each location, there was no significant difference in the number of bacterial OTUs or bacterial richness indices among the three cropping

systems across all 3 years (Figure S3a–i). Compared with the first year, the number of bacterial OTUs and both richness indices were significantly lower in the second and third years, but both diversity indices were significantly higher in the third year (Figure 4c,g,k). Of the six locations, the number of bacterial OTUs and both richness indices were consistently highest at HAN and ESC and lowest at LUX and ORG, whereas both diversity indices were highest at RHN and HAN, followed by ESC and LC, and lowest at LUX and ORG (Figure 4d,h,l). Interestingly, neither the number of bacterial OTUs nor the richness indices had a significant correlation with any soil C and N contents tested in this study; however, both diversity indices were negatively correlated with soil MBC (r values = -0.553 to -0.599 , p values ≤ 0.001) and MBN (r values = -0.527 to -0.609 , p values ≤ 0.001) (Table 3, Table S2).

Soils collected in 2018 were used to further evaluate the richness and diversity of the fungal community and the fungal/bacterial richness (F/B OTU) ratio (Table 2; Figure S4a–e). The fungi/bacteria richness ratio was calculated based on the number of OTUs, Chao1, and ACE index, respectively, and their trends were consistent. For example, the F/B OTU ratio was highest at LUX and ESC, followed by LC and RHN, and lowest at HAN. Of the tested soil properties, F/B OTU ratio had significant and positive correlations with soil NH_4^+ content, MBC and N, and soil moisture, but had negative correlations with soil NO_3^- content and C/N ratio (Table 3, Table S2).

3.3 | Soil C and N cycling-related enzymes activity under switchgrass cropping systems

The activities of five soil C and N cycling-related enzymes associated with switchgrass, including BG, CBH, UREA, NAG, and LAP, were measured in this study, thus we were able to evaluate the effects of N rate, year, and location on enzyme activities but not able to look at the effect of cropping system. Consistently, N rate did not impact the activity of any soil enzymes measured in this study, and neither year nor location affected the activities of BG and CBH (Table 2). Soil UREA and NAG activities were significantly affected by the interaction of year \times location ($p \leq 0.001$), whereas LAP activity was significantly affected by location ($p \leq 0.001$) (Table 2). Across the six locations, ESC and ORG had significantly greater BG, CBH, and UREA activities in soils associated with switchgrass compared with the other four locations (Figure 5a–i), whereas ESC and LC had significantly greater NAG and LAP activities than the other four locations (Figure 5j–o).

The activities of soil BG, CBH, UREA, and LAP associated with switchgrass were significantly correlated

TABLE 2 Significance of the interactions and main effects of cropping system, N rate, location, and year on soil physicochemical properties, microbial richness and diversity, enzyme activities, and aboveground plant biomass

Parameters	Cropping system	N rate	Year	Location	Cropping system × N rate
Soil physicochemical properties					
DOC	0.9365	0.5176	0.0009***	0.0018**	0.3002
TDN	0.5081	0.0003***	<0.0001***	0.0029**	0.0599
NH ₄ ⁺	0.4005	0.1499	0.0225*	0.0042**	0.0110*
NO ₃ ⁻	0.9870	0.0350*	0.3851	0.5038	0.2275
MBC	0.0018**	0.3390	<0.0001***	0.0008***	0.3309
MBN	0.0030**	0.0680	<0.0001***	<0.0001***	0.2585
C/N ratio	0.9352	0.1709	0.4079	0.9152	0.7702
pH	0.0021**	0.3732	NA	<0.0001***	0.5976
Soil moisture	0.1301	0.1257	0.3072	0.9167	0.0192*
Soil microbial community					
Number of OTUs	0.1512	0.8242	0.0015**	0.0001***	0.4413
Chao 1	0.4704	0.5988	0.0001***	0.0002***	0.4271
ACE	0.3541	0.5657	0.0001***	0.0011**	0.4429
Shannon	0.1430	0.8987	0.0001***	<0.0001***	0.8422
Invsimpson	0.1455	0.9191	<0.0001***	<0.0001***	0.8729
F/B ratio	0.0445*	0.0472	NA	<0.0001**	0.8238
Soil enzyme activity					
BG	NA	0.8326	0.3373	0.4713	NA
CBH	NA	0.7197	0.6474	0.6054	NA
UREA	NA	0.9599	0.1159	0.7618	NA
NAG	NA	0.9421	<0.0001***	0.0009***	NA
LAP	NA	0.8853	0.1297	0.0009***	NA
Aboveground plant biomass					
Fresh biomass	<0.0001***	0.0001***	0.2277	0.0004***	0.0950
Dry biomass	<0.0001***	0.0004***	0.6260	<0.0001***	0.2032

Note: Based on the Pillai's trace, no significant four-way interaction of year × location × cropping system × N rate was found at 0.05 significance level for all tested dependent variables; thus, a dropped model, by removing the four-way interaction, was used as the final model. Three-way interactions that have no significant effect on any variables included in this study were not shown in the above table. *, significant at the 0.05 probability level; **, significant at the 0.01 probability level; ***, significant at the 0.001 probability level. NA means data was collected only in 1 year or one cropping system, which was not allowed to get interaction or main effect data. DOC, dissolved organic C; TDN, total dissolved N; MBC, microbial biomass C; MBN, microbial biomass N. Number of OTUs at 97% similarity, Chao 1, ACE, Shannon, and Invsimpson were based on analysis of 16S rRNA sequences. Fungi/bacteria (F/B) ratio was based on the ratio of fungal OTU number to the bacterial OTU number. BG, 1,4-β-glucosidase; CBH, β-d-cellobiosidase; UREA, urease; NAG, N-acetyl-β-glucosaminidase; LAP, leucine aminopeptidase; ACE, abundance-based coverage estimator. The aboveground biomass of the trials in the present study and the overall GLBRC trials can be found in Jayawardena et al. (unpublished data).

with each other, whereas the activity of NAG was only significantly correlated with LAP (Table 3, Table S2). However, it is consistent that soil TDN positively affected the activities of all enzymes measured in this study. The activities of both C cycling-related enzymes (BG and CBH) were significantly and positively correlated with microbial biomass (MBC and MBN) and richness (Chao 1 and ACE), but significantly and negatively correlated with soil C/N ratio (Table 3, Table S2); the activities of all three N cycling-related enzymes (UREA, NAG, and LAP) were significantly and positively correlated with soil pH.

Meanwhile, variations in the responses of the N cycling-related enzymes to soil and microbial parameters were also observed. Soil UREA and LAP were significantly and positively correlated with soil MBC and MBN, whereas NAG had a negative correlation with MBC and no correlation with MBN; soil NAG and LAP were significantly and negatively correlated with soil C/N ratio, whereas UREA did not have a correlation with soil C/N ratio; soil LAP had significant and positive correlations with soil bacterial richness (Chao 1 and ACE), whereas UREA and NAG did not have a correlation with soil bacterial richness.

