INFLUENCE OF SWITCHGRASS MANAGEMENT ON SOIL MICROBIAL BIOMASS AND FATTY ACID METHYL ESTER CONTENT IN SOIL

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Title of Study: INFLUENCE OF SWITCHGRASS MANAGEMENT ON SOIL MICROBIAL BIOMASS AND FATTY ACID METHYL ESTER CONTENT IN SOIL

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Abstract: Switchgrass is a fairly new source of commercial biofuel feedstock. Its production can potentially preserve soil health and microbial functions in ecosystems. There is an increasing demand for large scale biomass production, making the relationship between crop production and soil health more important. Microbial biomass abundance and ratios can be an indicator of broader soil health, as they provide a measure of the living portion of soil organic matter. The goal of this study was to evaluate relationships between switchgrass production and the soil microbial community. Composite soil samples from three established switchgrass plots and adjacent uncultivated mixed grass plots were used for microbial biomass analysis, as well as fatty acid methyl ester (FAME) analysis. Compared to the adjacent unmanaged soils, switchgrass managed soils had a significantly lower microbial abundance indicated by the content of microbial biomass carbon, nitrogen and phosphorous, as well as total FAME. Microbial composition and community structure was also altered by switchgrass management. Data showed significantly lower microbial biomass C/N ratios and changes in the concentration of FAME indicator groups in the switchgrass managed soils than the adjacent unmanaged ones. More specifically, switchgrass management led to reduction in the abundance of gram positive bacteria, gram negative bacteria, actinobacteria, and saprophytic fungi, but promoted the growth of arbuscular mycorrhizal fungi.

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CHAPTER I

Introduction

The influence of soil microbes and their impact on soil health and crop production is well documented (Huang, 2016); there is a known relationship between plant type and soil microbial abundance and structure (Francini et al. 2018; Xiao et al. 2017). This relationship is important because knowing the state of a soils health may promote better management practices for producers.

As our world population grows and cropping systems become larger, the relationship between crop production and soil health becomes more important. There is an increasing demand for large scale biomass production as non-renewable resource consumption rises. Switchgrass is a fairly new source of commercial biofuel production. As a perennial grass, switchgrass production requires relatively low input and management from producers. When harvesting switchgrass, the root system and soil is left intact (Hartman, 2011). With its extensive fibrous roots; switchgrass can minimize soil erosion and may serve as a protectant to the soil structure and microbial community. As a no-till crop, switchgrass may have more potential than traditional biofuel crops to preserve soil health and microbial populations in soil.

Microbial community diversity and structure are often negatively influenced by cultivation, but disturbance is lower in no till systems (Gupta et al., 1988; Srivastava et al., 1989).

Microbial biomass abundance and ratios can be an indicator of broader soil health, as they provide a measure of the living portion of soil organic matter, and the ratio of microbial biomass carbon to nitrogen indicates community structure and diversity.

Data from previous studies suggest switchgrass management may lead to an increase in microbial biomass carbon and nitrogen when compared to other cropping systems. One study comparing switchgrass to a winter wheat cropping system concluded soils under switchgrass were found to have a higher soil C/N ratio and a significantly greater microbial biomass carbon percentage (Chatterjee et al., 2013). When comparing managed switchgrass to a traditional cropping system microbial biomass carbon was 200% greater in the soil under switchgrass cultivation (Al-Kaisi and Grote, 2007). However, there is contradicting information on how switchgrass managment specifically affects soil health and the microbial community.

The goal of this study was to evaluate relationships between switchgrass production and the soil microbial community. The specific objective was to determine the impact of the management of switchgrass on the microbial community abundance, diversity, and structure through evaluation of microbial biomass content and analysis of fatty acid methyl ester (FAME) profiles.

CHAPTER II

Review of Literature

Switchgrass cropping effects on soil microbial community

In cropping systems, microbes and plants coexist and influence each other. Many factors influence the dynamics of the soil microbial community such as the types of soil and plant, soil management practices (fertilizer input, tillage, cover crop, etc), geographical location, and macro- and micro-climate. Because the complexity of the system, experimental results on predominant factors influencing the microbial community are not always consistent.

Based on evaluations of chemical and microbial functional diversity under Switchgrass and sorghum production in different geographical locations, little significant differences were detected between soils under these biofuel crops and the native grassland (Watrud et al., 2013). Microbial genetic diversity was similar between the sites, but gene abundance was significantly lower in soils under biofuel crop cultivation than in the native grassland. A lower soil pH was observed in switchgrass than the native grassland soils. Four out of the six switchgrass sites evaluated had higher levels of active fungal biomass than the grassland. In two locations, there was a significantly lower gene diversity in switchgrass fields than in grassland. However, results are consistent that a significantly lower microbial abundance in soils under biofuel crops were found than in native grassland, and considerable variations were observed depending on the geographical location.

In a study looking into the effect of intercropping switchgrass into pine plantation on soil carbon and microbial activity, two-years of switchgrass cultivation greatly impacted soil properties, it led to a decrease in soil carbon and nitrogen content. There was an overall 21% decrease in total soil carbon from the upper 15 cm of the soil profile under switchgrass cultivation (Strickland et al., 2015). However, active microbial biomass was higher under switchgrass cultivation, and soil microbes are a dominant precursor of soil carbon formation. However, this was a short-term two years study. Caution should be exercised when extrapolating data for in-depth interpretation.

When comparing two switchgrass trials in differing ecotypes, microbial biomass and composition varied (Roosendaal et al., 2016). In soils under switchgrass grown in a lowland ecotype, a higher percentage of bacteria and higher overall microbial biomass was found, while soils under switchgrass grown in an upland ecotype had lower overall microbial biomass and a higher fungal to bacteria ratio. The switchgrass grown in the lowland soils had a higher total above and belowground biomass. In both sites, microbial biomass decreased with soil depth. Total soil carbon and nitrogen decreased and soil pH increased with soil depth in both sites.

Interestingly, geographical location had little influence on microbial biomass content in soils under switchgrass cultivation at seven sites and two different locations (Liang et al., 2016). The switchgrass has been established at least 10 years prior to sampling, and there were wide variations in the two locations, including soil type, years of switchgrass establishment, and management intensities. When microbial communities in the rhizosphere was compared with bulk soils, higher microbial biomass was found in the rhizosphere. Rhizosphere soils also had a higher microbial lipid abundance, particular those associated with fungi and gram-negative bacteria. It was concluded that switchgrass promoted rhizosphere microbial activity with fast nutrient cycling, potentially improving plant access to nitrogen and other nutrients. Gram negative bacteria were found at a higher rate in soils that had been cultivated for at least 10 years.

These results are consistent with another study using phospholipid- derived fatty acids (PLFA) to evaluate microbial profiles in rhizosphere soils under biofuel plant cultivation (switchgrass and jatropa). For both plants, rhizosphere soils contained a larger abundance of actinomycetes and fungi, as well as higher overall microbial biomass compared to bulk soil sample (Chaudhary et al., 2012). Data also suggested that switchgrass cultivation led to higher abundance of bacteria, while jatropa cultivation led to higher abundance of fungi. In this study, branched chain PLFAs indicated gram positive bacteria; monounsaturated PLFAs indicated gram negative bacteria; and polyunsaturated PLFAs indicated fungal population. It was concluded that the differences in microbial community compositions of the two plants were likely due to the differences in their root exudation.

