Soil microbial community structure and enzymatic activity responses to nitrogen management and landscape positions in switchgrass (Panicum virgatum L.)

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Abstract
Switchgrass (Panicum virgatum L.) is usually grown on marginal land for biofuel system, in which nitrogen (N) is an essential management practice, and landscape position is a key topographical factor in impacting the production. However, limited information is available regarding how the N application and landscape positions affect soil microbial communities and enzyme activities under switchgrass. Thus, the specific objective of this study was to evaluate the responses of N rate (high, 112 kg N/ha; medium, 56 kg N/ha; and low, 0 kg N/ha) and landscape positions (shoulder and footslope) on soil biological health under switchgrass field. Data showed that N addition significantly influenced carbon and N fractions. The hot water-soluble organic carbon (HWC) and nitrogen (HWN) fractions were significantly higher at footslope position than the shoulder position. The amount of total phospholipid fatty acid (PLFA), total bacterial, actinomycetes, gram-negative and gram-positive bacteria, total fungi, arbuscular mycorrhizal (AM) fungi, and saprophytes PLFAs were highest with medium and high N rates and footslope position. The N addition increased total PLFAs in N fertilizer treatments, viz. medium (5,946 ng PLFA-C/g soil) and high N rates (5,871 ng PLFA-C/g soil). Microbial biomass carbon and nitrogen and enzyme activities (urease, β-glucosidase, acid phosphatase and arylsulfatase) were significantly enhanced by N fertilization (medium and high N rates) compared to control (low N rates) under footslope position. The urease activity under medium (36.3 µmol N-NH₄⁺ g⁻¹ soil hr⁻¹) and high N rates (31.4 µmol N-NH₄⁺ g⁻¹ soil hr⁻¹) was 42.9% and 23.6% higher than low N rates, respectively. This study suggests that the application of medium N rate in footslope position to switchgrass can enhance the soil biological properties and hence can protect the environment from the excessive use of N fertilizer.

KEYWORDS
landscape positions, N rates, nitrogen management, soil microbial community structure, switchgrass, urease
INTRODUCTION

Interest in the production of renewable energy from plants has increased in the last decade due to substantial reduction in reserves of fossil fuel and increased emissions of greenhouse gases (GHG) (Owusu & Asumadu-Sarkodie, 2016). Crop biomass can be used as an alternate source of energy and may reduce GHG emissions. In the last decade, 14%–15% of the global energy use was satisfied by the energy produced from crop biomass (Venturi & Venturi, 2003). Perennial energy crops provide an opportunity to improve agricultural sustainability through crop diversification, reduced erosion, and improved soil and water quality. Switchgrass (*Panicum virgatum*) is a warm season C4-perennial crop gaining in popularity for biofuel and feedstock production on marginal lands (Gelfand et al., 2013). During summer, when cool-season grasses are dormant, switchgrass can provide forage for livestock (Wolf & Fiske, 2009). In addition, perennial bioenergy crops generally improve soil properties compared with row crops (Blanco-Canqui, Gilley, Eisenhauer, Jasa, & Boldt, 2014). However, their effects on soil health under different management practices have not been widely documented. In particular, little information is available on the response of soil microbial communities and soil enzyme activities to nitrogen rates and landscape positions. Proper nitrogen (N) management is required for efficient forage production (Obour, Harmoney, & Holman, 2017). Studies show that N is the most limiting nutrient for forage productivity, and application of N improves yield and nutritive value in warm season grasses (Agyin-Birikorang, Newman, Obour, & Kasozi, 2012; Silveira et al., 2015). Proper N management is crucial for sustainability and reducing environmental impact while landscape position (slope) is a key topographical factor in impacting the production of switchgrass (Lai, 2017; Lai, Kumar, Folle, & Owens, 2018; Mbomimpa et al., 2015). Deep roots of switchgrass add organic carbon to the soil and help in enhancing the soil health indicators through their influence on microbial communities (Liebig, Johnson, Hanson, & Frank, 2005).

Soil microbial communities are directly related to soil biogeochemical processes and play a prominent role in soil nutrient cycles and turnover (Zeng et al., 2016). The direct change in soil microbial community structure may affect the microbial functions and soil N and C dynamics (Leff et al., 2015; Männistö, Ganzert, Tiirola, Häggblom, & Stark, 2016). The changes in structure and composition of the microbial communities are considered as the strong indicators of soil biochemical process and essential ecosystem functions (Pardo et al., 2011). Soil enzymes are also crucial in soil functioning because they play a vital role in decomposing organic matter and transformation and cycling of nutrients (Jesus et al., 2016; Shereen, 2017). In general, these enzymes include hydrolases and oxidases that decompose substrates and release nutrients to the soil (Sinsabaugh, 2010). Urease enzyme is associated with microbial N acquisition, and it catalyses the decomposition of urea (Tabatabai & Brenner, 1972). β-1,4-glucosidase is a hydrolytic enzyme produced by soil microbes to decompose polysaccharides (Deng & Tabatabai, 1994). Acidic and alkaline phosphatase are enzymes associated with P acquisition, and they cleave PO₄³⁻ from phosphorus containing organic compounds (Hui, Mayes, & Wang, 2013). The enzyme arylsulfatase catalyses the hydrolysis of organic sulphate esters, releasing SO₂ that can be utilized by plants (Hai-Ming et al., 2014).