Cropping system × year	Cropping system × location	N rate × year	N rate × location	Year × location	Cropping system × year × location
0.3254	0.5424	0.2984	0.2267	0.0036**	0.0191*
0.7823	0.2208	0.4536	0.6922	0.0063**	0.3480
0.3124	0.7597	0.2891	0.4508	0.0008***	0.4433
0.7074	0.6211	0.9107	0.2513	0.2255	0.6164
0.8465	0.3802	0.6201	0.8277	0.0185*	0.7485
0.0121*	0.4050	0.8248	0.9979	0.1279	0.7971
<0.0001***	0.1251	0.1251	0.5286	0.1996	0.1925
NA	0.9002	NA	0.1164	NA	NA
0.9097	0.5377	0.7212	0.4018	0.0006***	0.6311
0.1457	0.0604	0.7545	0.1366	0.0018**	0.0026**
0.4498	0.2864	0.6620	0.1555	0.2970	0.0003***
0.4455	0.1894	0.5929	0.1676	0.2894	0.0003***
0.1889	0.0977	0.9397	0.2647	0.0004***	0.0900
0.3746	0.1490	0.5382	0.5596	<0.0001***	0.1288
NA	0.08924	NA	0.7832	NA	NA
NA	NA	0.5206	0.9825	0.2766	NA
NA	NA	0.7760	0.8455	0.9151	NA
NA	NA	0.7695	0.2833	0.0009***	NA
NA	NA	0.9875	0.8445	<0.0001***	NA
NA	NA	0.8407	0.9025	0.3842	NA
0.0149*	0.0004***	0.5140	0.1947	0.1080	0.2761
0.0343*	0.0006***	0.6622	0.9828	0.0268*	0.7837

3.4 | Plant traits and biomass yield under switchgrass cropping systems

Plant aboveground fresh and dry biomasses at harvest were significantly affected by the interactions of cropping system × year (p values ≤ 0.05) and cropping system × location (p values ≤ 0.001) and the main effect of N rate (p values ≤ 0.001) (Table 2, Jayawardena et al., unpublished data). Of the three cropping systems, plant aboveground biomass was significantly greater in the switchgrass system compared with the other two systems and the trend

was consistent in the 3 years (Figure 6a–c). Compared with 2016, the average biomass across locations was greater in 2017 but lower in 2018 (Figure 6a–c). Of the six locations, the average aboveground biomass of the 3 years was greatest at ORG, followed by RHN, ESC, LUX, and LC, and lowest at HAN (Figure 6a–c). Additionally, the application of 56 kg N ha^{-1} fertilizer significantly increased plant aboveground biomass compared with the unfertilized control (Table 2).

Switchgrass plant traits, including total shoot and root dry biomass, shoot dry biomass, root dry biomass,

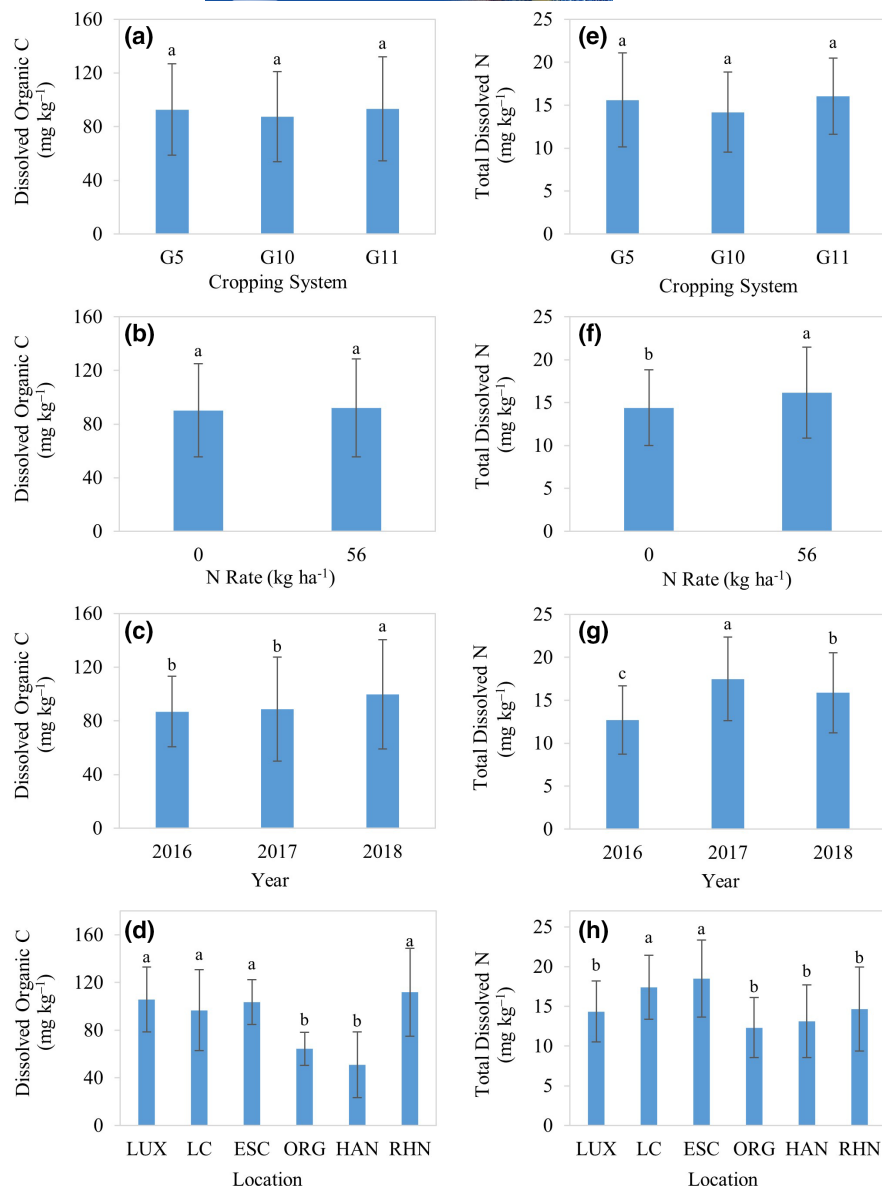


FIGURE 1 Effects of cropping system, N rate, year, and location soil dissolved organic C (mg kg⁻¹) and total dissolved N (mg kg⁻¹) contents. G5, switchgrass system; G10, restored prairie system; G11, undisturbed control system. LUX, Lux Arbor, MI; LC, Lake City, MI; ESC, Escanaba, MI; ORG, Oregon, WI; HAN, Hancock, WI; RHN, Rhinelander, WI. Bars with different letters mean that there is a significant difference between the two treatments (cropping systems, N rates, years, or locations) according to Tukey's HSD test at a significant level of 0.05

shoot/root biomass ratio, root length, root width, and the aboveground plant height, were further evaluated (Table 3, Table S2; Figure 7a–d). Both total dry biomass and shoot dry biomass were significantly affected by N rate ($p \leq 0.05$) and year ($p \leq 0.0001$), which were significantly higher in the fertilized soils than the unfertilized soils and significantly higher in the second and third years than the first year (Figure 7b,c). Total dry biomass and shoot dry biomass were significantly correlated with soil moisture (r values = 0.184–0.208, p values ≤ 0.05), F/B OTU ratio (r values = 0.590–0.699, p values ≤ 0.001), and UREA activity (r values = 0.405–0.571, p values ≤ 0.05) (Table 3, Table S2). By contrast, root dry biomass was not impacted by the main effect of N rate or year but it varied significantly across locations (Figure 7d). Root dry biomass was greatest at LUX and lowest at HAN across the six locations, and it was

positively correlated with soil NH_4^+ content ($r = 0.262$, $p \leq 0.001$), C/N ratio ($r = 0.349$, $p \leq 0.05$), and F/B OTU ratio ($r = 0.590$, $p \leq 0.001$) (Table 3). The shoot/root biomass ratio was consistently higher at ESC and ORG than the other four locations, whereas there were no clear trends of the shoot/root biomass ratio between two N rates or among the 3 years (data not shown). Root length and width had significant and positive correlations with root dry biomass (r values = 0.440–0.682, $p \leq 0.05$) (Table S2). Root length was positively correlated with soil DOC ($r = 0.441$, $p \leq 0.05$), TDN ($r = 0.540$, $p \leq 0.01$), soil moisture ($r = 0.311$, $p \leq 0.01$), NAG activity ($r = 0.342$, $p \leq 0.01$), but negatively correlated with bacterial richness (r values = -0.565 to -0.621 , $p \leq 0.01$); by contrast, root width was negatively correlated with soil moisture ($r = -0.225$, $p \leq 0.05$) (Table 3, Table S2). In addition, the aboveground plant height was significantly

