EFFECTS OF NUTRIENT SOURCES, HARVEST FREQUENCY, AND ENVIRONMENTAL CONDITIONS ON SWITCHGRASS PRODUCTION AND SOIL PROPERTIES

By

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Abstract: Switchgrass is considered a promising low-input biofuel feedstock. Previous development primarily focused on aboveground production, however, feedstock success will depend on identifying sustainable management practices that increase biomass yield for multiple purposes, while increasing soil quality. In this multi-site field study, effects of fertilizer and harvesting regimes on soil properties (Chapter 1) and above- and belowground biomass (Chapter 2) were assessed. A complementary greenhouse study assessed fertilizer management and environment (Chapter 3).

The field study evaluated soil organic matter (SOM), NO_3-N, plant-available phosphorous (P), soil aggregate stability and microbial communities following applications of organic (cattle manure, poultry litter) and inorganic fertilizers, and inter-seeded legumes. A control (no fertilizer) was also included. Harvesting regimes included single harvest in the fall, and two harvests per year. The study was conducted in 2011 and 2012 at two central Oklahoma sites with previously established switchgrass (Alamo). Regardless of harvest frequency, application of poultry litter increased aboveground biomass and soil NO_3-N, P, SOM, and arbuscular mycorrhizal fungal biomass, compared with the control. The use of organic fertilizer and inorganic NPK increased soil macroaggregates in the single harvest plots, compared to the control, which may improve water infiltration and decrease soil and nutrient loss due to water erosion. While the use of poultry litter increased aboveground biomass, soil P was markedly increased as well. Therefore, soil should be closely monitored to avoid P build-up and subsequent P loss to the environment.

The greenhouse study assessed above- and belowground parameters under different climatic conditions and fertilizer management. Increases in temperature and drought did not generally affect above- or belowground parameters. The only microbial community affected was mycorrhiza, with greater abundance under ambient, compared to warmer, drier conditions. This research supports switchgrass as a model perennial bioenergy crop producing high yields on marginal lands under wide environmental conditions.

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ABSTRACT

Switchgrass is a promising native, low-input biofuel feedstock. Switchgrass development has focused primarily on aboveground production, however, success will depend on identifying sustainable management practices that maintain or improve soil quality while increasing biomass yield. A field study was conducted to assess soil microbial communities, organic matter, NO_3-N, plant-available P, and aggregate stability following applications of organic (cattle manure, poultry litter, inter-seeded legumes) and inorganic (urea, chemical NPK) fertilizers. A non-amended (no fertilizer) control was also included. Harvesting regimes included single fall harvest and both summer and fall harvests per year. This study was conducted in 2011 and 2012 at two sites previously established to the cultivar Alamo in central Oklahoma, as a split plot design with four replications. Regardless of harvest management, application of poultry litter increased NO_3-N, P, organic matter, and mycorrhizal fungal biomass, as compared with the non-fertilized control. The use of organic and inorganic fertilizer increased soil macroaggregates under single harvest, but not following

twice per year harvests. While the use of poultry litter improved in soil aggregate stability, soil P was markedly increased as well. Therefore, soil should be closely monitored to avoid P build-up and subsequent P loss into adjacent ecosystems.

INTRODUCTION

The Energy Independence and Security Act (EISA) of 2007 mandates that the United States increase its biofuel production to 136 billion liters from renewable resources by 2022 and 60 billion liters of biofuel should come from lignocellulosic materials (Biomass Research and Development Board 2008) to reduce both fuel costs and dependence on foreign oil, and provide economic alternatives for farmers (Sanderson et al. 1999). Switchgrass (SG) has been recognized as a potential biofuel feedstock because it is a perennial plant, has high biomass production potential, averaging 14.6 tonnes ha^{-1} in a wide range of environments (McLaughlin and Kszos 2005), and can have a conversion of 0.34 L ethanol per kg of dry cellulosic biomass (Gutchick 2010). In addition, it can sequester large amounts of carbon into soils (Garten et al. 2010) because of its extensive roots that have been observed to grow deeper than 3 m (Ma et al. 2000); thus it has the potential to be more sustainable compared to corn-based ethanol production (Robertson et al. 2008).

Beef production is Oklahoma's largest agricultural sector and ranked 2nd in the US in 2010. In 2010, Oklahoma had an output of $3.9 billion in livestock production (NASS Survey 2010) making it the most important source of revenue for agriculture in the state. Switchgrass can be a good source of forage for haying or grazing during the early growth stage when its nutritive value is high. When allowed to re-grow and subsequently harvested at maturity, this crop can be used as a high quality biofuel feedstock (Guretzky et al. 2011). However, the use of switchgrass as a dual purpose crop may result in lower soil organic matter level due to large biomass removal (Balasko et al. 1984; Muir et al. 2001; Lee et al. 2007). In the long-term, this may reduce soil quality, consequently affecting the sustainability of biomass production. To achieve a sustainable dual purpose production of switchgrass, it is necessary to explore different nutrient sources and assess the resultant soil quality.

Nutrient sources can either be from organic amendments such as animal manure or plant residues which need to undergo decomposition to release nutrients, or from inorganic fertilizers. Inorganic fertilizers are readily available for plant uptake but require considerable fossil fuel inputs to produce. Poultry litter and cow manure are available in large quantities from many poultry and livestock operations in the US. Organic sources of nutrients add organic matter to the soil and can improve soil quality, which can be assessed by measuring physical, chemical, and biological properties of the soil. The concept of soil quality is based on the assumption that management can reduce, stabilize or increase ecosystem functions (Franzluebbers 2002). Thus, when assessing switchgrass production, the focus should not only be on aboveground biomass production, but what is happening belowground as well.

Soil microorganisms are critical to maintaining soil quality. They play an essential role in nutrient cycling, contributing to soil structure, aiding in the degradation of pollutants, etc. (Bloem et al. 1994; Sylvia et al. 2005). Bacteria are the most abundant microorganism in soils. Other microorganisms found in the soil include fungi, actinomycetes, protozoa, viruses, and nematodes. Plant-soil-microbe interactions are important in maintaining plant health, promoting plant communities (Wardle et al. 2004), soil fertility (Bargett et al. 2005), and carbon cycling (Kuzyakov et al. 2001; Johnson et al. 2002; Zhu and Miller 2003). Rhizosphere soil, the soil adjacent to the root, is characterized by close interactions between plant roots, soil microorganisms, nutrients, and water in the soil. This is a zone of high microbial activity and diversity (Jones and Hinsinger 2008) because plant roots secrete various organic exudates that stimulate the growth of soil microorganisms near the root surface. In a feedback mechanism, the soil microorganisms breakdown organic matter and make nutrients available for plant use, fix nitrogen and protect plant from pathogens (Jones and Hinsinger 2008).

Aggregate stability, the measure of soils' ability to retain its structure or resist disruption when there is a stress (Amezkéta 1999), is essential in minimizing erosion, enhancing infiltration and storage of water, maintaining or improving productivity due to improved water availability within the soil and providing a favorable environment for root growth. There are several factors affecting aggregate stability such as soil texture, tillage, soil amendments (Franzluebbers 2002; Nissen and Wander 2003), and microbial activities (Jastrow 1998).

Mycorrhizal fungi are known to form symbiotic relationship with plant roots. Arbuscular mycorrhizal fungi (AMF) of the Phylum Glomeromycota are important for agriculture (Schussler et al. 2001). AMF form a symbiotic association with more than 80% of land plant families including agricultural crops (Gosling et al. 2006). AMF produces extraradical hyphae that may extend several centimeters out into the soil from the roots and significantly increase the total effective surface area which can increase plant nutrient uptake, particularly of immobile nutrients such as P (Smith and Reid 2008), improve water uptake or help in the plant's tolerance to drought (Augé 2004), enhance plant resistance to root pathogens (Azcon-Aguilar et al. 1996), aid in soil aggregation (Tisdall and Oades 1982; Miller and Jastrow 1990) and C sequestration (Wilson et al. 2009).