Agricultural practices affect soil biodiversity and other microbiological properties (Groom, Gray, & Townsend, 2008). Soil microbial community structure and soil enzymatic activities can be affected by N application and landscape positions (Klaubauf et al., 2010; Shi et al., 2016). Inorganic fertilizers, especially N not only improves crop yields, but its application also directly or indirectly affects soil physical, chemical and biological properties. In long term, these changes in soil properties are believed to have significant influences on the quality of the soil (Acton & Gregorich, 1995). The sensitivity of soil microbial communities and enzymatic activities likely varies between agricultural and unmanaged ecosystems (Lladó, López-Mondéjar, & Baldrian, 2017). N addition in agricultural field exceeds rates of atmospheric deposition, and N fertilizer is often added in one or few large applications per year (Geisseler & Scow, 2014b). Furthermore, the increase in crop productivity due to fertilization activity in agricultural systems increases inputs of organic material in the form of root exudates, decaying roots and plant residues, and thus increases the pool of C sources for soil microorganisms’ growth (Geisseler & Scow, 2014b). Several field and laboratory studies have also identified major changes in the structure of soil microbial community and soil enzymatic activities under N addition treatments (Koyama, Wallenstein, Simpson, & Moore, 2014; Leff et al., 2015; Pan, Zhang, Liang, Liu, & Wang, 2018). The N availability is an important determinant of the soil microbial community (Leff et al., 2015). Landscape positions are also a key factor that can impact soil properties (Mwanjalolo Jackson-Gilbert et al., 2015). Footslope positions are generally rich in moisture, organic matter, clay content and thus tend to have higher primary productivity in comparison with upland areas. Evidently, footslope sites with more substrate, nutrients, and suitable environment can support a larger microbial community with greater microbial biomass (Liu, Zhang, & Wan, 2009). Soil microclimate and nutrients in footslope can favour different microbial communities with respect to those in upland sites and thus induce differences between footslope and upland in the microbial community (MacKenzie & Quideau, 2010). Soil enzymatic activities (urease, β-glucosidase, phosphatase and arylsulfatase) depend on landscape positions (Bergstrom, Monreal, & King, 1998; Decker, Boerner, & Morris, 1999).
Broughton and Gross (2000) found more productive and active microbial communities in footslope which is marked by the highest levels of soil moisture, plant biomass, N and C contents comparing with up and midslope positions. The total soil enzymatic activities are derived from the active microorganisms and stabilized pool in clay–humus complexes (Burns et al., 2013).

There is limited information available on the impact of N application rates and landscape positions on soil microbial communities and their enzyme activities in switchgrass. Understanding the effect of N on soil microbial communities and soil enzymatic activities is crucial for increasing bioenergy crop production as soil microbiological properties are an integral component of soil health and productivity. Thus, the objective of this study was to assess the responses of soil microbial communities and selected enzyme activities relevant to C, N, P and S cycling in soils to N management and landscape positions in switchgrass.

2 | MATERIALS AND METHODS

2.1 | Study site and experimental design

A long-term field experiment was initiated in 2008 near Bristol, South Dakota, USA (45°16′24.55″ N, 97°50′13.34″ W; altitude: 524.3 m above sea level). Soils of the site are dominated by loamy soils (Mbonimpa et al., 2015). The experiment was a split-plot design with four replications. The study comprised of three N fertilizer rates (low, 0 kg N/ha; medium, 56 kg N/ha; and high, 112 kg N/ha) and two landscape positions (shoulder, and footslope). Each treatment plot area was 21.3 m by 365.8 m with 2%–20% slope. Urea was applied as a N fertilizer. Switchgrass [cultivar: Sunburst; planting rate: 10 kg pure live seed (PLS)/ha] was planted on 17 May 2008. The previous crop grown at this location was soya bean (Glycine max L.). Switchgrass was harvested once annually around a killing frost from 2009 to 2018. Soil samples were collected from each plot during May 2018 at 0–5 cm depth. The samples were kept fresh and stored in a refrigerator for pending analysis. All the microbial parameters were analysed within 2–3 weeks of sampling. Samples were air-dried and ground to pass through a 2 mm sieve, and soil pH (1:1 soil/water) was determined using the procedure given by McLean (1982).

2.2 | Cold and hot water-soluble carbon and nitrogen fractions

Content of water extractable organic carbon and nitrogen fractions were carried out by schematic procedure described by Ghani, Dexter, and Perrott (2003). The extraction was performed with distilled water in a soil-to-solution ratio of 1:10. A 3 g of soil was poured with 30 ml of water and put for shaking on vortex and rotatory shaker for 10 s and 30 min at 40 rpm, respectively. After extraction, the suspension was centrifuged at 925.7 g for 25 min at 4°C. The filtrate obtained is cold water extractable organic carbon (CWC) and nitrogen (CWN). A further 30 ml of water is added to the remaining residue and put on a vortex shaker for 10 s. The suspension was left in hot water bath at 80°C for 12–15 hr. After extraction, the suspension was again put on vortex shaker for 10 s and then centrifuged at 3,000 rpm for 25 min at 25°C. The filtrate obtained is hot water extractable organic carbon (HWC) and nitrogen (HWN). The cold water and hot water carbon and nitrogen fractions were determined using the TOC-L analyzer (Shimadzu Corporation, model-TNM-L-ROHS).

2.3 | Microbial biomass carbon (MBC) and nitrogen (MBN)

MBC and MBN in soil were determined by the chloroform fumigation direct extraction method as described in Anderson and Domsch (1978), Gregorich, Wen, Voroney, and Kachanoski (1990). Ten grams of soil was placed into a 50 ml glass beaker for fumigation and non-fumigation analysis. Soil samples designated as fumigated were kept in a desiccator clouded with alcohol-free chloroform for 24 hr, evacuated, and extracted with 50-ml 0.5 M K₂SO₄. Similarly, the non-fumigated soil sample was prepared, and thereby both suspensions analysed for dissolved C and N. The MBC was calculated by the difference between carbon (C) in the fumigated and non-fumigated samples, and with a correction factor of 0.45 (Beck et al., 1997).

2.4 | Phospholipid fatty acid (PLFA) analysis

Phospholipid fatty acid subsamples were analysed at Ward Laboratories, Inc. (Lincoln, NE). These samples were analysed according to the method of Clapperton, Lacey, Hanson, and Hamel (2005). Total soil lipids were extracted in test tubes by shaking approximately 2 g (dry weight equivalent) of frozen soil in 9.5 ml dichloromethane (DMC):methanol (MeOH):citrate buffer (1:2:0.8 v/v) for 1 hr. Then, 2.5 ml of DMC and 10 ml of a saturated KCl solution were added to each tube and shaken for 5 min. Tubes were then centrifuged at 3,000 rev/min for 10 min. The organic fraction was transferred into clean vials. After drying under a flow of N₂ at 37°C, samples were dissolved in 2 ml of DCM and stored at −20°C for lipid-class separation.