Biomass removal and soil biogeochemical processes were compared under switchgrass and winter wheat cultivation. This study concluded that the introduction of switchgrass has an effect on soil nutrient dynamics; over a three year field study, switchgrass cultivation was found to have a higher soil carbon:nitrogen ratio (Chatterjee et al., 2013). This effect varied with net primary productivity (low, medium or high productivity of the crop) and soil depth (most significant in the 0-15 and 15-30 cm range) but soils under switchgrass showed to have a significantly greater microbial biomass carbon percentage. On average, soils under switchgrass cultivation had a 52% higher microbial biomass content than winter wheat. The soil nitrogen mineralization rate was also higher under switchgrass than under winter wheat.

When comparing managed switchgrass to a corn-soybean rotation, microbial biomass carbon was 200% greater in the soil under switchgrass cultivation (Al-Kaisi and Grote, 2007). Although the cropping systems in this study had been in place for 25 years, there was no significant difference in soil carbon content, which suggests that soil carbon accumulates very slowly compared to the quicker increases in soil microbial biomass carbon.

Switchgrass Carbon Sequestration

Bioenergy crops have the capacity to produce a large volume of biomass with high energy potential. Planting bioenergy crops in degraded soils has been shown to have a positive effect on soil carbon stock, with carbon sequestration rates ranging from 0.6 to 3.0 Mg C ha^{-1} yr^{-1}. (Lemus and Lal, 2005). Grasslands in particular have an underground biomass component as a carbon sink in their root systems. Switchgrass root systems increase the amount of soil organic carbon due to both the size of the root systems and the root secretions of organic compounds, which bind soil particles and stabilize soil organic carbon (Hartman et al., 2011). Root and crown biomass of switchgrass averages 84% of the total plant biomass, with crown tissue containing approximately 50% of the total biomass carbon (Frank et al., 2004).

In a four year field experiment conducted to evaluate soil carbon sequestration in switchgrass, it was found that soil carbon was sequestered at a rate of 2.4 ± 0.9 and 4.0 ± 1.0 Mg C ha^{-1} (Lee et al., 2007) This is consistent with another study comparing switchgrass carbon sequestration to that of corn and willow, where switchgrass had a significantly higher carbon accumulation than both of the other crops (Zan et al., 2001). Carbon sequestration was found here at rates of 1.7 to 3.0 Mg ha-1 yr^{-1}. Evidence shows that compared to other crops, switchgrass needs a longer period of time to accrue significant gains, and results vary significantly depending on establishment time. When looking at biomass and carbon partitioning in switchgrass, net system carbon gain increased over three years after seeding (Frank et al., 2004). Total carbon nearly doubled in this time and seemed to correlate most with the increase in crown biomass. A better baseline for assessing soil carbon sequestration in switchgrass is still needed to estimate the true amount of carbon being sequestered.

Soil microbial groups

Soils are a dynamic and ever changing environment that harbor countless microbes, it was estimated that a single gram of soil contains as many as 10^{10}–10^{11} bacteria (Horner-Devine et al., 2003). Several studies have evaluated the relationship between plant productivity and microbial community structure and diversity. Studies such as that by Maherali and Klironomos (2007), observed that plant productivity and nutrient acquisition were increased with a higher fungal diversity. One study by Van der Heijden et al. (1998) found that a grassland with the higher mycorrhizal fungal diversity had 42% higher plant productivity.

The composition of the microbial community was more important in cases where there was nutrient depleted soil, as found under cultivation. This is because plants were less dependent on the microbial community when nutrient availability was high and readily available in soil (Sprent & Platzmann, 2001). Johnson et al. (2001) proposed that fungal diversity was strongest when soils had a lower nutrient availability because different fungal species were able to obtain limiting nutrients through various soil sources. The ratio of bacterial communities to fungal communities were also important to plant productivity. Bacteria and fungi function differently in soil (Wardle et al., 2004); bacterial dominated microbial communities are often characterized by high levels of disturbance with high nutrient availability; these environments often have reduced soil organic matter content (Van der Heijden, 2008). Fungal dominated microbial communities were more common in soils with less disturbance and higher percentages of organic matter. Soil communities are quick to adapt and change, a bacteria-dominated soil could change to a fungal dominated soil with time and land use changes (Bardgett et al., 2005). A fungal dominated soil could also shift to bacteria dominated as with nutrient additions or cultivation.

Specific microbial communities have an effect on plant productivity and nutrient acquisition. Arbuscular mycorrhizal fungi (AMF) are particularly important in helping plants tolerate stress

conditions (Xavier and Boyetchko, 2002) and acquire nutrients, particularly phosphorous (Miransari, 2011). AMF has been found to enhance crop yield under stress which is found more commonly under field conditions (Daei et al., 2009).

Conclusions and objectives

Microbial community abundance and structure can be an indicator of broader soil health, as they provide a measure of the living portion of soil organic matter. In cropping systems there are many factors that influence the soil microbial community, including plant types, soil types, management practices, level of soil disturbance and time passed since crop establishment. Studies have found that switchgrass has the potential to better sequester carbon and improve soil health in the long term compared to traditionally cultivated crops. There is contradicting information involving the effects of switchgrass management on the microbial community. There are also very few studies comparing the effects of switchgrass management on the microbial community several years after establishment. The objective of this study was to examine relationships between switchgrass production and the soil microbial community. Specifically, to determine whether and to what extent switchgrass management influenced microbial content and composition in soils after nine years of crop establishment and management.

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CHAPTER III

Methodology

Soil Sampling

Soil samples were taken in 2018 from long-term switchgrass (*Panicum virgatum*) experimental sites. Soils from an adjacent area with mixed native vegetation were used to serve as controls. Three sites were used for this study and their respective soil types were Cow Creek (CC) (36°7’2.77”N, 97°5’52.06”W) on Easpur loam, EFAW (EF) (36°7’52.64”N, 97°6’16.75”W) on Easpur loam, and 40 North (4N) (36°8’21.48”N, 97°4’44.4”W) on Huska silt loam. Basic soil properties, such as soil series, subgroup, pH, organic carbon and total nitrogen are shown in Table 1. The switchgrass experimental sites were fertilized in the spring of each year with urea (46-0-0) fertilizer at 84 Kg ha^{-1}. Atrazine and metolachlor were also applied in the spring. At each site, there were three switchgrass experimental units. Five cores were composited to create a soil sample from each experimental unit at three soil depths, 0-10 cm, 10-20 cm and 20-30 cm.

Fresh soil samples were immediately processed and sieved with a 2-mm sieve. The processed samples were mixed thoroughly and were divided into three parts, a portion of the processed sample was freeze-dried and stored at -20^{o}C; a portion was air-dried and stored in sealed containers at 23^{o}C, and another portion was kept field-moist in sealed container stored at 0^{o}C. A portion of the

air-dried soil was ground to pass 80 mesh for determination of total carbon and nitrogen. Air-dried microbial biomass content, and freeze-dried soils were used for FAME (Fatty Acid Methyl Ester) analysis.