Switchgrass is known to have a symbiotic relationship with AMF, but to what extent AMF contributes to the resilience of switchgrass under stressful environment such as marginal soils and drought conditions has not been well-studied. However, AMF has been shown to respond differently under different management practices such as fertilizer application (Johnson et al. 1997). Thus, the objective of my project was to study the effects of inorganic and organic nutrient sources on biomass production of switchgrass for forage and biofuel feedstock. Because belowground properties are critical to sustainable biofeedstock production, yet are infrequently assessed, my study extends belowground to include soil biological, chemical and physical properties, following amendments with inorganic or organic fertilizer.

MATERIALS AND METHODS

Site Description

This study was conducted at Perkins and Lake Carl Blackwell (LCB) research stations near Stillwater, Oklahoma in 2011 and 2012. The switchgrass (SG) stands used for this study were established in 2008 at Perkins and 2009 at Lake Carl Blackwell.

Plots at Perkins were located at the Cimarron Valley Research Station on the upper terrace of the Cimarron River. The soils at Perkins consist of Teller (mixed, active, thermic Udic Argiustolls) (USDA-NRCS Soil Taxonomy) and Konowa (mixed, active, thermic Ultic Haplustalfs) (USDA-NRCS Soil Taxonomy) series. These soils are deep, well-drained and moderately permeable. Prior to implementation of treatments, baseline soil pH ranged from 5.1 to 6.5. The average annual precipitation on this site is 926 mm (36.5 inches) with an average daily high temperature of 32.8 ^{o}C (91 ^{o}F) in the summer and an average daily low of -3.3 ^{o}C (26 ^{o}F) in the winter. The soil texture is sandy loam.

The soil at Lake Carl Blackwell is classified as Port silt loam (fine-silty, mixed, thermic Cumulic Haplustolls) (USDA-NRCS Soil Taxonomy) which is very deep, well-drained, moderately permeable usually located on floodplains that may be subject to occasional or rare flooding. The average annual precipitation (2008 - 2010) was 937 mm (36.9 inches) with an average daily high temperature of 31.6 ^oC (89 ^oF) in the summer and an average daily low of -4.8 ^oC (23.4 ^oF) in the winter. Table 1.1 shows the annual rainfall and temperature of each switchgrass experimental site during the course of this study.

Experimental Design and Treatments

The experiment was a split plot with four replicates. Dual purpose switchgrass (dual) and sole purpose switchgrass (sole) treatments were assigned to main plots. Dual purpose switchgrass was harvested in July and November, whereas, the sole purpose was harvested in November only. Sub-plot treatments were nutrient sources: cow manure (CM), poultry litter (PL), chemical N fertilizer (N), chemical NPK fertilizer (NPK), a legume inter-seeded in switchgrass (L), and a non-fertilized control (C). The sub-plot area was 3.05 m wide and 4.6 m long. The main plot area was 6.1 m wide and 13.7 m long.

The sources of chemical fertilizers were urea, triple super phosphate (TSP) and potassium chloride (KCl) for N, P, and K, respectively (Table 1.2). The N treatment received 150 kg N ha^{-1}, and the NPK treatment received 150, 40, and 20 kg N, P_2O_5, and K_2O ha^{-1}, respectively. Cow manure and poultry litter were applied based on an N rate of 150 kg ha^{-1}. These fertilizer treatments were applied in spring 2009, 2010, and 2011. No fertilizers were applied in 2012. Nitrogen fertilizer applications were split, with 1/3 (50 kg N ha^{-1}) applied before greening and the remaining 2/3 (100 kg N ha^{-1}) applied eight days after the first harvest to stimulate regrowth. Table 1.2 shows the amount of N, P_2O_5, and K_2O applied for all treatments.

A lowland switchgrass variety ‘Alamo’ was planted in July 2008 at Perkins and in April 2009 at Lake Carl Blackwell at a seeding rate of 6 kg ha^{-1}. Alamo was used in the study as this is the most adapted cultivar for the south-central US (Muir et al., 2001). Seeds of switchgrass were drilled into a well-prepared seedbed with a conventional drill into 1.5 cm deep and 36 cm wide rows. Crimson clover (*Trifolium incarnatum* L.) was planted in spring 2009, 2010 and 2011 as one of the treatments at 12 kg ha^{-1} in between switchgrass rows and inoculated with appropriate *Rhizobia* spp. before planting to enhance symbiotic nitrogen fixation.

Soil Sampling

Soil samples (0-10 cm) were collected from each plot in spring (prior to fertilizer application) and fall of 2011 and 2012. Sampling depth was limited to the upper 10 cm of the soil profile where the majority of the soil microbial activity occurs (Eilers et al. 2012). Six core samples per plot were collected, and pooled as a composite sample. Soil samples were placed on ice and returned to the laboratory. Field moist samples were sieved through a 2 mm sieve. Analyses for NO_3-N, plant available P and K, pH, and OM were conducted on air dried soil samples. The remaining soil was freeze-dried and ground for microbial analysis.

Soil Chemical Analysis

Soil NO_3-N was extracted by 1M KCl solution and analyzed using the Lachat Quickchem 8000 Flow Injection Autoanalyzer (Kachurina et al. 2000). Two grams of soil were extracted with 20 ml Mehlich 3 solution for plant available P and K, and the concentrations of P and K in the extract were measured by an inductively coupled plasma emission spectroscopy (ICP) (Pittman et al. 2005). Soil pH was measured using a pH electrode in a 1:1 soil to water suspension. Soil organic matter (SOM) was determined by dry combustion using the LECO Truspec CN analyzer (Nelson and Sommers 1996).

Soil Aggregate Stability

Soil samples were collected using a spade at 30 cm depth and passed through an 8 mm sieve to remove large roots, and small stones. This soil was then further sieved through a 4 mm sieve. The soil remaining on the 8 mm and the 4 mm sieve were collected and air-dried overnight. Aggregates that remained on the 4 mm sieve were used for further analysis to assess water-stable aggregate stability. Geometric mean diameter and size distribution of aggregates were determined by the wet-sieving method using a modified Yoder machine (Yoder 1936; Low 1954). The Yoder machine consists of two columns of five sieves with different mesh sizes (4, 2, 1, 0.5 and 0.25 mm) held together by a spring in each column. Fifty grams of the air-dried sample were placed on the top sieve with the largest opening (4 mm). The column was slowly lowered into a 15 liter water-filled container until the samples were submerged. The samples were allowed to soak for 10 min, followed by 10 min of agitation cycle where sieves were moved vertically at a rate of 30 cycles per minute. Finally, soils were collected from each sieve, dried and weighed. The water stable aggregates and size distribution based on the amount of soil remaining on each sieve were calculated using the following equations:

$$GMD = \exp[\sum_i^n = \ wi \ logxi \ / \sum_i^n = \ wi] \qquad \text{[Eqn. 1]}$$

where GMD is the geometric mean diameter; x is the mean diameter for the i^{th} size; w is the aggregate weight for the i^{th} size class and $\sum_i^n = \ wi$ is the total weight of the sample.

$$\text{ASD (\%)} = \frac{\text{soil remaining in each individual sieve * 100}}{\text{total weight of soil used}} \qquad \text{[Eqn. 2]}$$

where ASD stands for the aggregate size distribution. Macroaggregates were those >0.25 mm and microaggregates were <0.25 mm in diameter (Six et al. 2004; Po et al. 2008).

Soil Microbial Biomass Analysis

The soil microbial biomass was determined using the phospholipid fatty acid analysis (PLFA) which is based on the extraction of lipids from the cell membranes and walls of microorganisms (Kaur et al. 2005). The lipids were extracted using five grams of freeze-dried soil mixed with chloroform:methanol: phosphate buffer (1:2:0.8 v/v/v). The soil-solvent mixture was separated by centrifugation (10 min at 1800 rpm) and decanted. Re-extraction was done by adding chloroform and repeating the centrifugation and collection of supernatant.