Lipid-class separation was conducted in silica gel columns. Samples were loaded onto columns, and the vials washed twice with a small amount of DCM using a pipette. Care was taken to keep solvent level above the silica gel at all times. The neutral, glyco- and phospholipids fractions were eluted by sequential leaching with approximately 2 ml
of DCM, 2 ml of acetone and 2 ml of methanol, respectively. The neutral and glycolipid fraction was discarded, and the phospholipids fractions were collected in separate 4 ml vials. These fractions were dried under a flow of N2 at 37°C in the fume hood. The dried fractions were dissolved in a few ml of MeOH for PLFA stored at −20°C.

Fatty acid methyl esters (FAMEs) were created through mild acid methanlysis. The phospholipids fractions were dried under a flow of N2 at 37°C in the fume hood. Half a Pasteur pipette full of MeOH/H2SO4 (25:1 v/v) was added to the vials, which were placed in an oven at 80°C for 10 min, cooled to room temperature before the addition of approximately 2 ml of hexane with a Pasteur pipette. Vials were vortexed during 30 s and left to settle for 5 min before the lower fraction was discarded entirely. Ten ml of methyl nonadecanoic acid (19:0; Sigma-Aldrich) was added. Samples were dried under a flow of N2 at 37°C in the fume hood, and vials were rinsed with 50 ml of hexane into 100 ml tapered glass inserts for gas chromatograph (GC) vial. Samples were analysed using an Agilent 7890A GC equipped with a CP-7693 auto-sampler and a flame ionization detector (FID). Hydrogen was the carrier gas (30 ml/min), and the column was a 50-m Varian Capillary Select FAME # cp7420. Sample (2 ml) injection was in 5:1 split mode. The injector was held at 250°C and the FID at 300°C. The initial oven temperature of 190°C ramped to 290°C over the course of 12 min.

Identification of peaks was based on the comparison of retention times to known standards (MID's Sherlock software system). The abundance of individual PLFAs was expressed as ng PLFA-C/g dry soil. Amounts were derived from the relative area under specific peaks, as compared to the 19:0 peak value, which was calibrated according to a standard curve made from a range of concentrations of the 19:0 FAME standard dissolved in hexane. Fatty acids were named according to the o-designation described as follows: total number of carbons followed by a colon; the number of double bonds; the symbol o; the position of the first double bond from the methyl end of the molecule. Cis and trans isomers are indicated with c or t, respectively. Methyl (meth) and hydroxy (OH) groups are labelled at the beginning of the name where appropriate. Iso and anteiso forms are indicated by i- and a-, respectively.

Individual fatty acids have been used as signatures for various functional groups of microorganisms (Bardgett, Lovell, Hobbs, & Jarvis, 1999; Bossio, Scow, Gunapala, & Graham, 1998; Grayston, Griffith, Mawdsley, Campbell, & Bardgett, 2001; Pankhurst, Pierrct, Hawke, & Kirby, 2002; Yao, He, Wilson, & Campbell, 2000). The i-15:0, a-15:0, i-16:0, i-17:0, and a-17:0 are classified as gram-positive bacteria while 16:1w7, 17:0cy, 2-OH 16:0, c18:1w7, and 19:0cy are classified as gram-negative bacteria, (Zelles, 1999). The 16:1w5 biomarker can also be found in gram-negative bacteria (Nichols, Mancuso, & White, 1987). The PLFA 18:2o6c was taken to indicate fungal biomass (Frostegård & Bååth, 1996; Petersen & Klug, 1994) and FAMEs 3OH‐12:0, a-12‐meth‐15:0, i-13‐meth‐15:0, 15:0, 2OH‐14:0, i-14‐meth‐16:0, 16:1o7c, i-15‐meth‐17:0, 10-methyl‐17:0o8c, 17:0 and 2OH-16:0 were chosen to represent bacterial PLFAs based on the bacterial standards used.

2.5 | Soil enzymatic analysis

2.5.1 | Urease assay

Urease (EC 3.5.1.5) enzyme activity was measured using the method provided by Kandler and Gerber (1988) and using urea as a substrate solution. Five grams of soil was taken into each of three 50 ml incubation flasks (two of them with substrate and one without the substrate), and 2.5 ml of urea solution and 20 ml of borate buffer were added and only 20 ml of borate buffer solution was added into the third flask (control). Soil samples were incubated for 2 hr at 37°C. After incubation, the control samples were treated with 2.5 ml of substrate solution and all the samples with 30 ml of potassium chloride. Samples were allowed for shaking for 30 min on a rotatory shaker, and the soil suspensions were filtered. To determine the released ammonium, 1 ml of filtrate was added with 9 ml of distilled water into a test tube. For colour reaction, 5 ml of sodium salicylate-sodium hydroxide solution and 2 ml of sodium dichloroisocyanurate solution were added and allowed for 30 min for colour development at room temperature. The ammonium content of the samples was determined spectrophotometrically at 660 nm, and the results were expressed as µmol N-NH4+ g−1 soil hr−1.

2.5.2 | β-Glucosidase assay

β-Glucosidase (EC 3.2.1.21) enzyme activity was assayed by the method of Eivazi and Tabatabai (1977), using the substrate 50 mM para-nitrophenyl-β-D-glucopyranoside (pNPG). One gram of moist soil was weighed into screw-cap glass tubes and incubated for 1 hr at 37°C with 4 ml of 0.05 M modified universal buffer (pH 6.0) and 1 ml of 50 mM pNPG dissolved in buffer. The reaction was terminated by adding 1 ml of 0.5 M CaCl2 and 4 ml of 0.2 M Tris-hydroxymethyl (aminomethane), adjusted to pH 12 with NaOH. The formation of
$p$-nitrophenol (pNP) was determined spectrophotometrically at 410 nm. Values were corrected for a blank (substrate added immediately after the addition of CaCl$_2$ and Tris–NaOH and for adsorption of para-nitrophenol (pNP) in the soil (Vuorinen, 1993). The β-glucosidase enzyme activity is expressed as μmol pNP released g$^{-1}$ soil hr$^{-1}$.