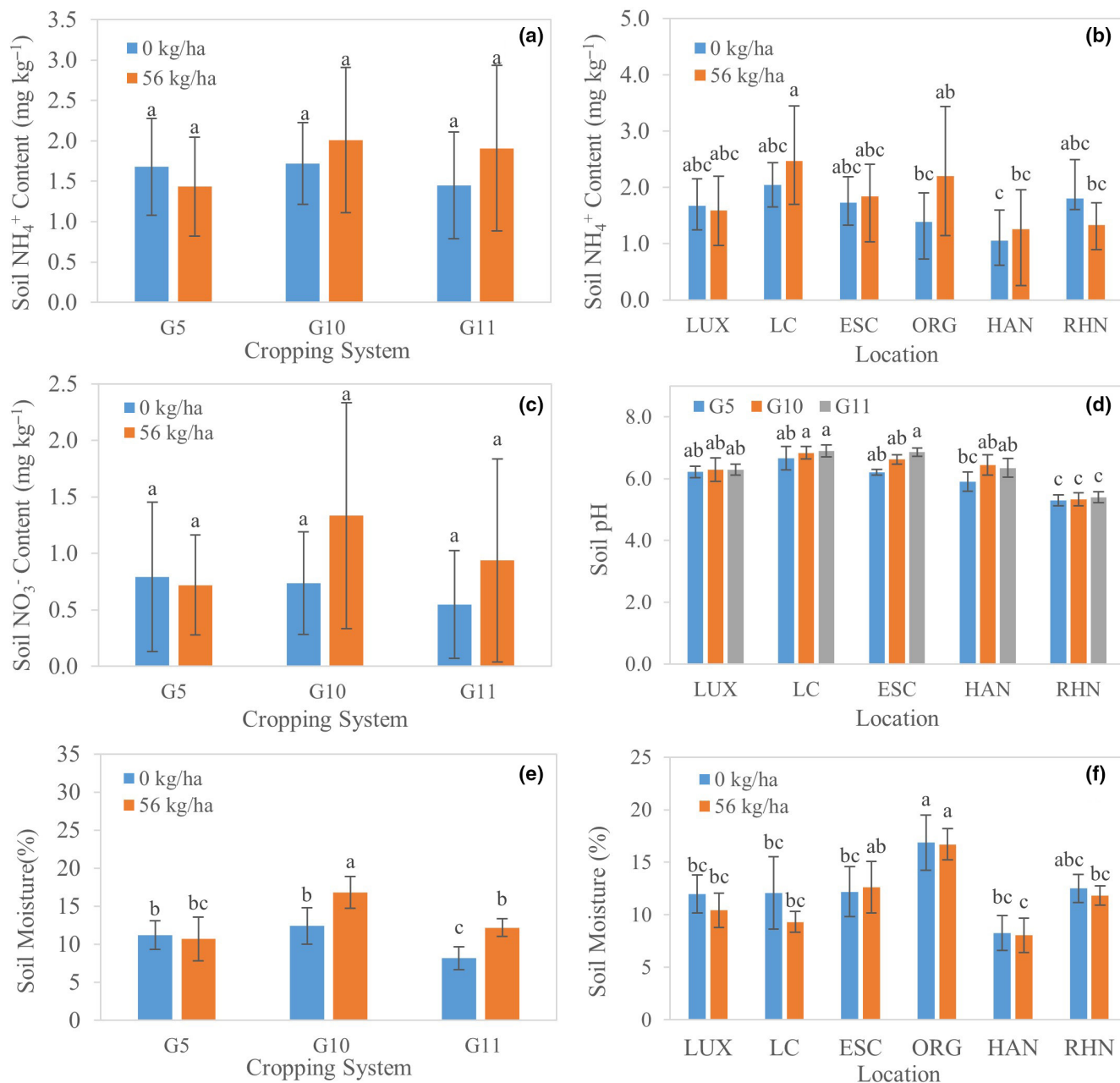


FIGURE 2 Effects of cropping system, N rate, year, and location on soil ammonium-N, nitrate-N, pH, and soil moisture. Soils collected in 2018 were used to determine soil pH. G5, switchgrass system; G10, restored prairie system; G11, undisturbed control system. LUX, Lux Arbor, MI; LC, Lake City, MI; ESC, Escanaba, MI; ORG, Oregon, WI; HAN, Hancock, WI; RHN, Rhinelander, WI. Bars with different letters mean that there is a significant difference between the two treatments (cropping systems, N rates, years, or locations) according to Tukey's HSD test at a significant level of 0.05

($p \leq 0.01$) affected by year; switchgrass was tallest in 2017 (153 cm), followed by 2018 (141 cm), and shortest in 2016 (130 cm) (Figure 7a). The plant height was positively correlated with soil MBC ($r = 0.423$, $p \leq 0.05$) and MBN ($r = 0.454$, $p \leq 0.01$) and the activities of soil BG ($r = 0.545$, $p \leq 0.001$), CBH ($r = 0.404$, $p \leq 0.001$), and UREA ($r = 0.511$, $p \leq 0.001$) but negatively correlated with soil microbial diversity indices (r values = -0.345 to -0.378 , p values ≤ 0.05).

4 | DISCUSSION

4.1 | Effect of switchgrass cultivation and N fertilization on soil fertility

As the components of soil labile C and N pools, DOC and TDN play crucial roles in soil C and N cycles and plant nutrient availability (Guicharnaud et al., 2010; Kalbitz et al., 2000; Rui et al., 2011). Compared with soil total

TABLE 3 Pearson's correlation (*r*) between selected soil nutrient contents, microbial richness and diversity, enzyme activities, and plant traits

Parameter	TDN	NH ₄ ⁺	NO ₃ [−]	MBC	MBN	pH	Soil moisture	OTUs	Chao1
DOC	0.36***	0.00	−0.09	−0.05	0.07	−0.44*	0.18***	−0.12	−0.09
TDN		−0.02	0.06	−0.11	0.04	0.60**	0.09	0.03	0.01
NH ₄ ⁺			0.24***	0.14**	0.12*	−0.12	−0.01	−0.04	0.01
NO ₃ [−]				0.01	0.02	0.30***	−0.24**	0.08	0.08
MBC					0.82***	0.29	0.13**	−0.16	0.02
MBN						0.04	0.27***	−0.17	−0.02
pH							−0.71***	0.54*	0.42*
Soil Moisture								−0.22***	−0.22***
OTUs									0.93***
Chao1									
Shannon									
F/B ratio									
BG									
CBH									
UREA									
LAP									
Field fresh biomass									
Shoot dry biomass									

Abbreviations: BG, 4-β-glucosidase; CBH, cellobiohydrolase; DOC, dissolved organic C; MBC, microbial biomass C; MBN, microbial biomass N; LAP, leucine aminopeptidase; TDN, total dissolved N; UREA, urease.