Soil analysis

Soil moisture content was determined gravimetrically from field moist samples by drying at 105°C for 48 hours. Soil texture was determined using composite samples by the hydrometer method (Gee and Or, 2002). This method consisted of adding 100 mL of HMP (Sodium Hexametaphosphate 5%) dispersing solution to samples and putting them on a horizontal shaker for sixteen hours. After sixteen hours the suspension was transferred to a sedimentation cylinder and deionized water was added to bring the final volume to 1.0 L. The suspension was then allowed to equilibrate to room temperature for two hours before using the hydrometer to take readings.

Total carbon and nitrogen were found through dry combustion analysis by placing soil samples wrapped in foil into a Leco TruSpec combustion analyzer (Bremner, 1996; Nelson and Sommers, 1996). Soil organic matter (SOM) was determined through the Walkley-Black titration method (Gavlak et al. 2003). This involved transferring 1 gram of air dried soil into a conical flask, and adding 10 ml 1N $K_2Cr_2O_7$ solution and 20 ml of concentrated H_2SO_4. The contents of the flask was swirled then allowed to stand for 30 minutes for the reaction to complete. Then 200 ml of distilled water was added to the flask to dilute the suspension. Then 10 ml of orthophosphoric acid and 1 ml of diphenylamine indicator was added. The solution was then titrated with 0.5 N ferrous ammonium sulphate until the color changed from violet to blue and finally to bright green. Soil pH was determined using a standard glass electrode (1:2) Soil/$CaCL_2$ solution (McLean, 1982). Soil property results are presented in Table 1.

Table 1 Physiochemical soil properties at three locations in Oklahoma, U.S.A. †

Soil location/series/subgroup	Vegetation	Soil Depth	pH‡	Organic C	Total N	Clay§	Sand§	Silt§
				------- g kg $^{-1}$ ----------		---------- % ----------		
Cow Creek/ Easpur/ Fluventic Halustolls	Bermuda grass	0-10cm	$6.4^{a}{}_{A}$	$14.9^{a}{}_{A}$	$1.4^{a}{}_{A}$	13.8	47.5	38.7
		10-20cm	$6.6^{a}{}_{A}$	$7.5^{a}{}_{A}$	$0.8^{ab}{}_{A}$	15.0	50.0	35.0
		20-30cm	$7.2^{a}{}_{A}$	$5.2^{a}{}_{A}$	$0.6^{b}{}_{A}$	13.8	47.5	38.7
	Switchgrass	0-10cm	$6.2^{a}{}_{A}$	$10.4^{a}{}_{A}$	$1.0^{a}{}_{B}$			
		10-20cm	$6.5^{a}{}_{A}$	$5.3^{b}{}_{B}$	$0.7^{b}{}_{A}$			
		20-30cm	$7.0^{a}{}_{A}$	$4.1^{b}{}_{A}$	$0.6^{c}{}_{A}$			
Efaw/ Easpur/ Fluventic Halustolls	Bermuda grass	0-10cm	$6.0^{a}{}_{A}$	$33.1^{a}{}_{A}$	$3.1^{a}{}_{A}$	21.3	26.3	52.4
		10-20cm	$6.3^{b}{}_{A}$	$17.6^{b}{}_{A}$	$1.8^{b}{}_{A}$	23.8	23.8	52.4
		20-30cm	$6.7^{b}{}_{A}$	$13.3^{b}{}_{A}$	$1.4^{b}{}_{A}$	25.0	22.5	52.5
	Switchgrass	0-10cm	$6.0^{a}{}_{A}$	$22.0^{a}{}_{B}$	$2.1^{a}{}_{B}$			
		10-20cm	$6.4^{b}{}_{A}$	$14.1^{b}{}_{A}$	$1.5^{b}{}_{A}$			
		20-30cm	$6.9^{c}{}_{A}$	$11.8^{b}{}_{A}$	$1.3^{b}{}_{A}$			
40 North/ Huska/ Mollic Notrustalfs	Indian grass	0-10cm	$5.2^{a}{}_{A}$	$26.9^{a}{}_{A}$	$2.5^{a}{}_{A}$	22.5	36.3	41.2
		10-20cm	$5.6^{ab}{}_{A}$	$16.7^{b}{}_{A}$	$1.7^{b}{}_{A}$	25.0	36.3	38.7
		20-30cm	$6.3^{b}{}_{A}$	$11.2^{b}{}_{A}$	$1.2^{b}{}_{A}$	23.8	35	41.2
	Switchgrass	0-10cm	$6.3^{a}{}_{A}$	$18.3^{a}{}_{A}$	$1.7^{a}{}_{B}$			
		10-20cm	$6.6^{a}{}_{A}$	$12.3^{a}{}_{A}$	$1.2^{a}{}_{A}$			
		20-30cm	$6.7^{a}{}_{A}$	$10.3^{a}{}_{A}$	$1.1^{a}{}_{A}$			

† Different letters indicate significantly different means according to least significant difference test at $P < 0.05$, where lower case letters (a–c) indicate comparisons within the same treatment at different soil depths, and capital letters (A–B) indicate comparisons within the same location and same depth at different treatments. ‡ Soil: Water ratio = 1:2. § Soil particle size distribution was the same for the same soil at the same location under all vegetation types.

Biomass Carbon and Nitrogen

Microbial biomass carbon and nitrogen were determined by the chloroform fumigation-incubation method (Vance et al., 1987). For this process, samples were weighed out according to their calculated soil moisture content and placed in desiccators. Samples that were to be fumigated were placed with a beaker of 50 ml of chloroform, a vacuum pump was then used to evacuate until the chloroform boiled. This process was repeated 3 times until the chloroform boiled, then air was let in to pass through the desiccator. The 4th time the desiccator was evacuated it was left to boil for 2 minutes then capped off, covered and kept in the dark for 24 hours. Un-fumigated samples were placed in a control desiccator without chloroform and followed the same process. After 24 hours, the chloroform was removed and the vacuum pump was used 8 times, 2 minutes each time, to remove traces of chloroform. The soil was then transferred to plastic bottles and 2 ml of NaOH was put into a glass bottle open at the top. Bottles were tightly closed and left to sit in the dark for 10 days. After the 10 day incubation period, the NaOH was transferred from the glass bottles to 125 ml flasks, 2 ml of $BaCl_2$ and one drop of phenolphthakin indicator was added to the mixture, then swirled together. Samples were then titrated with HCl until the solution turned from pink to clear.

Following incubation, samples were analyzed for inorganic N (NO_3^--N and NH_4^+-N) by the steam distillation method (Kenney and Nelson, 1982). Fumigated and non fumigated soils were extracted with 125 ml of 1M KCl. The soil/ KCl solution was put on a shaker for 30 minutes then left for 1-2 hours to settle. Once the solution settled it was filtered through a funnel and filter paper. 5 ml of H_3BO_3 indicator were added to a 50 ml flask and placed under the condenser of the steam distillation apparatus. 25 ml of extracted soil solution was pipetted into a distillation flask; 0.2 g of Devarda alloy and 0.6 g of MgO were added to the flask, immediately afterwards the flask was attached to the steam distillation apparatus. Once there was between 30-35 ml of distillate it was titrated with HCl until the color changed from green to a faint pink.