### 2.5.3 Acid and Alkaline phosphatase assay

Acid (EC 3.1.3.2) and alkaline (EC 3.1.3.1) phosphatase enzyme activity was assayed by the method of Tabatabai and Bremner (1969) and Eivazi and Tabatabai (1977) using $p$-nitrophenol phosphate, a synthetic compound as substrate. One g of moist soil was weighed into screw-cap glass tubes and incubated for 1 hr at 37°C with 4 ml of modified universal buffer (pH 6.0 for assay of acid phosphatase and pH 11 for assay of alkaline phosphatase) and 1 ml of $p$-nitrophenyl phosphate solution dissolved in buffer. By adding 1 ml of 0.5 M CaCl$_2$ and 4 ml of 0.5 M NaOH, the reaction was terminated. Both enzymes hydrolysed $p$-nitrophenyl phosphate to $p$-nitrophenol (pNP) and inorganic phosphate. The yellow colour intensity of $p$-nitrophenol was measured spectrophotometrically at 400 nm for both acid and alkaline phosphatase enzymes. Both acid and alkaline phosphatase enzyme are expressed as µg pNP g$^{-1}$ soil hr$^{-1}$.

### 2.5.4 Arylsulfatase assay

Arylsulfatase (EC 3.1.6.1) enzyme activity was determined according to the method of Tabatabai and Bremner (1970). One gram of moist soil was weighed into screw-cap glass tubes and incubated for 1 hr at 37°C with 4 ml of acetate buffer and 1 ml of $p$-nitrophenyl sulphate solution (0.05 M). By adding 1 ml of 0.5 M CaCl$_2$ and 4 ml of 0.5 M NaOH, the reaction was terminated. The yellow colour intensity of $p$-nitrophenol was measured spectrophotometrically at 420 nm. The arylsulfatase enzyme activity is expressed as µg pNP g$^{-1}$ soil hr$^{-1}$.

### 2.6 Statistical analysis

The statistical analysis of N rate and landscape position effects on soil microbial parameters were obtained using pairwise differences method to compare least-squares means estimated by a mixed model using the GLIMMIX procedure in SAS9.3 (SAS, 2012), where the N rate, position and N rate × position were considered as fixed effects and replication and replication × N rate as random effects. The analysis of variance (ANOVA) was used to test the fixed effects of the N rate and position on the soil microbial properties based on the mixed model. Data were transformed when necessary using the Box-Cox method (Box and Cox, 1981; Box and Cox, 1964). Significance was determined at $\alpha = 0.05$ (McLean, 1982).

### 3 RESULTS

#### 3.1 Soil pH, CWC, CWN, HWC and HWN

Data on soil pH, CWC, CWN, HWC and HWN as influenced by N rate and landscape position are presented in Table 1. The N rate did not influence the soil pH. However, landscape positions significantly impacted the soil pH. The pH at the footslope (7.63, slightly alkaline) was lower than the shoulder (8.12, alkaline) position. The pH values decreased with the decrease in slope. Further, the N rates significantly

| Treatment | Soil pH | CWC (µg C/g soil) | HWC (µg N/g soil) | CWN (µg C/g soil) | HWN (µg N/g soil) | MBC (µg/g soil) | MBN (µg N/g soil) |
|-----------|---------|------------------|------------------|------------------|------------------|----------------|------------------|
| N rate    |         |                  |                  |                  |                  |                |                  |
| High      | 7.81$^a$ | 25.9$^b$         | 63.2$^a$         | 3.72$^a$         | 6.96$^a$         | 230.2$^a^b$    | 22.0$^a$        |
| Medium    | 7.94$^a$ | 30.7$^a$         | 62.7$^a$         | 3.12$^a^b$       | 6.73$^a$         | 251.9$^a$      | 24.5$^a$        |
| Low       | 7.88$^a$ | 23.8$^b$         | 56.0$^a$         | 2.55$^b$         | 5.89$^a$         | 185.8$^b$      | 19.9$^a$        |
| Position  |         |                  |                  |                  |                  |                |                  |
| Shoulder  | 8.12$^a$ | 26.6$^a$         | 54.1$^b$         | 3.29$^a$         | 5.63$^b$         | 168.8$^b$      | 18.8$^b$        |
| Footslope | 7.63$^b$ | 27.0$^a$         | 67.2$^a$         | 2.97$^a$         | 7.42$^a$         | 276.4$^a$      | 25.4$^b$        |

Analysis of variance ($p > F$)

| N Rate (N) | 0.769 | 0.007 | 0.169 | 0.005 | 0.226 | 0.021 | 0.320 |
| Position (P) | 0.011 | 0.791 | 0.002 | 0.100 | 0.006 | <0.0001 | 0.009 |
| N × P | 0.612 | 0.498 | 0.172 | 0.080 | 0.207 | 0.571 | 0.466 |

Note. Mean values within the same column followed by different small letters are significantly different at $p < 0.05$ for each N rate and landscape position.
influenced the CWC and CWN fractions (Figure 1); however, landscape position did not impact the CWC and CWN. The CWC fraction was significantly higher with medium N rates (30.7 µg C/g soil) than the high (25.9 µg C/g soil) and low N rates (23.8 µg C/g soil). Further, landscape position only significantly impacted the HWC and HWN fractions. The HWC and HWN fractions at footslope position were 1.24 and 1.32 times higher than the shoulder position, respectively.

### 3.2 | Soil MBC and MBN

N rate significantly influenced the soil MBC and but did not influence the MBN (Table 1), whereas landscape positions significantly impacted the soil MBC and MBN. Soil MBC values were significantly higher in medium N rates (251.9 µg/g soil) compared to high (230.2 µg/g soil) and low N (185.8 µg/g soil) rates. For MBC and MBN, the footslope position (276.4 and 25.4 µg/g soil, respectively) had significantly higher values than the shoulder position (168.8 and 18.8 µg/g soil, respectively).