Phosphate buffer was added to the supernatant and was left overnight to allow the separation of the organic phase. The organic phase containing the lipids were recovered and concentrated by nitrogen gas flow at 50 ^{o}C. The total extracted lipids were separated with solvents such as chloroform, acetone and methanol into neutral lipids, glycolipids and phospholipids, respectively through silicic acid chromatography. Phospholipid fatty acid analysis was performed using Agilent 7890A gas chromatography with an Agilent 5975C series mass selective detector (GC-MS). Methyl nonadecanoate fatty acid (19:0) was used as the internal standard for all the samples. The following fatty acids were chosen to represent the gram positive bacteria: i15:0, a15:0, i16:0, i17:0, a17:0; for gram negative bacteria: 3-OH 14:0, 16:1_Δ9 (16:1ω7), 17:0_Δ9,10 (cy17:0), 2-OH 16:0, 19:0_Δ9,10 (cy19:0), 2-OH 12:0; for AMF: 16:1_Δ11(16:1ω5c), 20:1ω9, 22:1ω13; for saprophytic fungi: 18:1_ Δ9 (18:1ω9c), 18:2_Δ9,12 (18:2ω9) and for non-specific microbes 14:0, 15:0, 16:0, 17:0, 18:0, 20:0 (Zelles, 1999). The total microbial biomass or relative abundance of each functional group was expressed as nmol g^{-1} of soil.

Statistical Analysis

Data pertaining to soil properties were analyzed using the MIXED procedure of SAS (SAS Institute, 2008). Year was treated as a repeated measure, with averages overtime within a year were calculated and analyzed. The size distribution of soil aggregates in percentage were transformed using arcsine square root but the original means were presented. Harvest frequency, nutrient sources and year were considered fixed effects, whereas location and replication were considered as random effects. Location x harvest frequency, location x nutrient source interactions were not significant for the soil chemical, biological, and physical properties/variables ($P > 0.05$), but harvest frequency x year and nutrient source x year interactions were significant for some soil variables ($P < 0.05$). Mean comparisons were made using Fisher's protected LSD test ($\alpha=0.05$).

RESULTS

Soil Chemical Properties

Among the chemical properties tested, only soil NO_3-N was significantly different between dual (twice harvested) and sole purpose (single harvest) switchgrass treated with poultry litter in both years, and for those applied with cow manure in 2012 (Table 1.3). Switchgrass harvested twice had less soil NO_3-N than the single harvest. There was no significant difference in NO_3-N, P, or pH between dual and sole for the non-fertilized control, treatment with inter-seeded legumes, or treatments receiving inorganic fertilizers. Nutrient sources had significant effects on soil pH, NO_3-N, and available P in both years. In general, application of organic fertilizers increased soil pH (from moderately acidic to slightly acidic, pH range of 5.9-6.0 to 6.1-6.6). Plots receiving inorganic fertilizers remained moderately acidic (Table 1.3). Switchgrass plots receiving animal manure (poultry litter and cow manure) resulted in greater NO_3-N, available P and SOM. In fact, plots receiving organic fertilizer had three times as much plant-available P as the control or those receiving inorganic fertilizers in 2011, and nearly twice as much in 2012. SOM was significantly greater in treatments with organic fertilizer, as compared to those of inorganic fertilizer in 2012. Treatments with inter-seeded legumes had significantly lower NO_3-N, P, SOM and pH, as compared to those receiving organic fertilizers. However, there were no significant differences between the non-fertilized control and soil receiving applications of inorganic fertilizer (Table 1.3).

Soil Biological Properties

The evaluated soil biological properties were significantly different between single (sole) and twice (dual-purpose) harvested in 2011, but not in 2012 (Table 1.4). Soil from the sole purpose switchgrass plots that received applications of poultry litter contained greater abundances in total soil microbial biomass (TMB), AMF and saprophytic fungi, compared to soil from the dual-purpose switchgrass plots. Soil from the sole purpose switchgrass plots that received inorganic N also were characterized by greater TMB and saprophytic fungi, compared to soil from the corresponding dual purpose plots receiving N fertilization (Table 1.4).

Total microbial biomass, AMF, gram positive and gram negative bacteria, and saprophytic fungi were significantly affected by the nutrient sources in 2011. Total microbial biomass in soils following applications of cow manure was greater, as compared to the non-fertilized control, soil from the inter-seeded legume plots, or NPK fertilization for both dual (twice harvested) and sole purpose (single harvest) in 2011. In 2011, gram positive, gram negative and saprophytic fungi were significantly greater in sole purpose applied with poultry litter, cow manure and inorganic N than in the control and plots with inter-seeded legumes. AMF in sole purpose was greater in organic fertilizer treatments than the rest of the treatments. AMF and gram negative bacteria in twice harvested plots applied with cow manure were greater than the control and those that received poultry litter, nitrogen, NPK, and inter-seeded with legumes. In 2011, saprophytic fungi was greater in twice harvested plots applied with cow manure than control, nitrogen and NPK, but was not significantly different from those applied with poultry litter and inter-seeded with legumes. In 2012, saprophytic fungi were greater in single harvested plots applied with cow manure and poultry litter than control and plots inter-seeded with legume and applied with NPK.

Soil Physical Property

Single harvested switchgrass plots treated with cow manure, NPK and inter-seeded with legume had more water-stable macroaggregates than those in plots harvested twice a year. In general, the plots with single harvesting had more macroaggregates than those plots harvested twice a year (Table 1.5).

DISCUSSION

Management practices such as fertilization and harvesting frequency influence the soil chemical and biological properties. Dual-purpose switchgrass (harvested twice a year) depletes soil nutrients and moisture, used by the plants for regrowth, and this potentially impacts the ability for continuous productivity (Muir et al. 2001). This is presumably the reason that the dual purpose switchgrass plots (harvested twice a year) had lower soil NO_3-N than the corresponding sole purpose plots (harvested once a year). Guretzky et al. (2011) reported that nutrient removal was doubled when switchgrass was harvested twice a year compared to harvesting once a year. However, the biomass yields of dual-purpose switchgrass were equal (2012) to or slightly higher (2011) than the sole purpose in this study (see chapter 2), so the difference of nutrient removed between those two harvest frequencies was less than that of Guretzky et al. (2011). In my study, the use of organic fertilizers for sole purpose production was advantageous, as compared to inorganic amendments, as the organic amendments provided greater soil NO_3-N. Greater nitrate in the organic amended treatments may be the result of slow mineralization of the applied organic N. A long-term study on wheat receiving inorganic fertilizer or manure showed an increase of nitrate levels in the manure plots compared to the inorganic fertilized plots (Boman et al. 1996). Powlson et al. (1989) found a similar trend of increased nitrate levels on manured plots. Plant available P in organic fertilizer treatments was greater than the phosphorus levels in the inorganic treated plots. This is likely due to the high levels of P available in the manure applications. The amount of phosphorus applied depended on typical N applications for switchgrass feedstock production, and the phosphorus exceeded the amount required by switchgrass(Table 1.2). Therefore, the N-based manure application rate resulted in soil phosphorus accumulation. Furthermore, a study conducted in a low P soil also found little response in Alamo switchgrass biomass production following yearly applications of phosphorus (Muir et al. 2001). This supports my hypothesis that there is an innately low phosphorus requirement for this cultivar of switchgrass, and adding N-based organic fertilizer may exceed P requirement, resulting in P loss in erosion and run-off. This should be an important management consideration when applying organic fertilizer. This result indicates that soil P should be closely monitored following organic fertilizers, and applications of organic amendments may need to base on P requirements, rather than N requirements, to minimize P run-off and water quality deterioration (Sharpley 2006).

Addition of organic fertilizers in the form of animal manure increased SOM, as compared to soil from the inorganic or control treatments, although this was evident only after three years of continuous application. However, it has been reported that soil organic matter increased with manure application, compared with fertilizers in the first year following application (Edmeades 2003). In my study, soil pH was greater following applications of organic fertilizers. This is consistent with the findings of Fließbach and Mäder (2000). As expected, organic fertilizers added organic matter into the soil, thus improving soil buffering capacity. A study that compared conventional agriculture with organic farming management reported that an increase in soil pH in acidic soils is frequently observed (Clark et al. 1998; Fließbach and Mäder 2000; Das and Dkhar 2011). The application of inorganic fertilizers in this study decreased soil pH, making the soil more acidic. Similar results have been reported by Hopkins et al. (2011). The increases in urea applications acidify the soil through biological processes where the ammonium is oxidized to NO_3^-, releasing H^+ to the system (Brady and Weil 2008). In this study, all soil pH levels, however, were within the range of optimum growth of switchgrass (soil pH 5.0 – 7.0) (Lemus 2008).