Biomass Phosphorus

Microbial biomass phosphorus was determined following the method described by Brookes et al. (1982), using chloroform fumigation and extraction with $NaHCO_3$. Samples were weighed out according to their calculated soil moisture content and divided into three sets before being placed into desiccators, the first set was fumigated with chloroform and the second and third set were incubated aerobically for 24 hours at 25^{o}C. After incubation, the 1st and 2nd sets are transferred to 250 mL Erlenmeyer flasks, added 200 mL 0.5M $NaHCO_3$ (pH 8.5), and shaken for 30 min at 150 rev min^{-1} on an orbital shaker. 1.0 mL solution of KH_2PO_4 containing 250µg P was added to the 3rd set extractant and shaken with unfumigated soil under the same conditions as the other unfumigated and fumigated samples. The resulting solution was filtered through a Whatman No. 42 filter paper.

The Murphy and Riley method (1962) was used to determine phosphate content. 10 ml of solution was placed into a 25 mL volumetric flask containing 5 ml of trichloroacetic acid reagent, 2 ml of ammonium molybdate reagent and 5 ml of sodium arsenite - acetic acid reagent. The volume was then adjusted with water and after 15 minutes the absorbance of the heteropoly blue color was measured using a spectrophotometer adjusted to a wavelength of 700nm. The phosphate content of the aliquot analyzed by reference to a calibration graph plotted from the results obtained with standard phosphate stock solution containing 0, 5,10,15,20, and 25 ug of PO_4^{-3}

Ester-linked fatty acid methyl ester biomarkers

Soil microbial community composition was determined using fatty acid methyl ester (FAME) profiles based on an ester-linked (EL) method. FAMEs were extracted from soil samples at the 0-10cm depth following the extraction method by Shutter and Dick (2000).

The standard FAME protocol was applied to freeze dried soil samples in the following steps: ester-linked fatty acids were released and methylated of with 15ml of 0.2M KOH in methanol for 60min at 37°C; neutralized with 3ml of 1.0M acetic acid; and extracted with 3ml of hexane. This was followed by drying under N_2 flow. They were then re suspended in 100µl hexane containing 19:0 internal standard. The FAMEs were analyzed in a 6890GC Series II (Hewlett-Packard, Wilmington, DE) equipped with a flame ionization detector and a fused silica capillary column (25m by 0.2mm) using ultra high purity H2 as the carrier gas. The temperature program was ramped from 170 to 250°C at 5°C min for analysis, then heated to 310°C to clear the column. Fatty acids were identified and peak area quantified using the TSBA6 method from MIDI software (Microbial ID, Inc., Newark, DE). Fatty acid biomarkers were summed into microbial groups: AMF, saprophytic fungi, Gram-positive bacteria (GMP), Gram-negative bacteria (GMN), and actinobacteria (Li, 2018).

Individual peak data for each fatty acid were converted to nmol g^{-1} soil by referring to the internal standard (19:0 FAME) and molar percentages (mol%) by dividing the peak area by the fatty acid molecular weight, then dividing by the total molar area of all fatty acids identified in the sample (Zelles, 1996).

Data analysis

Data analysis was preformed using SAS 9.4 (SAS, 2015). One-way analysis of variance (ANOVA) was used to determine differences in soil FAME biomarkers, soil microbial biomass properties, and soil chemical properties. Comparison of treatment means was done using the least significant difference (LSD) test at a P-value of <0.05. Principal component analysis (PCA) was used to determine differences in soils samples tested with multiple variables from each sample.

Samples were grouped by unmanaged mixed grass and managed switchgrass as well as by location.

CHAPTER IV

Findings

Results

Soil properties tested include pH, content of organic carbon, and total nitrogen which are presented in Table 1. PH values ranged from 5.1-7.9. Organic carbon values ranged from 4.1-33.1 g kg-1 and total nitrogen ranged from 0.6-3.1 g kg^{-1}. Clay content ranged from 13.8-23.8%, silt 35-52.5% and sand 22.5-50.0%.

Microbial biomass carbon, nitrogen and phosphorous at three depths (0-10 cm, 10-20 cm and 20-30 cm) are shown in Figure 1. Microbial biomass carbon was significantly higher in the unmanaged mixed grassland sites in surface soils, ranging from 17.1-23.2 mg biomass-C 30 g^{-1} soil, compared to the managed switchgrass sites which ranged from 9.0-15.2 mg biomass-C 30 g^{-1} soil. Microbial biomass nitrogen was also significantly higher under native grassland in surface soils (3.6-4.6 mg biomass-N 30 g^{-1} soil) compared to switchgrass cultivated areas (1.8-3.7 mg biomass-N 30 g^{-1} soil). Microbial biomass phosphorous was higher in the unmanaged mixed grass sites which ranged from 17.6-39.1 mg biomass-P 30 g^{-1} soil, while managed switchgrass sites ranged from 12.2-20.9 mg biomass-P 30 g^{-1} soil in the top 0-10 cm of soil.

Total FAME indicators in surface soils (0-10 cm) are shown in figure 2, there were no significant difference between managed switchgrass sites (85-150 indicator in nmol g^{-1} soil) and unmanged

Mixed grass (125-177 indicator in nmol g^{-1} soil) although managed switchgrass sites did have lower amounts of biomass at every location.

Microbial biomass carbon/nitrogen and carbon/phosphorous ratios are shown in Figure 3. Microbial biomass C/N ratios in switchgrass cultivated sites ranged from 3.7-10.5, while native grassland sites ranged from 4.6-11.8. Microbial biomass C/P ratios ranged from 0.5-1.3 in switchgrass cultivated sites, and (0.4-1.5) in native grassland sites.

Gram positive, gram negative and acinobacteria FAME indicators, while not significantly different between the native grassland and switchgrass cultivated sites, show a general trend of being higher in surface soils at all three locations under native grassland (Figure 4). Arbuscular mycorrhizal fungi indicators were higher in surface soils at every location in the switchgrass cultivated sites. Protozoan and saprophytic fungi indicators were dependent on location.

Principal component analysis (PCA) of the FAME indicators showed that the first principal component (PC1) explained 43.4% of the variance while the second, (PC2) explained 21.0% and the third (PC3) 15.0%, for a total of 79.4% of the variability in the data being explained (Table 2). The PCA showed samples clustering into groups along the PC2 axis, separating by unmanaged and managed switchgrass samples (Fig.5 A-B) as well as clustering by location (Fig. 5 C-D). PC2 was loaded by many factors, but predominately by arbuscular mycorrhiza fungi, protozoan, actinobacteria, and microbial biomass nitrogen.