*, significant at the 0.05 probability level; **, significant at the 0.01 probability level; ***, significant at the 0.001 probability level. Fungi/bacteria (F/B) ratio, the ratio of ITS OTUs to 16S OTUs. The activities of the five enzymes were measured in the switchgrass system only. Due to the size of the table, plant-available N (PAN), MBC/MBN ratio, ACE, Invsimpson, N-acetylglucosaminidase (NAG), plant height, root length, root width, and total dry biomass were not shown in the table but can be found in Table S2.

organic carbon, which does not change very quickly (Trumbore, 1997), DOC is an organic fraction that is more sensitive to land management (Van Wesemael et al., 2019). The effects of N fertilization on soil C content are known to vary across systems (Bowsher et al., 2018; Khan et al., 2007; Lai et al., 2018; Schmer et al., 2011), depending on N fertilizer type, N fertilizer rate, soil properties, and environmental conditions.

In this study, the DOC content was not reduced by switchgrass cultivation compared with the restored prairie and the undisturbed control systems, and it was increased by N fertilization (Table 2; Figure 1a, Figure S1a–c). In addition, the average DOC content increased in the second and third years compared with the first year (Figure 1c). This is a very interesting result since the aboveground biomass of switchgrass was removed annually for bioenergy feedstock, and the harvest was supposed to remove a portion of C from the field; however, the DOC content increased in the second and third years, which suggested that switchgrass could act to improve or stabilize the soil organic C content. The results were supported by Dou et al. (2013), who also reported that switchgrass

cultivation increased soil organic C and MBC compared with conventional cropping systems. Jung and Lal (2011) reported that the impact of switchgrass cultivation on soil organic C sequestration is mainly through belowground biomass because the aboveground biomass is removed for bioenergy feedstock. In this study, we also found a significant and positive correlation between root length and soil DOC content (Table S2).

Soil TDN, which includes dissolved inorganic N (NH₄⁺, NO₃[−], and NO₂[−]) and DON, is an important component in soil N cycle and serves as a primary N source for microorganisms and plants. In this study, the average TDN was not reduced by switchgrass cultivation compared with the restored prairie and the undisturbed control systems. Instead, it was increased in the second and third years compared with the first year (Table 2; Figure 1e,g). This is a very encouraging result, and it suggests that switchgrass not only can improve or maintain soil C content but also can improve or maintain soil N contents even when the aboveground biomass is removed annually for bioenergy feedstock. As a dedicated bioenergy crop, it is of significance that switchgrass cultivation and production

Shannon	F/B ratio	BG	CBH	UREA	LAP	Field fresh biomass	Shoot dry biomass	Root dry biomass
0.12	0.08	0.10	0.09	0.14	0.12	0.11	−0.10	0.20
0.21*	0.18	0.21*	0.20*	0.19*	0.24**	0.12	0.16	0.13
−0.07	0.22*	0.15	0.09	0.04	0.17	−0.09	−0.02	0.26**
0.07	−0.27**	0.14	0.14	0.03	0.16	−0.09	0.04	0.03
−0.55***	0.21*	0.41***	0.43***	0.33**	0.29**	0.13	0.15	0.06
−0.53***	0.38***	0.61***	0.58***	0.45***	0.46***	0.15	0.30	−0.12
0.50**	−0.29	0.20	0.05	0.39*	0.82***	−0.29	0.07	0.03
−0.29***	0.51***	0.07	0.03	0.14	−0.05	0.16**	0.21*	0.04
0.72***	−0.36***	0.10	0.06	0.06	0.15	−0.01	−0.24	−0.18
0.51***	−0.43***	0.26**	0.20*	0.17	0.19*	−0.02	−0.19	−0.29
	−0.23*	−0.11	−0.11	−0.13	0.05	−0.09	−0.31	0.11
		−0.29	−0.39	−0.13	−0.44**	0.29**	0.70***	0.32*
			0.92***	0.87*	0.45***	0.11	0.39*	−0.16
				0.79***	0.55***	0.03	0.22	−0.28
					0.54***	0.08	0.48*	−0.01
						0.05	0.10	0.06
							0.38*	0.06
								0.23

can improve or at least maintain soil fertility. To replace the N taken away by biomass harvest, a 56 kg N ha^{-1} urea was added to the fields annually. The average TDN content was significantly improved by fertilization (Table 2; Figure 1f), but the inorganic N contents (NH_4^+ and NO_3^-) in the switchgrass system were not affected by fertilization (Figure 2a,c). This suggests that organic N instead of inorganic N was boosted by switchgrass cultivation. The result also indicates that switchgrass cultivation could play an important role in long-term sustainability of cropping systems. In addition, switchgrass cultivation can form mutualistic associations with N-fixers in soil and on the root surface and contribute fixed N to soils (Roley et al., 2018, 2019; Smercina et al., 2020). Across the six locations, the average TDN was significantly higher at the locations (ESC and LC) with higher soil pH (Table 1; Figures 1h and 2d). Li et al. (2020) and Parham et al. (2002) reported that soils with low pH could reduce microbial diversity and enzyme activity, which could lead to a decreased TDN content. The results were further evidenced by the positive correlations between soil TDN and soil pH, between soil TDN and enzyme activity, between soil TDN and microbial diversity

(Shannon index), and between soil TDN and root length (Table 3, Table S2).

4.2 | Effect of switchgrass cultivation and N fertilization on soil microbial biomass C and N

Soil microorganisms are the key players in decomposing organic matter to mineral forms that are available to crops (Kaschuk et al., 2010; Li et al., 2021), which was also supported by the significant correlations between microbial biomass (MBC and MBN) with enzyme activities, microbial diversity, and plant height in this study (Table 3, Table S2). Soil MBC and MBN contents were highly correlated, and their responses to cropping system, N rate, year, and location are consistent (Tables 2 and 3). Soil microbial biomass under monocropping of switchgrass, especially MBN, was lower than those in the restored prairie and the undisturbed control systems with diverse plant species (Figure 3a,e), which indicates that plant diversity might be an important factor impacting soil MBC and MBN. This