The soil microbial community composition can be influenced by several confounding factors such as management, environment, and their interactions with each other, plants, and soil micro- and macrofauna. In my study, soil applied with animal manure, particularly cow manure, had the greatest microbial biomass. This is similar to the findings of Kong et al. (2011). Both in this study and Kong et al. (2011), gram positive bacteria dominated the microbial communities in soils from all nutrient sources. Fungal:bacterial ratios observed in this study ranged from 0.28 to 0.45 for all fertilization treatments, which were close to those of de Vries et al. (2006), but slightly higher than the ratio reported by Kong et al. (2011).

Gram positive and gram negative bacteria were not affected by switchgrass harvest frequency, but were significantly affected by nutrient sources

(Table 1.4). This could be due to the increased availability of N in the organic fertilizer amended soils, as these amendments could be utilized for microbial metabolism. Mycorrhizal and saprophytic fungi were significantly affected by both inorganic and organic nutrient sources, and also by harvest frequency. Sole purpose switchgrass harvest had greater fungal biomass than those harvested twice each year. This could be attributed to a more extensively established root system and a continuous source of photosynthates from the aboveground biomass, compared to that of the dual harvest treatment. Switchgrass is noted for deep extensive roots, beneficial in storing carbon (Ma et al. 2000). Single harvest of switchgrass will allow continuous growth throughout the year, providing the photosynthesized carbon to the roots, increasing root biomass and providing carbon and essential nutrients to symbiotic organisims such as arbuscular mycorrhizal fungi. Indeed, greater fungal and bacterial biomass has been reported in soils treated with animal manure (Tu et al. 2006). Manure application not only provided an abundance of carbohydrates readily available to soil microorganisms, but these organic amendments added additional bacteria contained within the manure, as well. Harris et al. (1994) found that N mineralization was the greatest in manure amended plots, suggesting that the microorganisms are either active or abundant. As organic matter can be decomposed more rapidly in the presence of greater microbial biomass, it is likely that the greater amounts of nitrate and phosphorus can be attributed to the increased decomposition of organic matter, with a concomitant continuous release of nutrients. Addition of inorganic fertilizers, which are highly soluble, have been reported to increase soil N availability, resulting in decreased microbial respiration (Ramirez et al. 2010). While it is not clearly understood why microbial respiration decreased with the addition of N-based inorganic fertilizer, Ramirez et al. (2010) suggest that it could be strongly related to the direct application and the fertilizer concentration. Ramirez et al. (2010) however, did not compare this effect with organic fertilizers. Ladd et al. (1994) also reported inorganic or mineral fertilizers suppressed soil microbes in an agricultural setting.

Soil aggregation is a result of interactions of internal physical, chemical and biological properties of the soil. Soil organic carbon acts as a binding agent and a basis in the aggregate formation. Soil microorganisms and their organic by-products, root exudates and the rate of decomposition of roots and organic matter all can affect aggregate formation. Aggregates are typically grouped into macroaggregates (>0.25 mm) and microaggregates (<0.25 mm) by their water-stable sizes (Tisdall and Oades, 1982). Macroaggregates can develop from and around particulate organic matter (POM) (Bronick and Lal 2005). The percent of water-stable macroaggregates from soil collected from sole purpose switchgrass plots that were treated with inorganic (NPK), organic (manure), or seeded with legumes were greater than those of corresponding dual purpose plots. This could be an interaction among the plant-soil-microbes and the harvesting management. When switchgrass is harvested once a year, its growth cycle is uninterrupted and the plant is able to continue growth and expand biomass production both belowground and aboveground. With the continuous supply of photosynthates, increased root exudates produce additional binding agents for the soil particles to adhere, forming water-stable macroaggregates. Hutch et al. (2002) reviewed several studies on plant rhizodeposition and found that, typically, plant species release about 20% of carbon used in photosynthesis as root exudates. Paynel et al. (2001) and Aulakh et al. (2001a and 2001b) demonstrated that different plant species and cultivars have different root exudation patterns. Increases in root exudates may be important to soil quality, as Traore et al. (2000) found that root mucilage and polygalacturonic acid were effective in increasing soil aggregate stability. _ Polygalacturonic acid may stabilize aggregates by increasing bond strength (Csarnes et al. 2000). Studies by Shepherd et al. (2001) and Debosz et al. (2002) reported an increase in concentration of carbohydrates as well as an increase in macroaggregation, following the addition of manure and use of cover crops. When microbes decompose particulate organic matter, they produce polysaccharides that act as a binding agent (Jastrow 1996). Humic substances from added organic matter can also increase aggregate stability and reduce dispersion due to wet-dry cycles (Piccolo et al. 1997). Increases in macroaggregates are associated with increases in fungal activities and fresh residues (Denef et al. 2001). Wilson et al. (2009) found soil aggregation was tightly correlated with the abundance of AM fungal hyphae. Aggregate formation and stabilization is promoted by AM fungi hyphae and fibrous root growth (Jastrow 1987; Miller and Jastrow 1990; Jastrow et al. 1998).

In this study, macroaggregate formation was not greater in soils amended with organic fertilizer, as compared to corresponding plots receiving inorganic fertilizer amendments. Previous studies have shown increases in macroaggregate formation following organic fertilizer application, compared to inorganic fertilizer amendments (Shepherd et al. 2001; Debosz et al. 2002; Wortmann and Shapiro 2008). In a 5 year field experiment, Celik et al. (2004) found that water stable aggregates were 65% greater with the application of manure or compost than treatments without manure or compost applied. Soil water holding capacity was also increased by 85 and 56% compared to the control with compost and manure applied, respectively. Wortmann and Shapiro (2008) found over 200% increase in large macro-aggregates (>2 mm) in both manure and compost applications within 15 days of application which was attributed to the consolidation of micro-aggregates. This is in contrast to the findings of Foster (2011) where long-term applications of manure increased the amount of microaggregates due to drying of the soil, following an increase in biomass yield. Similar results to this study were however observed by Whalen and Chang (2002) where long-term applications of cattle manure did not improve macroaggregates due to the presence of dispersing agent in the manure. Reduction of macroaggregates can also be due to the process of rewetting the dry soil that can cause the destruction of aggregates due to build-up of air pressure inside the aggregates (Singer et al. 1992). Rewetting of moist soil results in less destruction of the aggregates compared to rewetting of dry soil (Denef et al. 2002). In a long-term study in Kansas, application of inorganic fertilizer in an irrigated corn field did increase soil organic carbon (SOC) but did not improve aggregate stability (Blanco-Canqui and Schlegel 2013).

Sole purpose (harvesting once a year) resulted in a positive effect on soil chemical, biological and physical properties, presumably due to improved plant-soil-microbe interactions. Similarly, adding organic fertilizer such as cow manure and poultry litter increased soil pH, NO_3-N, plant-available P, SOM, total microbial biomass, gram positive, gram negative, AMF, saprophytic fungi and fungal:bacterial ratio. Use of organic fertilizer particularly animal manure has a potential to maintain soil fertility because of the slow release of nutrients but one should be cautious of soil P build-up and future problems of P loss to water quality when manure application rate is based on N requirement of the crop.

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Table 1.1 Total annual rainfall and mean annual air and soil temperature (12.7 cm soil depth) of Perkins and Lake Carl Blackwell, Oklahoma in 2011 and 2012.

	Perkins	**Lake Carl Blackwell**
Mean annual rainfall (mm)	893	937
2011	668	584
2012	611	524
Mean annual air temperature (^{o}C)	15.7	14.7
2011	16.5	15.9
2012	17.5	16.8
Mean annual soil temperature under 12.7 cm sod (^{o}C)	16.7	15.0
2011	17.4	14.8
2012	18.7	15.0

Table 1.2. Amount of N, P_2O_5 and K_2O applied, using different organic and inorganic amendments. These amendments were applied spring of each year (2009, 2010, 2011).