Figure 1

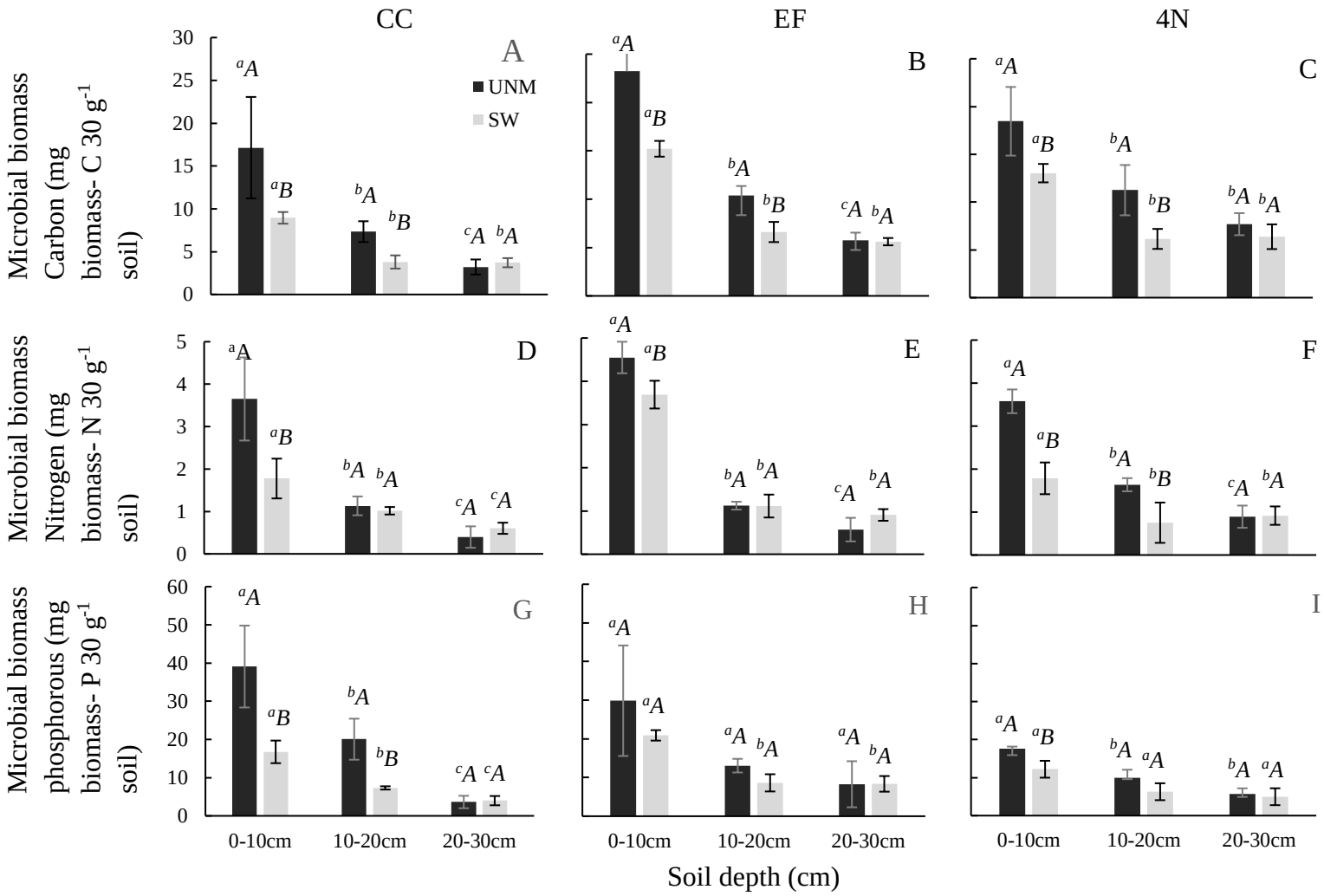

Fig. 1. Microbial biomass carbon, nitrogen and phosphorous content in soils of three depths at the studied locations (CC, EF, and 4N are locations as defined in Table 1), separated by unmanaged mixed grass (UNM) and switchgrass soils (SW). Different letters in italics indicate significantly different means according to the least significant difference test at $P < 0.05$, where lower case letters (*a*–*c*) indicate comparisons within the same treatment at different soil depths, and capital letters (*A*–*B*) indicate comparisons within the same location and same depth at different treatments. Error bars indicate standard deviations of averaged samples.