### 3.3 | Soil microbial community structure

PLFA analysis revealed that long-term N fertilization and landscape positions had a significant impact on soil microbial community structure (Tables 2 and 3). The microbial biomass represented by total PLFAs was significantly higher in N applied treatments and footslope position compared with the treatment that did not receive N fertilizer and shoulder position. Total PLFAs biomass was significantly higher with medium (5,946 ng PLFA-C/g soil) and high N rates (5,871 ng PLFA-C/g soil) compared to low N rates (4,274 ng PLFA-C/g soil). Under different slope positions, significantly higher total PLFAs was observed at footslope (7,054 ng PLFA-C/g soil) compared to shoulder position (3,673 ng PLFA-C/g soil). The mean abundance of total bacterial, actinomycetes, gram-negative and positive bacteria, AM fungi, protozoa and undifferentiated PLFAs were significantly increased in the N-amended plots and footslope position. Significantly higher total bacterial PLFAs was observed with high (3,106 ng PLFA-C/g soil) and medium N rates (3,042 ng PLFA-C/g soil) than low N rates (2,146 ng PLFA-C/g soil). Significantly higher concentration of total bacterial PLFAs was recorded at footslope slope position (3,700 ng PLFA-C/g soil) than shoulder position (1,830 ng PLFA-C/g soil). Concentration of actinomycetes PLFAs was significantly higher with medium (544 ng PLFA-C/g soil) and high N rates (534 ng PLFA-C/g soil) compared to low N rates (376 ng PLFA-C/g soil). Among the slope positions, significantly higher concentration of actinomycetes PLFAs was recorded at footslope slope position (657 ng PLFA-C/g soil) compared to shoulder position (313 ng PLFA-C/g soil).

With regard to gram-negative bacterial PLFAs, significantly higher concentration was observed with medium N rates (1,457 ng PLFA-C/g soil) compared to low N rates (998 ng PLFA-C/g soil). Application of N fertilizer had a positive impact on gram-positive bacterial PLFAs. Higher gram-positive bacterial PLFAs was observed under high N rates (1,667 ng PLFA-C/g soil), and comparatively lower PLFAs was recorded in low N rates (1,148 ng PLFA-C/g soil). Further, total fungi biomass PLFAs concentration was significantly higher with medium N (1,004 ng PLFA-C/g soil) than low N rates which has 726 ng PLFA-C/g soil. Furthermore, long-term N fertilization had a positive impact on AM fungi PLFAs in the following order with medium N > high N > low N. However, these parameters were not significantly different between medium and high N rate. The landscape position also had a significant effect on AM fungi PLFAs in the order of footslope (509 ng PLFA-C/g soil)>shoulder (405 ng PLFA-C/g soil) position (Table 2).
The saprophytes PLFAs concentration was 36.3% higher in medium N rates than low N rates, respectively (Table 3). Among the landscape positions, the higher saprophytes PLFAs of 93.5% observed under footslope than shoulder position. Significantly higher protozoa PLFAs were observed with medium N rates, which was 1.44 and 1.49 times higher than low and high N rates, respectively. Both high and medium N rates increased significantly higher undifferentiated PLFAs compared to lower N rate. The N rate and landscape positions (except for fungi:bacteria ratio) did not significantly influence the fungi:bacteria ratio, predator:prey ratio, and gram-positive:gram-negative bacteria ratio.

3.4 Soil enzyme activities

Difference in enzyme activities was observed under different nitrogen application rates and landscape positions. In general, results showed comparatively higher enzymatic activities in the medium N rates than high and low N rates. Under different slope positions, the higher enzymatic activities were observed under footslope position than shoulder position (Table 4). The urease enzyme activity was significantly affected by N rates and landscape positions. The urease activity under medium (36.3 µmol N-NH<sub>4</sub><sup>+</sup> g<sup>-1</sup> soil hr<sup>-1</sup>) and high N rates (31.4 µmol N-NH<sub>4</sub><sup>+</sup> g<sup>-1</sup> soil hr<sup>-1</sup>) were 42.9% and 23.6% higher compared to low N rates (25.4 µmol N-NH<sub>4</sub><sup>+</sup> g<sup>-1</sup> soil hr<sup>-1</sup>). The urease enzyme activity at the footslope position (33.2 µmol N-NH<sub>4</sub><sup>+</sup> g<sup>-1</sup> soil hr<sup>-1</sup>) was significantly higher than the shoulder position (28.9 µmol N-NH<sub>4</sub><sup>+</sup> g<sup>-1</sup> soil hr<sup>-1</sup>).

Nitrogen fertilization and landscape positions significantly affected the β-glucosidase enzyme activity in soil. The high (95.0 µmol pNP g<sup>-1</sup> soil hr<sup>-1</sup>) and medium N rates (90.9 µmol pNP g<sup>-1</sup> soil hr<sup>-1</sup>) showed a higher β-glucosidase enzyme activity of 25.8% and 20.4% than low N rates (75.5 µmol pNP g<sup>-1</sup> soil hr<sup>-1</sup>). Under different landscape positions, the β-glucosidase enzyme activity under footslope position (96.6 µmol pNP g<sup>-1</sup> soil hr<sup>-1</sup>) was significantly higher than shoulder position (77.7 µmol pNP g<sup>-1</sup> soil hr<sup>-1</sup>). The acid phosphatase enzyme activity did not change significantly among N rates. The landscape positions had a significant effect on acid phosphatase enzyme activity. Significantly higher acid phosphatase enzyme activity was recorded at footslope position (1.35 µg pNP g<sup>-1</sup> soil hr<sup>-1</sup>) than shoulder position (0.91 µg pNP g<sup>-1</sup> soil hr<sup>-1</sup>). The alkaline phosphatase enzyme activity was significantly affected by different N rates and landscape positions. The results showed that alkaline phosphatase enzyme activity was significantly higher with medium N rates (1.65 µg pNP g<sup>-1</sup> soil hr<sup>-1</sup>) than high (1.48 µg pNP g<sup>-1</sup> soil hr<sup>-1</sup>) and low N rates (1.28 µg pNP g<sup>-1</sup> soil hr<sup>-1</sup>). Significantly higher alkaline enzyme activity was observed at shoulder position (1.60 µg pNP g<sup>-1</sup> soil hr<sup>-1</sup>) than footslope position (1.34 µg pNP g<sup>-1</sup> soil hr<sup>-1</sup>). As shown in Table 4, the arylsulfatase enzyme activity in the soil applied with medium (10.1 µg pNP g<sup>-1</sup> soil hr<sup>-1</sup>) and high N rates (9.45 µg pNP g<sup>-1</sup> soil hr<sup>-1</sup>) was significantly higher than low N rates (6.48 µg pNP g<sup>-1</sup> soil hr<sup>-1</sup>). Landscape positions also significantly impacted arylsulfatase
enzyme activity. The arylsulfatase enzyme activity at the footslope position (9.52 µg pNP g\(^{-1}\) soil hr\(^{-1}\)) was significantly higher than the shoulder position (7.80 µg pNP g\(^{-1}\) soil hr\(^{-1}\)).