Nutrient Source	Year	N	P_2O_5	K_2O
			kg/ha	
Cow manure	2009	150	78	102
	2010	150	129	134
	2011	150	55	213
	Total	**450**	**262**	**449**
Poultry Litter	2009	150	150	171
	2010	150	140	149
	2011	150	138	150
	Total	**450**	**428**	**470**
Nitrogen	2009	150	0	0
	2010	150	0	0
	2011	150	0	0
	Total	**450**	**0**	**0**
NPK	2009	150	40	20
	2010	150	40	20
	2011	150	40	20
	Total	**450**	**120**	**60**

Table 1.3. Mean soil pH, nitrate, available P and soil organic matter (SOM) of experimental plots at Lake Carl Blackwell and Perkins, Oklahoma. These values represent the combined means of these two locations.

	Soil pH				Soil NO_3-N				Soil Test P				SOM			
					mg kg^{-1}								%			
	2011		2012		2011		2012		2011		2012		2011		2012	
	dual	sole	dual	sole	dual	sole	dual	sole	dual	sole	dual	sole	dual	sole	dual	sole
C	6.0b	5.9b	6.0bc	5.9c	2.6bc	2.5c	1.8	2.1b	56b	52c	83b	68b	1.15	1.35	1.35b	1.41b
CM	6.6a	6.4a	6.3a	6.4a	4.4a	5.7b	2.4+	4.4a+	145a	153b	114a	125a	1.54	1.61	2.09a	2.17a
PL	6.4a	6.3a	6.3a	6.2ab	5.2a+	7.9a+	3.2+	4.9a+	177a	198a	151a	137a	1.47	1.57	2.29a	2.01a
N	5.9b	5.9b	5.8cd	5.9c	3.9ab	3.0c	2.6	2.2b	53b	55c	71b	76b	1.21	1.25	1.51b	1.50b
NPK	5.8b	5.8b	5.7d	5.8c	3.6abc	3.2c	2.5	2.7b	62b	66c	82b	104ab	1.31	1.27	1.53b	1.60b
L	5.9b	5.9b	6.1ab	6.0bc	2.2c	2.2c	2.0	1.8b	57b	53c	83b	75b	1.25	1.14	1.40b	1.44b

Different letters within each column indicate sub-plot (nutrient sources) means are significantly different at alpha = 0.05. + indicate significant difference between dual and sole purpose switchgrass within a year and nutrient source at alpha = 0.05. C = control/unfertilized, CM = cow manure, PL = poultry litter, N = nitrogen (urea), NPK = nitrogen, phosphorus, potassium (urea, triple superphosphate, potassium chloride), L = inter-seeded with legumes, dual indicates plots were harvested twice a year, sole indicates plots were harvested once a year.

Table 1.4. Mean soil microbial communities of experimental plots at Lake Carl Blackwell and Perkins, Oklahoma. These values represent the combined means of these two locations.

Nutrient Source	Total Microbial Biomass (TMB)				AMF				Gram Positive Bacteria			
	nmol g^{-1} of soil											
	2011		2012		2011		2012		2011		2012	
	dual	sole	dual	sole	dual	sole	dual	sole	dual	sole	dual	sole
C	54.0c	55.7c	37.3	39.4	3.0b	3.5bc	1.6	2	16.9	16.5b	12.8	13.3
CM	68.6a	66.2a	43.6	45.3	4.4a	4.1ab	2.4	2.3	20.4	19.3ab	13.8	14.7
PL	59.1bc+	68.3a+	40.5	44.8	3.2b+	4.5a+	1.8	2.4	18.2	20.5a	13.3	13.9
N	55.0bc+	63.1ab+	38.5	41.3	3.0b	3.5bc	1.6	1.9	17.2	18.9ab	13	13.4
NPK	56.1bc	56.4bc	35.4	42.2	2.9b	2.9c	1.4	2	17.6	17.2b	11.7	13.9
L	60.97b	56.12c	39.2	37.7	3.6b	3.8ab	1.6	1.8	18.4	16.7b	13.3	12.3

Nutrient Source	Gram Negative Bacteria				Saprophytic Fungi				Fungi:Bacteria Ratio			
	nmol g^{-1} of soil											
	2011		2012		2011		2012		2011		2012	
	dual	sole	dual	sole	dual	sole	dual	sole	dual	sole	dual	sole
C	5.9c	6.5b	2.8	3.3	5.2b	5.9b	2.5	2.4b	0.38	0.42	0.28bc	0.28b
CM	9.5a	8.7a	4.4	4.4	7.8a	8.1a	3.9	4.6a	0.42	0.45	0.37a	0.38a
PL	7.2b	8.8a	3.6	4.6	6.6ab+	8.5a+	3.4	4.5a	0.39	0.45	0.33ab	0.38a
N	6.5b	8.3a	3.4	4.1	5.5b+	7.6a+	2.5	3.2ab	0.36	0.42	0.26c	0.31b
NPK	6.5b	7.3b	2.9	3.8	5.7b	5.7b	2	3.6ab	0.38	0.37	0.25c+	0.35ab+
L	7.3b	6.5b	3.1	3.1	6.6ab	5.8b	3.1	2.5b	0.41	0.42	0.31abc	0.30b

Different letters within each column indicate sub-plot (nutrient sources) means are significantly different at alpha = 0.05. + indicate significant difference within dual and sole purpose switchgrass within a year and nutrient source at alpha = 0.05. C = control/unfertilized, CM = cow manure, PL = poultry litter, N = nitrogen (urea), NPK = nitrogen, phosphorus, potassium (urea, triple superphosphate, potassium chloride), L= inter-seeded with legumes, dual indicates plots were harvested twice a year, sole indicates plots were harvested once a year.

Table 1.5. Mean water-stable macroaggregate (%) following three years of nutrient amendments on experimental plots at Lake Carl Blackwell and Perkins, Oklahoma. These values represent the combined means of these two locations.

Macroaggregates in %	2012	
	dual	sole
C	55.06	60.98
CM	60.32 +	72.55 +
PL	64.24	67.01
N	56.99	62.17
NPK	53.85 +	68.32 +
L	52.92 +	66.7 +

+ within each row indicate that main plot (dual vs sole) means with the same treatment are significantly different at alpha = 0.05. C = control/unfertilized, CM = cow manure, PL = poultry litter, N = nitrogen (urea), NPK = nitrogen, phosphorus, potassium (urea, triple superphosphate, potassium chloride), L= inter-seeded with legumes, dual indicates plots were harvested twice a year, sole indicates plots were harvested once a year.

CHAPTER II

THE EFFECTS OF NUTRIENT SOURCES AND HARVEST FREQUENCY ON SWITCHGRASS ABOVE- AND BELOWGROUND BIOMASS

ABSTRACT

Switchgrass has been identified as a potential low-input crop for bioenergy. In Oklahoma, cattle and poultry industries are important sources of livelihood, allowing for the use of animal waste to reduce cost of production in switchgrass. Switchgrass can be produced for use as a biofuel feedstock, forage, or both. The continuous harvesting of aboveground biomass, however, will entail nutrients being extracted from the soil. Thus, it is necessary to explore different nutrient sources that can sustain the production of switchgrass. A field study was conducted at two sites in Oklahoma (2011, 2012) in a split plot design with four replications. These plots were harvested once a year (Fall) for sole purpose biofuel feedstock or twice a year for dual purpose (feed and biofuel) (Summer and Fall). Cow manure, poultry litter, nitrogen, or NPK were applied in plots with established switchgrass (Alamo) and inter-seeded legumes. A non-fertilized control was also included. In the dual purpose, the first harvest comprised

approximately 75-80% of the total biomass, with the remaining 20-25% collected on the second harvest. These data indicate forage harvest should be conducted earlier in the season to allow more time for regrowth for biofuel feedstock and for better forage quality for livestock feeding. The application of animal manure, particularly poultry litter in the dual purpose treatment, increased aboveground biomass when compared to N, NPK, inter-seeded legume, or the control. For the single harvest treatment, poultry litter applications resulted in greatest aboveground biomass among all nutrient treatments in both years. Root biomass was not different among nutrient sources or between harvest frequencies. Poultry litter or animal manure are viable nutrient sources for increasing aboveground biomass and reducing cost of production as these products are readily available in agricultural areas.