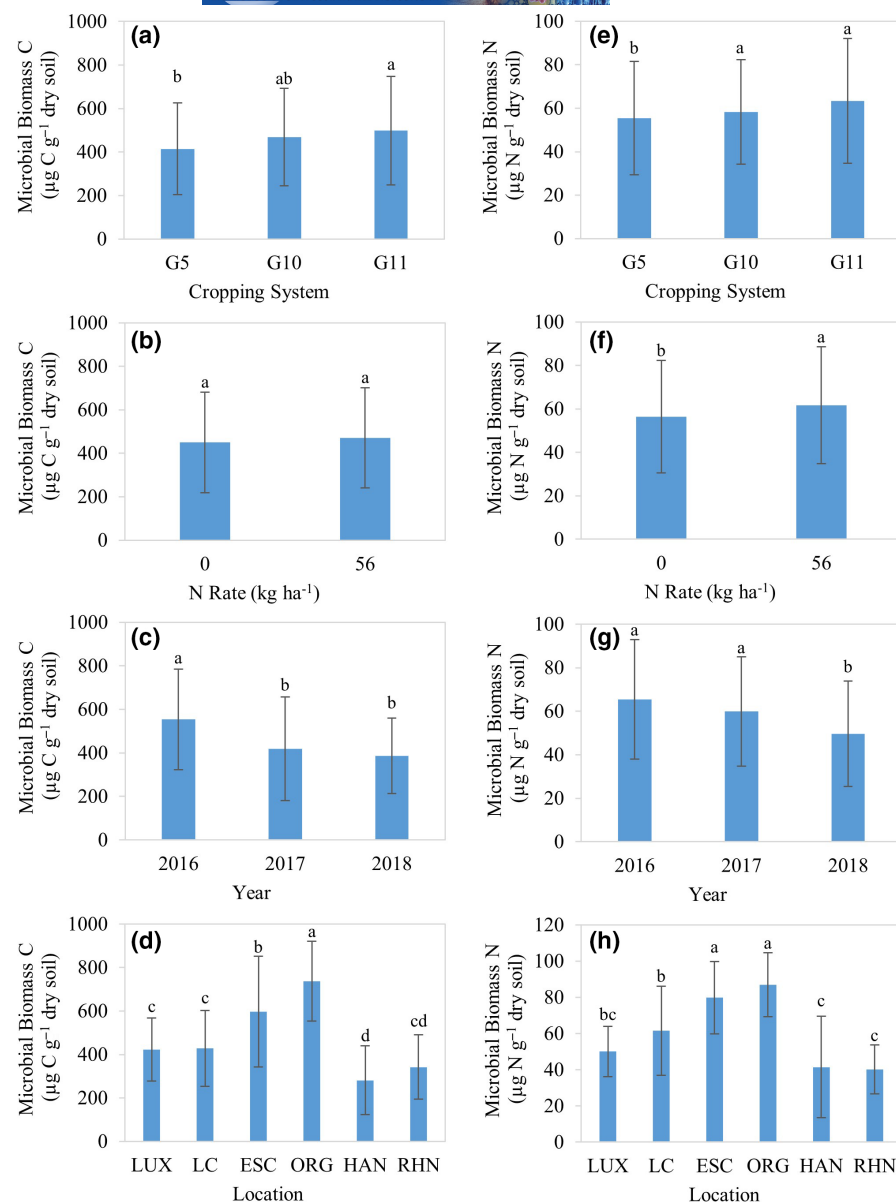


FIGURE 3 Effects of cropping system, N rate, year, and location on soil microbial biomass C ($\mu\text{g C g}^{-1}$ dry soil) and microbial biomass N ($\mu\text{g N g}^{-1}$ dry soil) contents. G5, switchgrass system; G10, restored prairie system; G11, undisturbed control system. LUX, Lux Arbor, MI; LC, Lake City, MI; ESC, Escanaba, MI; ORG, Oregon, WI; HAN, Hancock, WI; RHN, Rhinelander, WI. Bars with different letters mean that there is a significant difference between the two treatments (cropping systems, N rates, years, or locations) according to Tukey's HSD test at a significant level of 0.05

result is supported by Li et al. (2021), who evaluated soil MBC and MBN under different cover crop mixtures and reported that soil with more diverse species of cover crops had greater MBC and MBN contents. Compared with the first year, the average MBC and MBN decreased in the second and third years, which is interesting because DOC and TDN increased in the second and third years (Figures 1c,g and 3c,g). In contrast, both MBC and MBN were highest at locations (ORG and ESC) with the richest background organic C and total N contents and lowest at locations (RHN and HAN) with lower background organic C and total N contents (Table 1; Figure 3d,h). Fierer et al. (2009) and Kallenbach and Grandy (2011) reported that the quantity and quality of soil organic carbon (C) and nitrogen (N) are the dominant controls on soil microbial biomass and activities. The variation in the present study, however, suggests

the important roles of soil moisture, microbial diversity, and soil fungi/bacteria OTU ratio in impacting soil MBC and MBN contents, which was evidenced by their significant correlations (Table 3, Table S2).

4.3 | Effect of switchgrass cultivation and N fertilization on soil microbial richness and diversity

The soil microbial community plays a critical role in soil nutrient acquisition, cycling, and availability; thus, it has often been used as an important component of soil fertility (Nogueira et al., 2006; Upchurch et al., 2008). Promoting a soil microbial community for high plant productivity requires managing microbial abundance,

community composition, and microbial activity and functions (Bonanomi et al., 2018; Chaparro et al., 2012).

In this study, the number of bacterial OTUs and all richness and diversity indices were significantly affected by year and location (Tables 2 and 3, Table S2). Compared with the first year, bacterial richness decreased in the second and third years (Table 2; Figure 4g), and the trend was consistent with the changes of MBC and MBN with year (Figure 3c,g). By contrast, bacterial diversity was significantly higher in the third year than in the first year (Figure 4k). All bacterial richness and diversity indices were positively correlated with soil pH while negatively correlated with soil moisture; the Shannon index was also positively correlated with soil TDN (Table 3, Table S2). The results were consistent with previous studies (Cui et al., 2018; Li et al., 2017, 2020; Sun et al., 2015), which also reported that soil nutrient availability, especially C and N, and soil acidity were main factors affecting soil microbial population, richness, and structure.

Interestingly, neither richness indices (Chao1 and ACE) had significant correlations with any soil C and N contents tested in this study, whereas both diversity indices (Shannon and Invsimpson) were negatively correlated with MBC and MBN (Table 3, Table S2). The results indicated that a higher MBC and MBN is not always linked with a higher microbial diversity in every cropping system. In this study, a decrease in MBC and MBN in the second and third years was correlated to a more diverse microbial community and a greater content of TDN. There is no 'ideal' soil microbial community structure, and we should not expect healthy soils to have a single 'optimal' community structure or that more microbial richness/diversity is always better (Fierer et al., 2021). The decreased richness but increased diversity in the second and/or third year could be partially due to the selection pressure caused by the continuous monocropping of switchgrass. Under continuous monocropping of switchgrass, the root exudates and chemicals released by switchgrass can limit the size of microbial community and number of species in the community, which was evidenced by the reduced MBC, MBN, OTUs, and richness indices in the second and third year (Figures 3c,g and 4c,g). Because diversity is impacted by the evenness of a community as well as the number of taxa present, this is consistent with

switchgrass recruiting and maintaining a more even community over time, possibly due to multiple distinct niches present in the rhizosphere. The decreased richness but increased diversity under monoculture of switchgrass compared with prairie mix was also observed by Revillini et al. (2019).