### 3.5 Principle component analysis (PCA)

The PCA results showed that N and landscape positions had a significant influence on soil microbial and enzymatic activities (Figure 2). The first principle component explains 76% of total variation and the second one 14% variation. The PCA results showed that medium and high N rates under footslope position had a significant influence on soil microbial communities, viz. total fungi, AM fungi, protozoa, gram-negative, saprophytes, total PLFA, bacterial, actinomycetes and gram-positive PLFAs. The medium and high N rate with footslope position also clustered with urease, beta-glucosidase, acid phosphatase, arylsulfatase enzymes, MBN, MBC, CWC and HWC. Alkaline phosphatase and pH were clustered under medium N rate with shoulder position, suggesting that alkaline phosphatase and pH were more influenced by N fertilization and shoulder position.

### 4 DISCUSSION

The finding from this study showed that N rate did not significantly influence the pH in the switchgrass field, whereas the pH values were influenced by landscape positions. The pH values were alkaline at the shoulder compared to the footslope position. Loeppert and Suarez (1996) reported that inorganic carbonates are associated with increased pH in shoulder position. Similar trend of increased pH at shoulder position compared to footslope position was also found by Rhanor (2013) and Mbonimpa et al. (2015). Further, labile soil C pools (CWC and HWC) of soil organic carbon are considered as sensitive indicators of changes in soil biological conditions caused by soil management practices (Choudhary & Gill, 2013). The concentrations of C were always higher in the HWC than in the CWC fraction, reflecting different chemical compositions and stability of water extracts (Figure 1). The concentration of CWC very closely reflects dissolved organic carbon (Ostrowska, Porębska, & Kanafa, 2010). The labile soil N pools (CWN and HWN) largely represent degraded plant material together with microbial tissues (Six, Conant, Paul, & Paustian, 2002). In this study, N rate significantly impacted the CWC and CWN fractions, whereas HWC and HWN fractions were not influenced by the N rate. The landscape position had a significant impact on HWC and HWN fractions and not significantly influenced the CWC and CWN fractions. The higher level of water-soluble fractions under N fertilization at footslope position may be due to the addition of higher amount of plant residues and microbial activity. Increased microbial activities through N fertilization may accelerate soil carbon and nitrogen
decomposition and increased the water-soluble fractions of C and N (Chaudhary, Dheri, & Brar, 2017). Water-soluble fractions of organic C is a C source for soil microbes, and it is also believed to be microbially mediated (Christ & David, 1996); the flow of water-soluble C fractions in soil supplies substrate for microbial biomass turnover (Benbi, Kiranvir, & Sharma, 2015).

In this study, N rates significantly impacted soil MBC, but did not influence MBN. The soil MBC and MBN values were increased with medium N addition, but decreased with high N rates. The higher N addition decreased soil MBC and MBN, likely due to decreased soil pH. With increasing rates of N application, the decrease in pH unit of N is well pronounced (Geisseler & Scow, 2014b). Studies carried out in a different ecosystem have shown that pH exerts a strong influence on soil microbial biomass (Geisseler & Scow, 2014a). Tian et al. (2017) reported that the increased addition of N decreased the soil MBC and MBN due to reduction in soil pH. The decrease in pH results from nitrification, the oxidation of ammonium to nitrite and then to nitrate, which increases protons and thereby increases soil acidity (Geisseler & Scow, 2014b).

In this present study, soil MBC and MBN were significantly influenced by landscape positions. Soil MBC and MBN values were 56.7% and 35.1% higher at footslope position than shoulder position, respectively. Khalili-Rad, Nourbakhsh, Jalalian, and Eghbal (2011) reported greater microbial biomass at footslope slopes than more elevated landscape positions in a semi-arid wheat system. Similar trend has also been confirmed in non-agricultural habitats such as pasture and forest systems (Askin & Kizilkaya, 2009; Kussainova, Durmuş, Erkoçak, & Kızılkaya, 2013). Increased soil MBC

### Table 4

| Treatment | Ureaase (µmol N-NH₄⁺ g⁻¹ soil hr⁻¹) | β-glucosidase (µmol pNP g⁻¹ soil hr⁻¹) | Acid phosphatase (µg pNP g⁻¹ soil hr⁻¹) | Alkaline phosphatase (µg pNP g⁻¹ soil hr⁻¹) | Arylsulfatase (µg pNP g⁻¹ soil hr⁻¹) |
|-----------|-----------------------------------|--------------------------------------|----------------------------------------|------------------------------------------|----------------------------------|
| **N Rate** |                                   |                                      |                                        |                                          |                                  |
| High      | 31.4b                             | 95.0a                                | 1.19a                                  | 1.48ab                                   | 9.45a                            |
| Medium    | 36.2a                            | 90.9a                                | 0.95a                                  | 1.62a                                    | 10.6a                            |
| Low       | 25.4b                            | 75.5b                                | 0.91b                                  | 1.54b                                    | 8.68b                            |
| **Position** |           |                                      |                                        |                                          |                                  |
| Shoulder  | 28.9b                            | 77.7b                                | 0.91b                                  | 1.35b                                    | 7.31b                            |
| Footslope | 33.2a                            | 96.6a                                | 1.35a                                  | 1.85a                                    | 10.6a                            |

### ANOVA (p>F)

- Position (P): <0.0001, Position(P): 0.008, Position(N): 0.04, Position(...): 0.01, Position(...): 0.01, Position(...): 0.01

**Note:** Mean values within the same column followed by different small letters are significantly different at p < 0.05 for each N rate and landscape position.