INTRODUCTION

Switchgrass (*Panicum virgatum* L.) is a perennial warm-season grass. It has a broad distribution in North America covering areas with mean annual temperatures ranging from 5-25 ^{o}C and mean annual precipitation ranging from 300-1500 mm (Hartman et al. 2011). It is one of the dominant species in the tallgrass prairie in the Central Great Plains. It has been used for grazing, forage and groundcover. Switchgrass can be productive for 10 or more years after its establishment (Sokhansanj et al. 2009). Recently, switchgrass has gained recognition as an ideal bioenergy crop for ethanol production (Sanderson et al. 2006, Bagley et al. 2013).

Switchgrass can be grouped into two categories: the upland and lowland varieties. The upland varieties are shorter than its lowland counterparts and are highly acclimated to well-drained soils on mid to high latitude. Lowland varieties are adapted to lower latitudes and moist conditions (Porter 1966). McLaughlin and Kszos (2005) reported switchgrass yields ranged from 4.5 Mg ha^{-1} in northern plains to 23.0 Mg ha^{-1} in Alabama, with a US average yield of 11.2 Mg ha^{-1}. In Iowa, yield ranged from 6.9 to13.1 Mg ha^{-1} from a test of 20 switchgrass varieties (Lemus et al. 2002). Switchgrass is responsive to N application but not to P and K (Muir et al. 2001, Haque 2012). Fertilizer recommendation varies in different regions because of variation in soil, weather, and management (Parrish and Fike 2005).

In Oklahoma, switchgrass has the potential to be grown for dual-purpose, i.e., for both forage and biofuel feedstock. The early harvest can be used for hay and the regrowth for biofuel feedstock. However, the large biomass removal from a dual purpose crop would leave behind few residues on the soil to build organic matter (Balasko et al. 1984; Muir et al. 2001; Lee et al. 2007). This may in the long-term reduce soil quality, consequently affecting biomass production of the grass. Sanderson et al. (1999) recommended a single harvest in the fall for south central US for maximum biomass yield. Madakadze et al. (1999) agreed with the findings of Sanderson et al. (1999) in research conducted in Quebec, Canada. Fike et al. (2006) documented that in southeastern US, single harvest was the best for lowland cultivars of switchgrass but harvesting twice yearly resulted in greater biomass for the upland cultivars. Adler et al. (2006) and Mulkaney et al. (2006) reported that a single summer harvest reduced the continuous productivity of the plant because of the stress of regrowth during the hot and dry months; single harvesting in November, however, allowed the plants to reallocate the nutrients to the roots.

To achieve a sustainable dual purpose production of switchgrass, it is necessary to explore different nutrient sources that can sustain the production of this perennial grass. The use of legumes and organic fertilizers such as animal manure is one way by which nutrients can be provided for the growing switchgrass and at the same time improve soil quality (George et al. 1995; USDA 1998; DeLuca and DeLuca 2013). The use of organic amendments will provide nutrients to the crop steadily over the growing season (Vogel et al. 2002). The slow release of nutrients helps to minimize potential environmental problems and if the organic amendments are available on the farm, this could further reduce fertilizer expenses (Brejda 2000). In addition, organic fertilizers such as animal manure consist of both macro- and micronutrients (Stevenson et al. 1998) that are needed by plants. The effectiveness of organic fertilizers may not be fully utilized in the first year of application but can have residual effects in subsequent years due to the slow release of nutrients and increase of organic matter in soil that can improve soil quality. Application of fresh manure has been shown to increase soil organic matter (Dick 1992). Edmeades (2003) reported that a typical application of 35 tonnes ha^{-1} fresh manure contribute 7000-8000 kg ha^{-1} organic matter, and this increase in organic matter contributes to sustained productivity of crops. Sakar et al. (2003) reported that in the first 6 years of their 9 year study when comparing application of straw, farmyard manure or inorganic fertilizer alone or in combinations, the grain yield of rice applied with inorganic fertilizer alone were 10-17% greater than those applied with organic fertilizer and/or in combination with inorganic fertilizer. However, from the 7th year to the 9th year (end of the study), those applied annually with wheat straw and farmyard manure had greater yield. There have been studies reviewing fertilizer and crop production, however these focus on different rates of inorganic fertilizers. To date few studies have documented the impacts of different nutrient sources (inorganic vs inorganic) and harvest frequencies on switchgrass biomass yield. Thus, this study was initiated to evaluate aboveground effects of organic and inorganic nutrient sources on dual and sole purpose switchgrass production.

MATERIALS AND METHODS

Site Description and Sampling

The experiment was conducted at Cimarron Valley Research Station, Perkins, OK and Lake Carl Blackwell Research Station, near Stillwater, OK during 2009-2012. The soils at the sites were classified as deep, well-drained and moderately permeable Teller sandy loam (mixed, active, thermic Udic Argiustolls) and Port silt loam (fine-silty, mixed, thermic Cumulic Haplustolls), respectively. A lowland variety Alamo switchgrass (SG) was planted in 2008 at Perkins and 2009 at Lake Carl Blackwell (LCB). The experiment was a split plot with four replicates. Dual purpose switchgrass (dual) and sole-feedstock switchgrass (sole) treatments were assigned to main plots. Dual purpose switchgrass was harvested in July and November to simulate grazing or harvesting for hay and later at maturity for biofuel feedstock, whereas, the sole purpose was harvested in November only to simulate the sole purpose as biofuel feedstock. The subplot were the six different nutrient sources (cow manure [CM], poultry litter [PL], urea [N], urea + triple superphosphate + potassium chloride [NPK], control [C] or non-fertilized and inter-seeded with crimson clover (*Trifolium incarnatum* L.) which is a legume as alternative N source [L]) as the sub-plot. For the nutrient sources, an equivalent nitrogen rate of 150 kg ha^{-1} (for CM, PL, N and NPK) was applied in 2009, 2010, and 2011. No fertilizers were applied in 2012. Cow manure and poultry litter were applied based on an N rate of 150 kg ha^{-1}. These fertilizer treatments were applied in spring 2009, 2010, and 2011. Nitrogen fertilizer applications were split, with 1/3 (50 kg N ha^{-1}) applied before greening and the remaining 2/3 (100 kg N ha^{-1}) were applied eight days after the first harvest to stimulate regrowth.

Above- and Belowground Biomass

Aboveground biomass was sampled from an area of 1 m^2 on each plot by clipping 5 cm above the ground. Dual purpose switchgrass plots were first harvested in late June/early July and again in November of 2011 and 2012. Sole purpose switchgrass plots were harvested only in November 2011 and 2012. Aboveground plant materials were dried at 60 ^oC and weighed to determine dry mass per unit area. Belowground biomass was collected in November 2011 and 2012 in the same area where aboveground biomass was collected using a coring device (12.7 cm diameter) to a depth of 10 cm. Soils were washed off to collect roots, then the roots were dried and weighed similar to the aboveground biomass.

Statistical Analysis

Data pertaining to crop biomass were analyzed using the MIXED procedure of SAS (SAS Institute, 2008). Year was treated as a repeated measure, with averages overtime within a year were calculated and analyzed.

Harvest frequency, nutrient sources and year were considered fixed effects, whereas location and replication were considered as random effects. Location x harvest frequency, location x nutrient source interactions were not significant for the crop biomass ($P > 0.05$), but harvest frequency x year and nutrient source x year interactions were significant for the aboveground biomass ($P < 0.05$). Mean comparisons were made using Fisher's protected LSD test ($\alpha = 0.05$).

RESULTS

Aboveground Biomass

Nutrient sources had significant effects on aboveground biomass for dual purpose switchgrass in both years and sole purpose only in 2011 (Table 2.1). In 2011, dual purpose switchgrass applied with poultry litter had 66% greater biomass than the non-fertilized plots and 39% greater than the NPK treatment. Application of poultry litter in 2011 dual purpose showed significantly greater biomass compared to application of cow manure, urea, NPK and inter-seeded with legumes. Other nutrient sources were not significantly different from each other for dual purpose. In 2011, sole purpose, applied with poultry litter, cow manure and NPK had greater biomass than the control by 44%, 26% and 22%, respectively. In 2012, dual purpose switchgrass total biomass was significantly greater when applied with poultry litter and cow manure compared to inter-seeded legumes by 82% and 47%, respectively. However, single harvesting of switchgrass in 2012 did not show significant differences among the control, and plots treated with poultry litter, NPK, N, or inter-seeded with legumes.