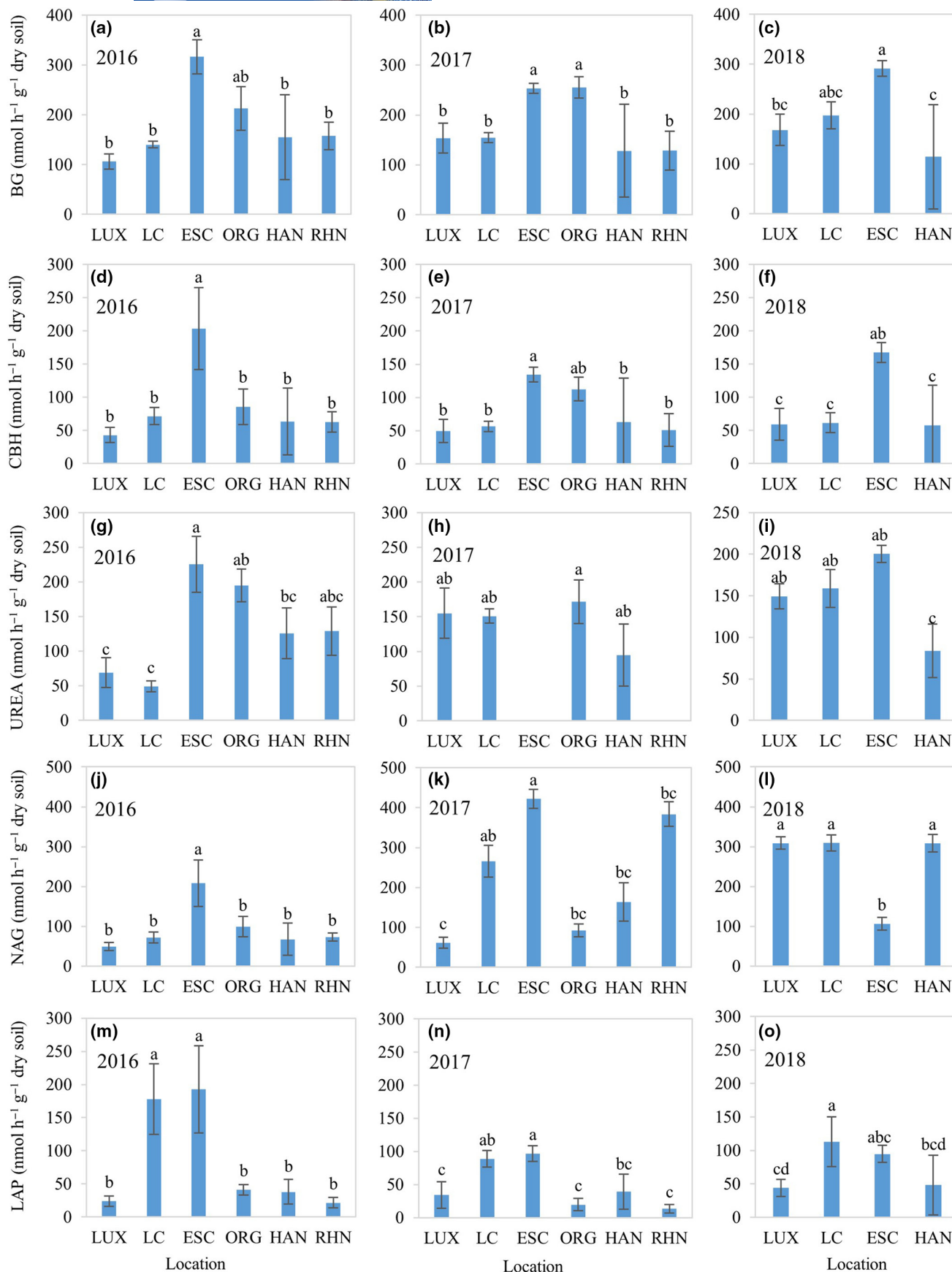
Soil fungi and bacteria play different roles in organic matter decomposition; fungi are primary degraders of particulate, predominately terrigenous C, to contribute litter C for the microbial loop, whereas bacteria are rapid recyclers of simple nutrient-rich organic compounds (Fabian et al., 2017; Krauss et al., 2011). A shift toward a fungal-dominated community was thought to enhance organic C accumulation (Six et al., 2006). In this study, the average F/B richness ratio in the switchgrass system was higher than that in the undisturbed control system but was lower than that in the restored prairie system, which indicated that switchgrass cultivation may benefit organic C accumulation.

4.4 | Effect of switchgrass cultivation and N fertilization on the activities soil C and N cycling-related enzymes

Soil enzymes are mainly produced by microorganisms; enzymatic activities are the indicators of microbial functions and are closely related to microbial abundance, biomass, richness, and diversity (Meena & Rao, 2021; Trasar-Cepeda et al., 2008). Soil enzymes are directly involved in organic matter decomposition and their activities in key nutrients (C, N) cycling have been widely used as potential indicators for evaluating the effects of land use and management practice on soil health (Acosta-Martínez et al., 2007; de Medeiros et al., 2015; Pandey et al., 2014).

In this study, the activities of all five enzymes (BG, CBH, UREA, NAG, and LAP) associated with switchgrass were significantly and positively correlated with soil TDN content (dissolved inorganic N + DON) content but not correlated with soil inorganic N contents (NH_4^+ and NO_3^-) (Table 3, Table S2). This is an interesting result and indicates that soil DON, instead of inorganic N, could be an important factor affecting the activities of the organic C decomposition-related enzymes (BG and CBH), organic N mineralization-related enzymes (NAG

FIGURE 5 Effects of N rate, year, and location on soil C-cycling and N-cycling enzyme activities in the switchgrass system. BG, β -glucosidase; CBH, cellobiohydrolase; UREA, urease; NAG, N-acetyl- β -glucosaminidase; LAP, leucine aminopeptidase. LUX, Lux Arbor, MI; LC, Lake City, MI; ESC, Escanaba, MI; ORG, Oregon, WI; HAN, Hancock, WI; RHN, Rhinelander, WI. Bars with different letters mean that there is a significant difference between the two treatments (N rates, years, or locations) according to Tukey's HSD test at a significant level of 0.05