Figure 2

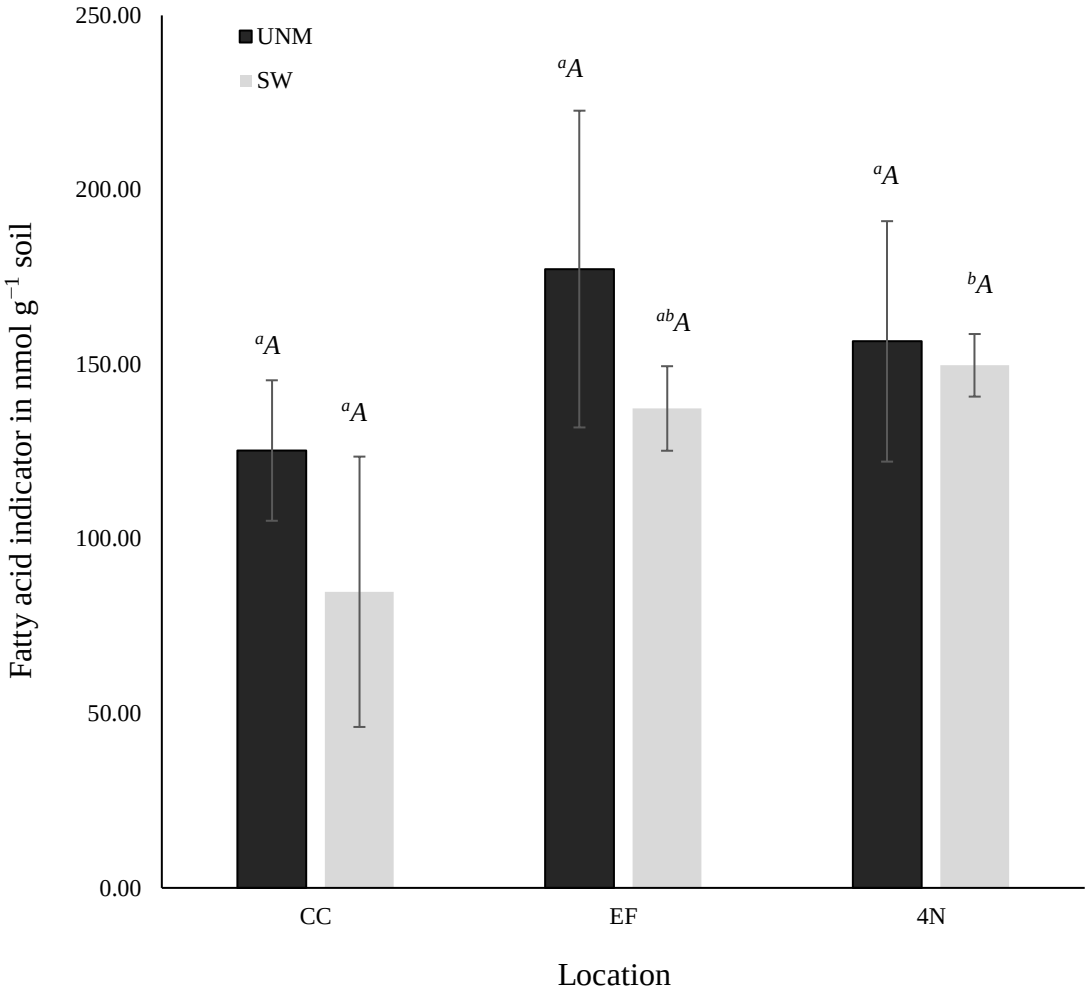

Fig. 2. Abundance of total fatty acid methyl ester (FAME) indicators. Indicated by FAME concentrations in surface soils at the studied locations (CC, EF, and 4N are locations as defined in Table 1), separated by unmanaged mixed grass (UNM) and switchgrass soils (SW). Individual peak data for each fatty acid was converted to nmol g^{-1} soil by referring to the internal standard (19:0 FAME). Different letters in italics indicate significantly different means according to the least significant difference test at $P < 0.05$, where lower case letters (*a*–*c*) indicate comparisons within the same treatment at different soil depths, and capital letters (*A*–*B*) indicate comparisons within the same location and same depth at different treatments. Error bars indicate standard deviations of averaged samples.

Figure 3

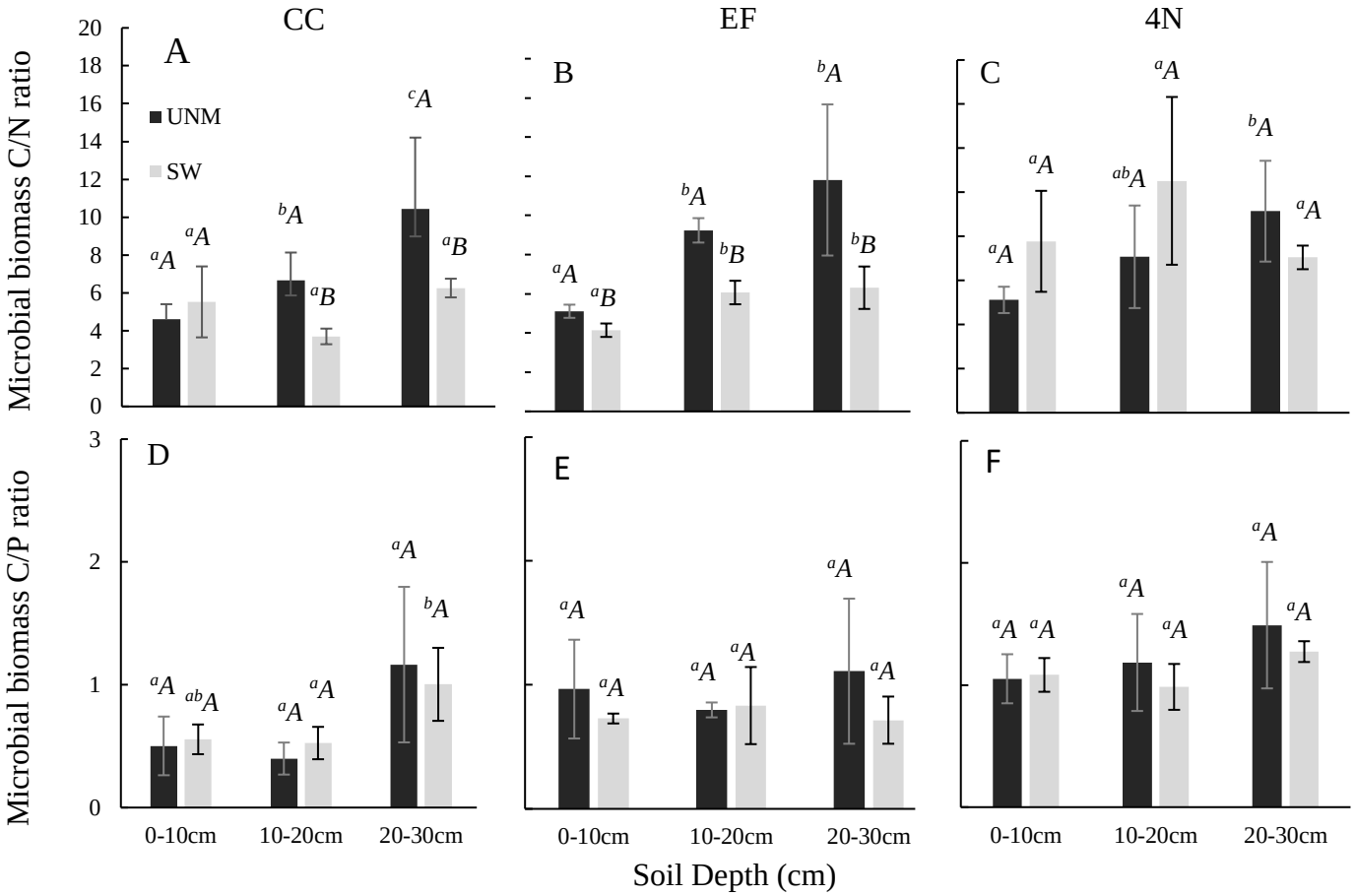

Fig. 3. Microbial biomass C/N and C/P ratios in soils of three depths at the studied locations (CC, EF, and 4N are locations as defined in Table 1), separated by unmanaged mixed grass (UNM) and switchgrass soils (SW). Different letters in italics indicate significantly different means according to the least significant difference test at $P < 0.05$, where lower case letters (*a*–*c*) indicate comparisons within the same treatment at different soil depths, and capital letters (*A*–*B*) indicate comparisons within the same location and same depth at different treatments. Error bars indicate standard deviations of averaged samples.