When comparing harvesting frequencies, aboveground biomass production in 2011, single harvested switchgrass (sole purpose) plots treated with cow manure had 53% greater biomass than dual (Table 2.1). There were no significant differences among other nutrient sources. Comparing harvesting frequencies in 2012, there were no significant differences within any nutrient sources including the control.

For the dual purpose harvesting, biomass was greater in the first harvest (late June/early July) than the second harvest (November). In 2011, the first harvest of poultry litter treatment had significantly greater biomass than those applied with cow manure, N, NPK, inter-seeded with legumes or the control (Table 2.1). Those applied with poultry litter had 63% greater biomass than the unfertilized control plot. In 2012, the first harvest, the biomass yields of poultry litter and cow manure treatments were 65% and 49% greater than the plot inter-seeded with legume. Application of cow manure, however, did not result in significantly different production as those applied with inorganic fertilizers or the non-fertilized plots. The first harvest comprised approximately 75-80% of the total biomass, with the remaining 20-25% collected at the second harvest.

Belowground Biomass

Root density was not significantly different among nutrient sources or harvesting frequency in either year (Table 2.2).

DISCUSSION

Few studies have documented the impacts of nutrient sources and harvest frequencies on switchgrass biomass yield (Muir et al. 2001). Dual purpose switchgrass can be advantageous to the cattle industry but continuous biomass removal can deplete soil nutrients. Farmers depend on fertilizers to restore soil nutrients. But the use of inorganic fertilizers requires fossil fuel for its production which can negate the advantage of using switchgrass to minimize carbon emission.

In my study single harvesting of switchgrass had greater biomass than those harvested twice a year (dual purpose) in 2011, these results were similar to the findings of Fike et al. (2006), as they found for lowland cultivars such as Alamo, single harvesting a year maximized biomass yield. Upland cultivars yield greater biomass when harvested twice a year than lowland cultivars (Fike et al. 2006) which indicates that upland cultivars may be more beneficial for dual purpose production. Muir et al. (2001) reported biomass yields of 10.7 to 14.5 Mg ha^{-1} with inorganic fertilizer application rates of 168 kg N ha^{-1} across 3-6 years of research on Alamo harvested once a year in Texas. Muir et al. (2001) reported a maximum yield of 22.5 Mg ha^{-1} with fertilizer rate of 168 kg N ha^{-1} in one year. Cassida et al. (2005) found that Alamo yielded close to 15.0 Mg ha^{-1}

under one harvest per year with 150 kg N ha^{-1} in Texas and Louisiana and 160 kg N ha^{-1} in Arkansas. Guretzky et al. (2011) reported obtaining a yield of 12.2 and 22.8 Mg ha^{-1} with harvesting twice with 0 and 180 kg N ha^{-1}, respectively. In my study, biomass yields varied from 8.7 to 16.6 Mg ha^{-1} and 8.3 to 21.1 Mg ha^{-1} in dual and sole harvest, respectively at a rate of 150 kg N ha^{-1}. For the sole purpose, greater biomass were obtained from those applied with poultry litter, cow manure and NPK compared with the control, N and inter-seeded with legume in 2011, and the biomass may have been influenced by the smaller amounts of phosphorus in the control, N and inter-seeded legume plots. In 2012, there were no significant differences in biomass yield among nutrient sources. This could be due to the drier year, since mineralization and movement of nutrients, particularly N, may have been limited due to less precipitation and soil moisture. There was a general trend of increased biomass in switchgrass plots harvested twice with poultry litter treatment. In Chapter 1 Table 1.3, soil NO_3-N and plant available P were greater in the plots applied with poultry litter, which may be responsible for the increased yields. Regrowth were observed to be less when harvested in late June/early July. Therefore, harvesting earlier to allow more time for regrowth is needed for biofuel feedstock in a dual purpose system.

The amount of precipitation and temperature differences among years caused variability in biomass yields. The year 2012 was drier and warmer compared to 2011 which may explain the decrease in biomass yield in switchgrass. Guretzky et al. (2011) reported similar effect of precipitation in the variability of yield in different years of their study. In addition, no fertilizers were applied in 2012 to find out the residual effect of prior years of fertilizer application.

There were no significant differences in the root density of switchgrass in this study which could be due to the limited depth (10 cm) sampled. Frank et al. (2004) found that 50% of root biomass of switchgrass was at the top 30 cm from an observation of 1.1 m soil depth. This suggests our sampling depth was inadequate for an accurate assessment of root biomass. The root density from 2011 to 2012 was almost doubled for both dual and sole purpose which could be attributed to its root development as a response to drier, warmer conditions. Lloret et al. (1999) studied seedling survival in a drought condition of shrubland species and found a positive correlation between the seedling survival and its allocation to root development.

In the dual purpose, the first harvest comprised approximately 75-80% of the total biomass and the remaining 20-25% on the second harvest, implying that harvesting for forage should be done earlier to allow more time for regrowth for biofuel feedstock. In dual purpose the application of animal manure, particularly poultry litter, increased aboveground biomass when compared to N, NPK or plots inter-seeded with legumes and an impressive 66% and 70% over the control. In sole purpose, those applied with poultry litter had the greatest aboveground biomass, compared to any other treatments in either years. When comparing harvest frequencies, single harvest for sole purpose had the greater total aboveground biomass in 2011. In 2012, aboveground biomass was lower compared to the previous year and there were no significant differences between sole and dual and among nutrient sources which could be attributed to a warmer and drier environment and no fertilizer were applied that year. Root biomass was not different among nutrient sources or between harvest frequencies. Utilizing poultry litter or animal manure is a viable nutrient source for increasing aboveground biomass and reducing the cost of production if already available within the farm.

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Table 2.1. Effects of different nutrient sources and switchgrass production (dual – harvested twice a year vs sole – harvested once a year) on switchgrass aboveground biomass at two locations Perkins and Lake Carl Blackwell, Oklahoma in 2011 and 2012.

	Aboveground biomass (Mg ha^{-1})							
	2011				2012			
	Dual			Sole	Dual			Sole
	1st harvest	2nd harvest	Total		1st harvest	2nd harvest	Total	
Control	7.6 b	2.4	10.0 b	14.7 c	7.0 bc	0.7	7.7 bc	7.4
Cow manure	8.9 b	3.2	12.1 b+	18.5 ab+	9.7 ab	0.9	10.6 ab	10.5
Poultry litter	12.4 a	4.2	16.6 a	21.2 a	10.7 a	2.4	13.1 a	11.6
Nitrogen	9.6 b	2.7	12.3 b	15.5 bc	8.1bc	0.7	8.8 bc	8.4
NPK	9.1 b	2.8	11.9 b	18.0 abc	8.8 bc	0.7	9.5 bc	10.3
Legume	7.7 b	3.0	10.7 b	10.5 d	6.5 c	0.7	7.2 c	7.4

Different letters within each column indicate means are significantly different at alpha = 0.01. + within each row indicate that main plot (dual vs sole) means with the same nutrient source are significantly different at alpha = 0.05.

Table 2.2. Effects of different nutrient sources and switchgrass production (dual – harvested twice a year vs sole – harvested once a year) on switchgrass belowground biomass at two locations Perkins and Lake Carl Blackwell, Oklahoma in 2011 and 2012.