### Figure 2

Principal component (PC) analysis scores (1 and 2, square symbols) for soil characteristic as determined at two positions Shoulder and Footslope (FS) under three levels (0, 56 and 112 kg/ha) of nitrogen (N) fertilization. The eigenvectors (of PC1 and PC2) of the soil characteristics (circles) are also super imposed with the PC scores biplot at a similar scale reflecting their association. The eigenvectors were multiplied by five to obtain a clear and superimposed figure.
and MBN in footslope positions of switchgrass fields may lead to more rapid biological turnover of plant matter and integration of new residues into soil organic matter (Tiemann, Grandy, Atkinson, Marin-Spiotta, & McDaniel, 2015).

PLFAs are the key components of microbial cellular membranes of all living organisms (Kaur, Chaudhary, Kaur, Choudhary, & Kaushik, 2005). Microorganisms produce varieties of PLFAs, which act as microbial biomarker. The PLFAs extracted from soils through analysis can provide an information about the overall microbial community structure of terrestrial ecosystem (Quideau et al., 2016). In general, nitrogen application decreases soil microbial biomass (Wei et al., 2013), but in our present study, N addition (medium and high N rates) significantly increased total PLFA content, total bacterial, actinomycetes, gram-negative, gram-positive, total fungi, AM fungi, saprophytes, protozoa and undifferentiated PLFAs biomass concentration. In the present study, significantly lower concentration of total PLFAs was recorded in low N rates. The observed increase in the soil microbial communities may be due to the availability of more substrate for microbial growth. Lv, Xue, Wang, and Zhang (2017) also observed that application of N increased the soil microbial communities. Many studies have observed that fertilizers application can alter the microbial community composition which can be measured by PLFA patterns (Böhme, Langer, & Böhme, 2005; Rousk, Brookes, & Bååth, 2011; Zhong et al., 2010). In a literature review, Allison and Martiny (2008) also found that 84% of the studies reported that microbial community composition is sensitive to N, phosphorus (P) and potassium (K) fertilization. Some long-term experiments in Tennessee, USA (Peacock et al., 2001), and at Bad Lauchstadt (Böhme et al., 2005) and Halle, Germany (Langer & Klimanek, 2006) observed that gram-positive bacteria tended to increase under N fertilization. These findings are corroborated with the results of the present study where higher gram-positive bacterial PLFAs biomass recorded in high N fertilization. According to Kirchmann, Schön, Börjesson, Hammér, and Kätterer (2013), the possible reason behind the increase in bacteria with N fertilization could be due to repeated addition of more N-rich residues to the soil. The increase in root exudation, exudate sugar, sugar alcohol and phenolic content under N fertilization promote a more bacterial community in soil (Zhu, Vivanco, & Manter, 2016). Increasing the N addition from medium to high inhibited the overall microbial growth. If adding N relieves the bacterial growth limitation, it is possible that the competitive potential of the bacterial community increases relative to that of fungal community, resulting in a decrease in fungal growth. Mille-Lindblom and Tranvik (2003) found an interaction between fungal and bacterial growth on submerged plant material. The results revealed that the presence of bacteria inhibited the fungal growth. Compared to gram-negative bacteria, gram-positive bacteria biomass was higher in the present study and showed a shift in community structure. Landscape positions also significantly influenced the soil microbial community structure. Higher concentrations of PLFAs of microbial communities were observed on the footslope position with an increasing trend from shoulder to footslope position. Such variations of PLFA biomass as affected by landscape positions have also been related to variations of organic matter and soil moisture content (Khalili-Rad et al., 2011). Nahidan, Nourbakhsh, and Mosaddeghi (2015) and Feng, Su, Zhang, Chen, and He (2015) also found that microbial biomass affected by the landscape positions; and higher microbial biomass was recorded in the footslope position due the presence of higher organic matter content.

Nitrogen mineralization is an important process in soils because it supplies sufficient amount of N for plant growth and development (Chang, Chung, & Tsai, 2007). In this present study, the highest urease enzyme activity was observed with the application of medium and high N rates and the lowest was observed in the low N rates. The results also revealed that increasing rate of N decreased urease enzyme activity especially with high N rate treatment. Application of medium N rate might have promoted higher root growth by stimulating the N demand (Stewart et al., 2016) and consequently increased urease enzyme activity in soil. Perennial bioenergy crops contribute substantially more belowground biomass compared to annual crops and increase the activity of soil microbes with a consequent increase in the soil enzymatic activity (Johnson, Archer, Weyers, & Barbou, 2011). In this study, urease enzyme activity increased with N application, reached optimum value and decreased with increasing N application (Jingjing, Mijia, Xiaojia, Chi, & Jun, 2015; Thenabadu & Dharmakeerthi, 1996). The results from the present study observed significant differences in urease enzyme activity among landscape positions. The urease enzyme activity on footslope position was significantly higher than the footslope position, which was in good agreement with the work of Asskin and Kizilkaya (2006). Higher microbial biomass and deposition of soil organic carbon might have enhanced urease enzyme activity at the footslope position. According to Bo-Jie, Shi-Liang, Li-Ding, Yi-He, and Jun (2004), shoulder soils were the most affected by erosion, and footslope soils showed the higher organic matter and clay content. Because of the high in organic matter and microbial biomass contents, it was assumed that organic matter, microbial biomass and clay content might be affecting the urease enzyme activity in the soil (Dengiz, Kizilkaya, Gö, & Hepsen, 2007).