Figure 4

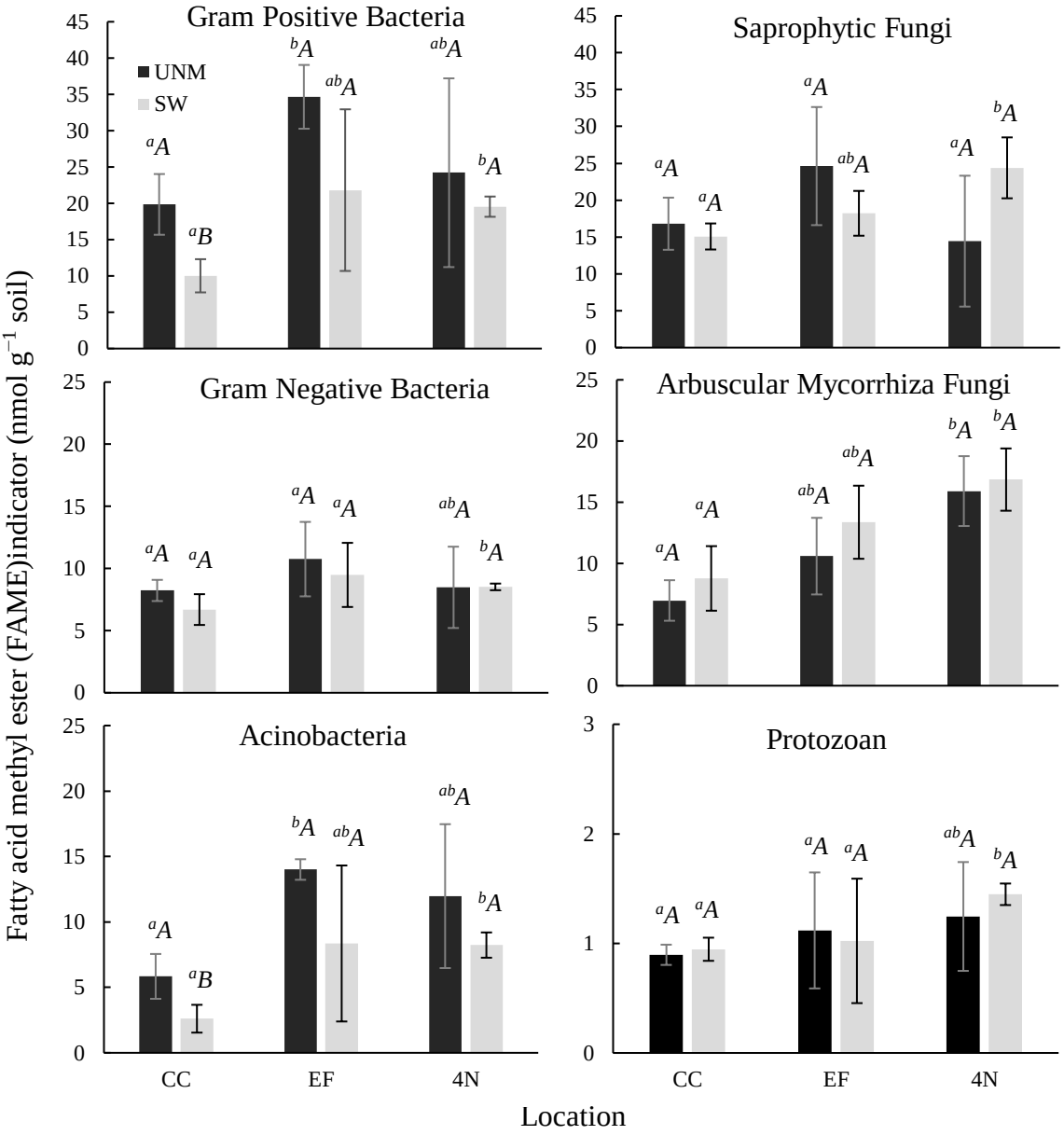

Fig. 4. Fatty acid methyl ester (FAME) indicators of selected microbial groups. Indicated by FAME concentrations in surface soils at the studied locations (CC, EF, and 4N are locations as defined in Table 1), separated by unmanaged mixed grass (UNM) and switchgrass soils (SW). Individual peak data for each fatty acid was converted to nmol g^{-1} soil by referring to the internal standard (19:0 FAME), fatty acid biomarkers were summed into microbial groups and averaged by indicator. Different letters in italics indicate significantly different means according to the least significant difference test at $P < 0.05$, where lower case letters (*a–c*) indicate comparisons within the same treatment at different soil depths, and capital letters (*A–B*) indicate comparisons within the same location and same depth at different treatments. Error bars indicate standard deviations of averaged samples.

Table 2 Principle component (PC) loadings between soil fatty acid methyl ester biomarkers (FAMEs), soil chemical properties, microbial biomass properties, soil texture and the first three PCs.

Parameter	PC1	PC2	PC3
Saprophytic Fungi	0.23	0.27	0.28
Arbuscular Mycorrhiza Fungi	0.21	0.34	-0.25
Protozoan	0.23	0.34	0.10
Actinobacteria	0.27	0.37	0.09
Gram Negative Bacteria	0.31	0.14	0.30
Gram Positive Bacteria	0.35	0.00	0.23
pH	-0.16	0.19	0.36
Total Nitrogen	0.34	-0.21	-0.12
Organic Carbon	0.34	-0.20	-0.13
Microbial Biomass Carbon	0.28	-0.30	0.07
Microbial Biomass Nitrogen	0.22	-0.43	0.12
Microbial Biomass Phosphorus	0.06	-0.16	0.56
Microbial Biomass C:N Ratio	0.00	0.33	-0.12
Clay %	0.29	0.09	-0.39
Sand %	-0.30	0.07	0.20
Eigenvalues	6.52	3.15	2.25
Total variance (%)	43.4	21.0	15.0
Cumulative variance (%)	43.4	64.4	79.4