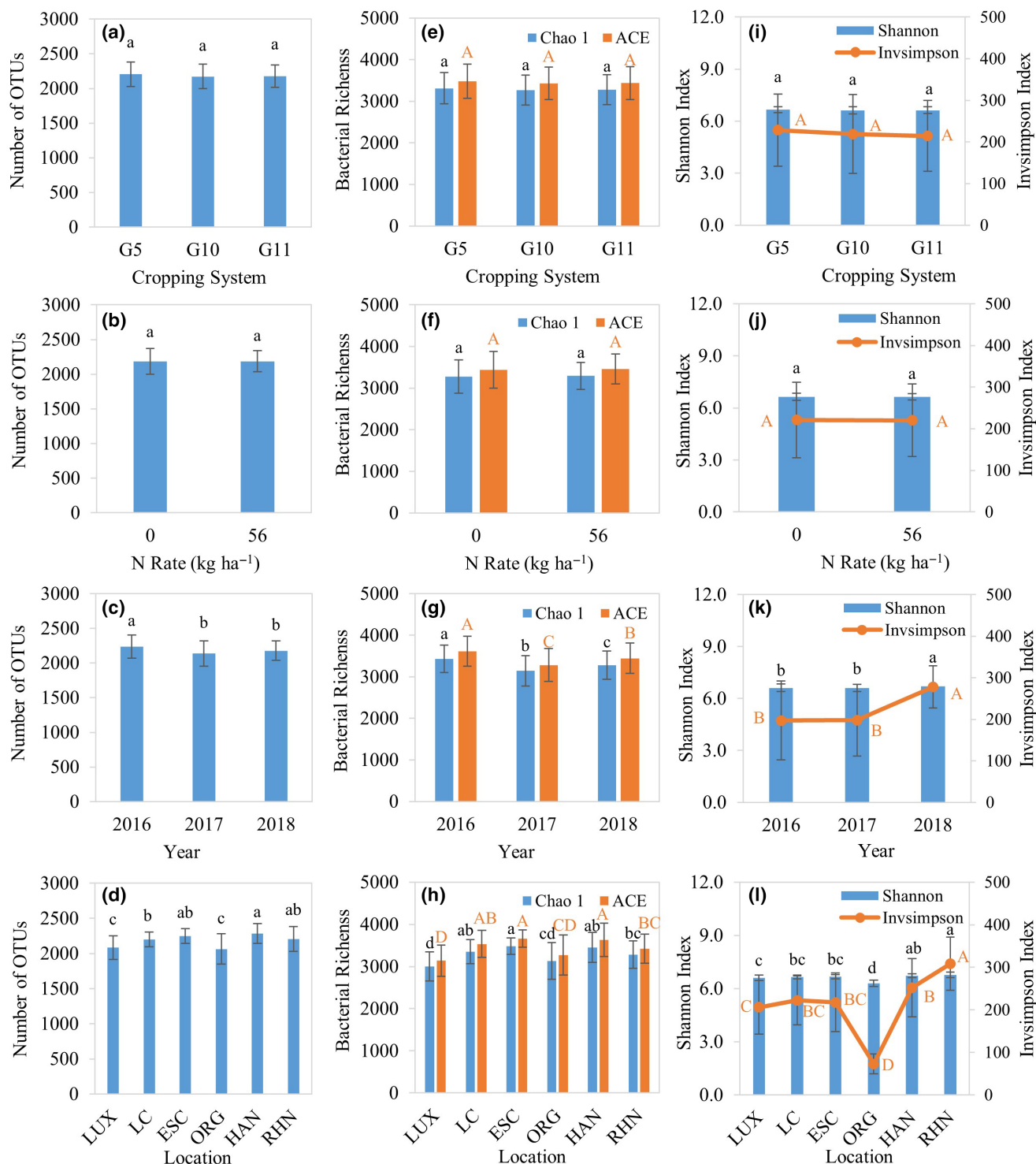


FIGURE 4 Effects of cropping system, N rate, year, and location on the richness and diversity indices of the soil bacterial community. LUX, Lux Arbor, MI; LC, Lake City, MI; ESC, Escanaba, MI; ORG, Oregon, WI; HAN, Hancock, WI; RHN, Rhinelander, WI. Bars with different letters mean that there is a significant difference between the two treatments (cropping systems, N rates, years, or locations) according to Tukey's HSD test at a significant level of 0.05

and LAP), and even inorganic N transformation enzyme (UREA).

Soil enzyme activity also depends on the abundance and diversity of the microbial community (Zhang et al., 2017).

In this study, the activities of BG, CBH, UREA, and LAP associated with switchgrass were significantly and positively correlated with each other, and their activities were positively correlated with microbial biomass (MBC and MBN)

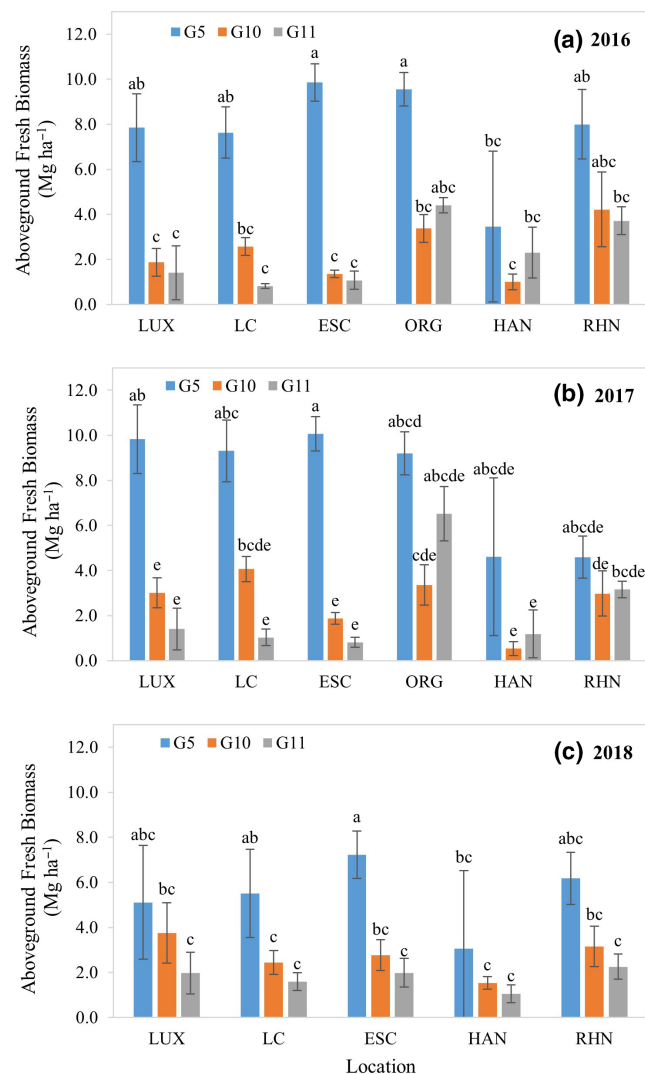


FIGURE 6 Effects of cropping system, N rate, year, and location on plant aboveground biomass. G5, switchgrass system; G10, restored prairie system; G11, undisturbed control system. LUX, Lux Arbor, MI; LC, Lake City, MI; ESC, Escanaba, MI; ORG, Oregon, WI; HAN, Hancock, WI; RHN, Rhinelander, WI. Bars with different letters mean that there is a significant difference between the two treatments (cropping systems, N rates, years, or locations) according to Tukey's HSD test at a significant level of 0.05

(Table 3, Table S2). Furthermore, the activities of both C cycling-related enzymes (BG and CBH) were significantly and positively correlated with microbial richness (Chao 1 and ACE) but significantly and negatively correlated with soil C/N ratio, and the activities of all three N cycling-related enzymes (UREA, NAG, and LAP) were significantly and positively correlated with soil pH (Table 3, Table S2). These results align with previous studies (Meena & Rao, 2021; Parham et al., 2002; Sinsabaugh et al., 2008; Vinhal-Freitas et al., 2017) and confirm the importance of soil N availability, C/N ratio and pH in impacting soil microbial richness, diversity, and enzymatic activities. We also observed variation in enzyme activities across the six locations that may

be related to soil properties (Table 1; Figure 5a–o). Among the six locations, soil BG, CBH, and UREA activities were significantly greater at locations (ESC and ORG) with higher soil organic C and total N contents as well as lower C/N ratio, and soil NAG and LAP activities were significantly greater at locations (LC and ESC) with higher soil pH.