In this present study, N application increased soil β-glucosidase enzyme activity partially due to the accumulated microbial biomass especially fungi. Application of N might have enhanced the fungi population in soil and in turn they produced β-glucosidase enzyme. Soil microbes allocate more N under high soil N availability towards the production of enzymes used for acquiring energy, among other nutrients.
Soil β-glucosidase is a kind of enzyme that mainly originates from fungi (Elíasides, Rojas, Cabello, Voget, & Saparrat, 2011). The application of N fertilizer to switchgrass enhanced the fungi population in soil in turn produced more β-glucosidase enzyme. Zhang et al. (2015) reported that long-term addition of N increased β-glucosidase enzyme activity in soil. In addition, Lupwayi, Kanashiro, Eastman, and Hao (2018) found that under long-term manure and fertilization, soil microbial properties increased and improved the β-glucosidase enzyme activity in soil. Improvement in soil microbial biomass enhances the substrate availability and increases the activity of β-glucosidase enzyme in soil (Sinsabaugh et al., 2008). In this present study, the landscape positions had a significant impact on β-glucosidase enzyme activity. It could be attributed to organic matter build-up and deposition, as well as more biological activity at the lower landscape positions which promote more organic matter build-up and more substrate availability and improved β-glucosidase enzyme activity in soil. Khalili-Rad et al. (2011) reported greater glutaminase activity at toe slopes than more elevated landscape positions in a semi-arid wheat system. In another study, low-slope (depression) contained higher β-glucosidase enzyme activity than summits and slopes (Wickings, Grandy, & Kravchenko, 2016). It can be hypothesized that higher content of organic matter in footslope positions has provided more substrate and energy for microbial biomass and consequently has resulted in higher β-glucosidase enzyme activity.

In soil, phosphatase enzyme catalyzes the hydrolysis of organic phosphorus to inorganic phosphorus (Nannipieri, Giagnoni, Landi, & Renella, 2011). Results from this present study indicated that N fertilization and landscape positions affected activity of acid and alkaline phosphatase enzymes in soil. The results show that application of medium and high N rates had a significant influence on acid and alkaline phosphatase enzymes activity in soil. Significantly higher acid phosphatase enzyme activity was observed at footslope position, whereas significantly higher alkaline phosphatase enzyme activity was observed at shoulder position. The footslope soils have lower pH values compared to shoulder position. The significant effect of N fertilization on activities of acid and alkaline phosphatase in soils can be attributed to lower and higher pH values at footslope and shoulder positions, respectively. This favours the acid phosphatase enzyme activity because it has been shown that acid phosphatase is predominant in acid soils and alkaline phosphatase enzyme activity is predominant in alkaline soils (Juma & Tabatabai, 1978; Šarapatka, Dudová, & Kršková, 2004). The current findings are in accordance with the previous reports of the importance of pH in regulating activity of acid and alkaline phosphatase enzyme in soil (Eivazi & Tabatabai, 1977). The rate of synthesis and release of acid phosphatase enzyme by soil microorganisms and its stability in soils increases with lowering pH (Tabatabai, 1994). Dodor and Tabatabai (2003) and Dick, Cheng, and Wang (2000) also reported that N fertilization and pH affected the activities of acid and alkaline phosphatase enzyme activity in soil.

Arylsulfatase enzyme activity is a measure of the inherent capacity of a soil to catalyse the hydrolysis of ester sulphates (Whalen & Warman, 1996). Soil filamentous fungi play an important role in mobilization of sulphate esters (Baum & Hrynkiewicz, 2006). Active microorganisms are associated with the enzymatic activities in soil because the microbial biomass is considered as the primary source of soil enzymes (Klose, Moore, & Tabatabai, 1999). In this present study, application of N fertilizer and landscape positions had a significant impact on arylsulfatase enzyme activity. The enhanced population of bacteria and fungi could have increased the production of arylsulfatase enzyme activity due to the N fertilizer application. Ekenler (2002) also found a positive relationship with soil microbial biomass and arylsulfatase enzyme activity in soil.

The PCA summarized the analyses and placed shoulder and footslope positions in totally opposite sides on the quadrants, as well as, the N fertilization clustered with soil carbon pools, microbial communities and soil enzymes, showing that medium to high N fertilization under footslope position significantly influenced soil microbial parameters. The medium N rate at the footslope position might create relatively stable environment for microbial activity, which in turns enhances the carbon pool, MBC, MBN, soil microbial communities and soil enzyme activities that are considered as a sensitive indicators of soil biological health (Lv et al., 2017; Pan et al., 2018).

Soil microbial community and soil enzymatic activities are the foundation of enhanced nutrient availability and organic matter decomposition processes which leads to a sustainable crop production. Responses of soil microbial community structure and soil enzymatic activities to N fertilization and landscape positions were evaluated in this study. The N rate and landscape positions were significantly influenced the soil carbon pools, soil microbial communities and soil enzymatic activities. The overall results show that increasing N rates up to medium level N increased most of the soil biological properties and altered the microbial community structure and composition over low N rates. A declining trend on soil microbial communities and soil enzymatic activities was observed with the application of medium N to higher N application rate. The higher concentration of microbial PLFAs and soil enzyme activities was found at footslope position. All the soil enzymes except alkaline phosphatase enzyme were increased under footslope position and decreased in the shoulder position. The results also revealed that application of medium N rate at footslope position increased the soil biological health under switchgrass. This implies that application of medium level N under footslope position created more favourable
environment to the growth of microorganisms and plant roots that secrete enzymes into the soil. Since the microbial biomass is considered to be the primary source of soil enzymes, and the application of medium level N treatment under footslope position recognized to be an environment that promotes the large portion of soil microbes, we assume that the higher enzyme activity may be explained in terms of higher population of microbes metabolizing the amino acids, sugars and organic acids and other compounds of plant roots and organic matter of soil. Thus, a long-term addition of medium level N fertilization under footslope position to switchgrass can improve soil microbial communities and soil enzymatic activities which are the indicators of soil biological health.

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