Figure 5

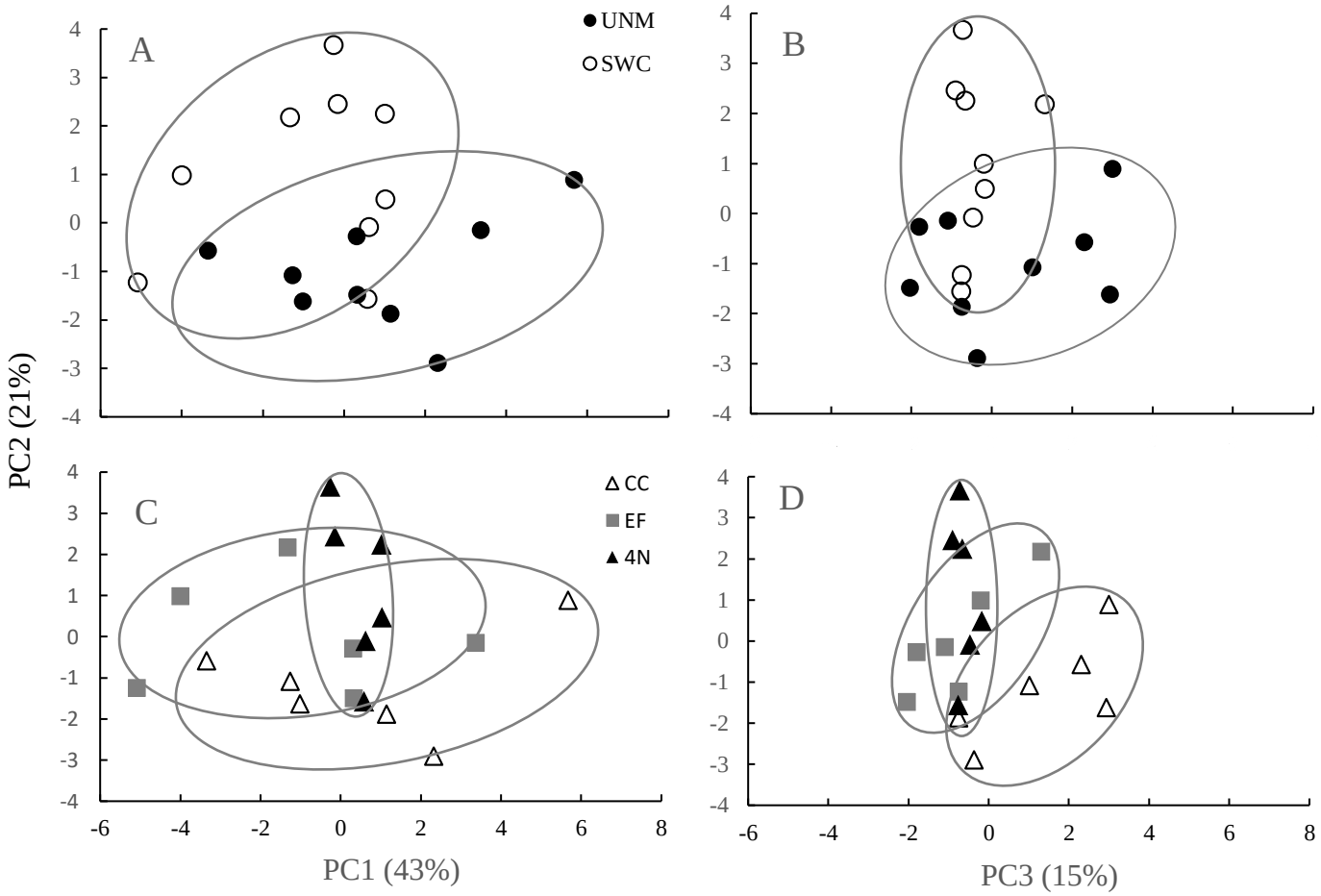

Fig. 5 Principal component analysis (PCA) of fatty acid methyl ester biomarkers (FAMEs), chemical properties, Microbial biomass properties, and texture of soils sampled. Samples were collected at 0-10cm at the studied locations (CC, EF, and 4N are locations as defined in Table 1), against the first and second principal components (A and C), as well as the second and third components (B and D). All 18 soils were included, data presented differentiates between unmanaged mixed grass soils (UNM) and switchgrass soils (SW) (A and B) as well between sampling sites (C and D).

Discussion

The primary purpose of this study was to evaluate relationships between switchgrass production and the soil microbial community. More specifically, the main objective was to determine the impact of managed switchgrass on microbial biomass abundance, as well as community structure and diversity.

Microbial biomass abundance were all higher in the unmanaged mixed grass plots compared to managed switchgrass plots (Fig. 1). This suggests the management of switchgrass led to a reduction in microbial biomass. With the exception of one plot (Fig.1 H) the top 0-10 cm of unmanaged soils contained a significantly higher amount of microbial biomass carbon, nitrogen and phosphorous at every location. As soil depth is increased, microbial biomass in general is reduced. Still, there were significant differences in the microbial biomass abundance of several plots in the 10-20cm range (Fig.1 A-C, F and G) where unmanaged mixed grass soils had a higher microbial biomass abundance. While there were no significant differences found in the abundance of total fatty acid methyl ester (FAME) indicators (Fig. 2), this data also shows lower microbial abundance in all locations in managed switchgrass sites.

Microbial biomass ratios are significant because they give an indication of microbial diversity in the soil. Soils with a higher C/N ratio indicate a more diverse microbial community and, in general, a healthier soil. At the Cow Creek and Efaw locations, microbial biomass C/N ratios in soils under managed switchgrass plots were significantly lower than those under unmanaged mixed grass vegetation, while the opposite trend was observed at the 40 North site (Fig. 3 A-C). This observed discrepancy between sites may be attributed to differences in the native vegetation. Bermuda grass was the predominant plant in the unmanaged mixed grass plots at the Cow Creek and Efaw locations. While at the 40 North location the unmanaged mixed grass plots were predominantly Indian grass and big blue stem. Microbial biomass C/N ratios increased with

increasing soil depth, which may signify increasing dominance of the fungal community in deeper soil profiles.

While there were few significant differences in specific microbial groups, there was a general trend of higher microbial abundance in the uncultivated mixed grass samples (Fig.4). All bacterial groups, including Gram positive, Gram negative and Actinobacteria were higher in uncultivated mixed grass species at every location, including a significantly higher abundance of Gram positive and Actinobacteria in the Cow Creek location. The opposite trend was found in abundance of Arbuscular mycorrhizal fungi (AMF). AMF are known as a fungi that develop a symbiotic relationship with plant root systems, under stress conditions and poor soil nutrition these fungi can help host plants perform better (Daei et al., 2009; Miransari, 2011). Switchgrass cultivated samples in every location had a higher abundance of AMF (Fig. 4).

Reduction of microbial abundance will result in reduced capacity of soils to cycle nutrients. However, the enrichment of AMF can potentially strengthen a plants' ability to uptake nutrients and water. The lost function resulting from an overall reduced microbial abundance may be partly compensated by improved efficiency in nutrient and water uptake.

Differences in managed switchgrass and unmanaged mixed grass were also shown through principle component analysis (Fig. 5). Groupings along the PC2 axis (which accounted for 21% of the total variance) showed clustering separated by cultivated and uncultivated as well as clustering by location. PC2 was loaded by many factors, including specific microbial groups, microbial biomass data and soil chemical properties, but predominately by arbuscular mycorrhiza fungi, protozoan, actinobacteria, and microbial biomass nitrogen.

Our results are consistent with a study by Watrud et al. (2013) where microbial biomass was compared in soils under switchgrass cultivation to those under native grassland. A significantly lower microbial abundance, lower gene diversity, and lower soil pH were all found under

switchgrass. Another study looking into the effect of switchgrass on soil carbon and microbial activity showed two-years of switchgrass cultivation led to an overall 21% decrease in total soil carbon (Strickland et al., 2015).

Microbial communities are influenced by time: a well-established root system will have different microbes present than a newly rooting system in its early stages of establishment. Data suggests switchgrass management during its first few years of the establishment has a negative effect on microbial biomass populations in soil. Our study took place only nine years after switchgrass cultivation and was consistent with this data. One limitation to our study was that experimental sites were only sampled once, this provides a snapshot of microbial activity at the time of sampling but further research is still needed to determine how switchgrass cultivation affects the microbial population further from establishment time. The combination of management involving fertilizer applications,

CHAPTER V

Conclusion

Comparing with the adjacent unmanaged fields, soils under switchgrass management had significantly lower content of microbial biomass carbon, nitrogen and phosphorous and total FAME.

Switchgrass management led to significantly lower microbial C/N ratios, lower content of FAME indicating gram-positive bacteria, gram-negative bacteria, actinobacteria, and saprophytic fungi, but higher content of FAME indicating arbuscular mycorrhizal fungi.

Reduction of microbial abundance will result in reduced capacity of the soil to cycle nutrients. However, the enrichment of arbuscular mycorrhizal fungi can potentially strengthen plants' ability to uptake nutrients and water. Therefore, the lost function resulting from reduced microbial abundance may be partly compensated by improved efficiency in nutrient and water uptake.

Restoring microbial biomass can take years after an initial disturbance by planting, the switchgrass fields used for this study were established nine years before sampling. For sustainable bioenergy feedstock production, it is imperative to continue monitoring the site to ensure in depth understanding on the long-term impact of switchgrass cultivation to soil health.

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