4.5 | Effect of switchgrass cultivation and N fertilization on plant traits and biomass yield

Optimizing biomass yield while minimizing environmental impact is important in the successful development of a bioenergy industry. Plant aboveground fresh and dry biomasses were significantly affected by cropping system, N rate, year, and location (Table 2; Figure 6a–c; Jayawardena et al., unpublished data). The aboveground biomass in the switchgrass system was 2.9–3.3 times higher than those in the restored prairie and the undisturbed control systems, and it increased 1.2 times by adding 56 kg N ha⁻¹ of fertilizer. The variation in the aboveground biomass was significantly associated with variations in soil moisture and fungal/bacterial OTU ratio (Table 3). Switchgrass shoot and root responded differently to N rate and year; the total dry biomass and shoot dry biomass of switchgrass were significantly higher at 56 than 0 kg N ha⁻¹ and significantly higher in the second and third years than in the first year; in contrast, root dry biomass was not impacted by the main effects of year or N rate (Table 3; Figure 7b–d). Previous studies also reported that inorganic N fertilization increased the aboveground biomass of perennial warm-season grasses (Heggenstaller et al., 2009; Lemus et al., 2008; Vogel et al., 2002), but its effects on root biomass, SOC stocks, and other soil properties were unclear (Heggenstaller et al., 2009). Ma et al. (2000) and Kibet et al. (2016) both found that there was no significant difference in switchgrass root biomass between fertilized and unfertilized treatments. The shoot/root biomass ratio was greater at ESC and ORG than the other locations, whereas there were no clear trends of the shoot/root biomass ratio among 3 years or between two N rates. The greater shoot/root biomass ratio at ESC and ORG could be a result of multiple soil properties and enzyme activities. ESC and ORG had considerably higher background organic C, total N, CEC, Ca, and Mg contents but a lower C/N ratio; ESC and ORG also had greater BG, CBH, and UREA activities than other locations (Table 1; Figure 5a–i).

5 | CONCLUSIONS

In conclusion, switchgrass cropping system yielded an aboveground biomass 2.9–3.3 times higher than the restored

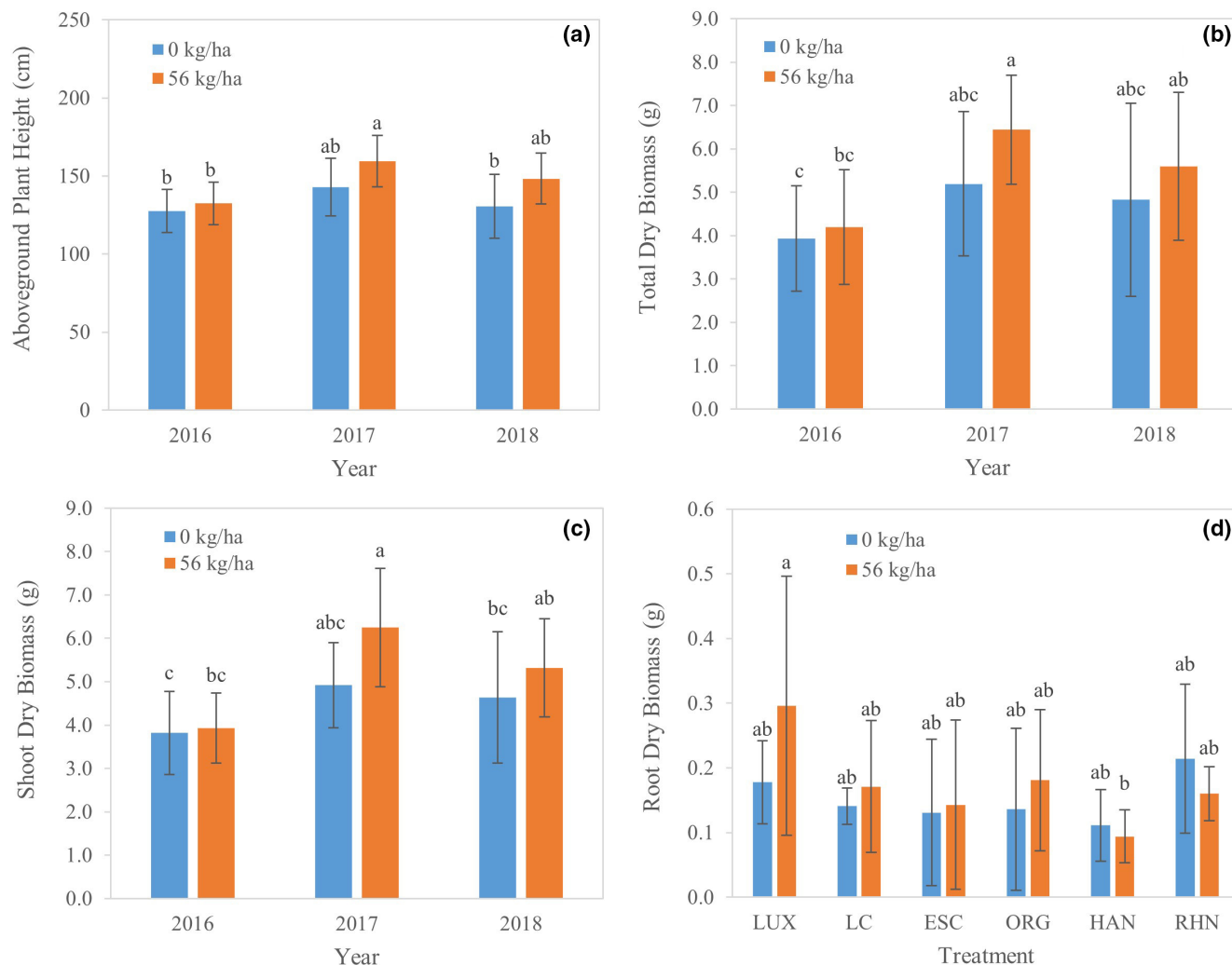


FIGURE 7 Effects of N rate, year, and location on plant traits of switchgrass. LUX, Lux Arbor, MI; LC, Lake City, MI; ESC, Escanaba, MI; ORG, Oregon, WI; HAN, Hancock, WI; RHN, Rhinelander, WI. Bars with different letters mean that there is a significant difference between the two treatments (cropping systems, N rates, years, or locations) according to Tukey's HSD test at a significant level of 0.05

prairie system and the undisturbed system (Jayawardena et al., unpublished data) but did not reduce soil DOC, TDN, or bacterial diversity. The annual aboveground biomass removal for bioenergy feedstock, however, reduced soil MBC and MBN and bacterial richness in the second and third years; however, continuous monocropping of switchgrass still improved soil TDN, inorganic N, bacterial diversity, and shoot biomass in the second and/or third years compared with the first year. N fertilization increased aboveground biomass yield by 1.2 times (Jayawardena et al., unpublished data) and we found that it significantly increased soil TDN, MBN, and the shoot biomass of switchgrass compared with the unfertilized control. Locations with higher C and N contents and lower C:N ratio had higher aboveground biomass, MBC, MBN, and the activity of BG, CBH, and UREA enzymes; by contrast, locations with higher pH had higher soil TDN and activity of NAG and LAP enzymes.

The comprehensive data can inform future biogeochemical models to successfully implement switchgrass for bioenergy production and inform decision-makers, researchers, county agents, and producers to make improved decisions about sustainability and soil health when considering introducing switchgrass.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

All authors provided critical feedback and helped shape the research, analysis, and manuscript. Conceptualization, X.L., S.E., L.T., and M.F.; methodology, X.L., L.T., S.E., and M.F.; investigation, X.L., Y.L., H.V.S., L.B., A.A., and C.B.; software, X.L. and L.B.; formal analysis, X.L., R.H., and M.F.; visualization, X.L., Y.L., and A.A.; validation, X.L., R.H., and D.S.; resources, L.T., S.E., and M.F.; data curation, X.L., H.V.S., and M.F.; writing—original draft preparation, X.L. and M.F.; writing—review and editing, all authors; supervision, M.F.; project administration, H.V.S. and A.A.; funding acquisition, S.E., L.T., and M.F. All authors have read and agreed to the published version of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in data dryad at <http://doi.org/10.5061/dryad.547d7wmbf>.

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