	Root Density			
	mg cm^{-3}			
	Fall 2011		Fall 2012	
	dual	sole	dual	sole
C	14	14	41	37
PL	19	19	45	43
N	14	19	39	41
NPK	16	15	34	48
L	16	12	43	32

CHAPTER III

EFFECTS OF ELEVATED TEMPERATURE AND DRIER CONDITION ON SWITCHGRASS PRODUCTION

ABSTRACT

Based largely on extrapolations of models and field observations, switchgrass (*Panicum virgatum*) has been proposed to be a model bioenergy crop. As a native perennial, warm-season grass, switchgrass has adaptability to a wide range of environments and low fertilizer requirement. In this study, I provide empirical data to assess the effectiveness of switchgrass for low-input sustainable feedstock production, under changing climates. This study assesses the interactions of fertilizer amendment (organic and inorganic amendments) and elevated temperature and increased drought on above- and belowground components influencing switchgrass performance in controlled greenhouse environments. Production was not significantly different between non-fertilized control, applications of inorganic fertilizers N or NPK, or organic amendment (applied as poultry litter). Furthermore, increased temperatures (typical daily high temperature of 53 ^{o}C) and drought conditions (maintained at slightly above the permanent wilting point) did not affect biomass possibly due to lack of stress under any treatment. Soil microbial communities were also not affected by any

treatment, with the exception of arbuscular mycorrhizal fungi which were more abundant under ambient climatic conditions than warmer, drier conditions. However, overall microbial biomass was not affected by fertilizer or climate. This study indicates switchgrass can be a productive bioenergy crop if North American climates becomes warmer and drier, and is able to produce high quality biomass (based on cellulose:lignin ratios) under an array of fertilizer amendments. Importantly, switchgrass biomass quality and quantity were similar in treatments receiving no fertilizer applications, compared to either organic or inorganic fertilizations.

INTRODUCTION

The growing concern over climate change and energy security was brought about by the increasing carbon dioxide in the atmosphere, reliance on fossil fuel and increasing cost of foreign fuel which prompted the US government to explore alternative renewable sources of energy. In 2006, global atmospheric CO_2 concentrations were already 380 parts per million (ppm), compared to 280 ppm before the industrial revolution (IPCC 2007). One of the primary contributors of the increased global carbon emission is fossil-fuel combustion contributing 8.6 Pg C yr^{-1} (Lal 2008). Increases in greenhouse gases and the resultant changes in global climate is a phenomenon recognized by 160 countries that adapted the Kyoto Protocol in 1997 (Breidinich et al. 1998). In the Kyoto Protocol, it was agreed that there is a need to reduce greenhouse gas emissions to slow atmospheric accumulation.

In recent years, the use of biofuel has increased rapidly in the United States. Jessup (2009) reported that biofuel production between 2005 to 2008 averaged 2.4 billion gallons per year compared to 150 million gallons per year from 1978

through 2004. Furthermore, the projected rate of biofuel production between 2009 to 2022 is 1.9 billion gallons per year (Biomass Research and Development Board 2008).

The US Department of Energy Herbaceous Energy Crops Program (HECP) screened more than 30 herbaceous species for their potential as biofuel feedstocks (Parrish and Fike 2005). Native switchgrass was selected as a model herbaceous species because of its perennial growth habit, high yielding potential on marginal lands, wide environmental tolerance, compatibility with conventional farming practices, and its potential to revegetate drastically disturbed sites for reconstructed grasslands (Parrish and Fike 2005; Wright and Turhollow 2010). When harvested biomass is burned directly or converted to ethanol, prairie grasses can have a negative C balance compared to fossil fuels (Garten et al. 2010). Using established technology, prairie hay can be pelletized and burned for distillation heat in corn ethanol production or co-fired in coal power plants for electricity. It can also be converted to next-generation liquid biofuel using emerging technology for cellulosic ethanol or synthetic fuel (Tilman et al. 2006).

For ethanol production, corn is the current dominant feedstock for biofuel in the US (Hoekman 2009). Production by first generation feedstocks like corn requires large quantities of nitrogen (N) and phosphorus (P) fertilizer for optimal yields. Production of N fertilizer accounts for a major portion of agricultural fossil fuel use (Vitousek et al. 1997). Also, fertilizer usage is a barrier to achieving C neutrality because it reduces the functioning of mycorrhizas and other plant-soil-microbe interactions that generate biological soil fertility and increase soil C sequestration (Zhu and Miller 2003; Welbaum et al. 2004). Switchgrass is highly dependent upon symbiotic associations with arbuscular mycorrhizal fungi (AMF) (Wilson and Hartnett 1998). Because of their reliance on beneficial interactions with soil microorganisms, and in particular, their strong symbiotic relationships with mycorrhizal fungi, switchgrass has been shown to have considerably higher nutrient and water use efficiency than corn (Beale and Long 1997; Heaton et al. 2004).

Mycorrhizas are a key mechanism for the high nutrient and water-use efficiencies and large biomass yields of native prairie grasses in low-input systems. Mycorrhizal function is largely based on reciprocal transfer of photosynthate from the plant and P, N, and other nutrients from the fungus. AM fungi can exert a sizable C sink that causes plants to increase their rates of photosynthesis (Wright et al. 1998; Miller et al. 2002). Furthermore, AM fungal hyphae can replace root hairs and root epidermal cell surfaces as sites of P and N uptake (Smith et al. 2003). Mycorrhizal symbioses also confer other plant benefits including improved plant-water relations, tolerance to soil contaminants including herbicides, and resistance to pathogens (Smith and Read 1997; Miller and Jastrow 2000). At the ecosystem scale, AM fungi play a critical role in the formation of soil structure (Jastrow et al. 1998; Miller and Jastrow 2000) and regulating C flux from plants to the soil (Zhu and Miller 2003). Mycorrhizas enhance C sequestration because they transfer C away from root surfaces where microbial metabolism is greatest into the soil matrix, including aggregates (see reviews by Rillig and Mummey 2006, Treseder and Allen 2000). AM fungi directly contribute to soil aggregation through the physical entanglement of soil particles with external hyphae (Tisdall and Oades 1982). Sizeable amounts of these hyphae have been reported for prairie soils (Eom et al. 1999, Rillig and Allen

1999; Wilson et al. 2006), with a peak length of 111 m hyphae cm^{-3} soil in a prairie community (Miller et al. 1995). As soil aggregation is thought to protect C rich detritus from microbial degradation, increased aggregate stability could increase C sequestration (Jastrow and Miller 1998; Miller and Jastrow 2000, Six et al. 2000). Switchgrass feedstock is comprised of shoots so it is understandable that the focus of these breeding programs has been aboveground. In fact, the major goal of the switchgrass breeding to date has been to develop cultivars that produce high biomass per unit area without increased cultural input; ultimately reducing cost per unit biofuel (Muir et al. 2001; Christian et al. 2002; Thomason et al. 2004; McLaughlin and Kszos 2005; Parrish and Fike 2005; Guretzky et al. 2011). However, there are potentially significant indirect benefits from the ecological services generated from sustainable use of switchgrass, including ground water purification, reduced soil erosion, improved soil tilth, pasture-based animal production, endangered species and wildlife habitat, and recreation. These services should be included in a comprehensive assessment of biofuel feasibility.

Switchgrass has also been proposed as a model crop for bioenergy due to its growth in a wide range of climatic conditions. Indeed, few other herbaceous crops are as broadly adapted across the range of growing conditions found in North America as is switchgrass. Switchgrass is a better selection than corn for biofuel since it requires less water and less fertilizer, thus improving soil conservation compared to row crops such as corn. Switchgrass also has higher energy output than the same amount of corn biomass when converted to ethanol.

Corn based ethanol has 21% net energy gain while switchgrass based ethanol has 343% net energy gain (McLaughlin and Walsh 1998). Prediction of switchgrass performance under global climate change is largely based on modeling studies (Barney and DiTomaso 2010).

Experiments to assess effects of climate change can be conducted along a latitudinal gradient, allowing predictions of plant and soil responses with increasing temperature and drought. However, field studies are complicated by the effect of soil type and land-use history such that the effect of climate is difficult to disentangle from the effect of soil at each site. Therefore, a greenhouse study was conducted to assess the effects of a warmer, drier climate on annual biomass production of switchgrass.

Previous research has shown AMF can significantly improve water uptake by increasing root absorptive surface area and hydraulic conductivity (Augé 2001, 2004; Allen 2011) and/or increasing the water-holding capacity of soil (Miller and Jastrow 2000; Jastrow et al. 1998; Wilson et al. 2009). A number of recent studies have explored the role of AM fungi on plant water relations at the cellular to organism levels, indicating that the root exudates and signaling molecules that are emitted by plants to stimulate AM colonization are produced in higher quantities when plants are water-stressed (Horii et al. 2009; Akiyama et al. 2005), and that AM-derived benefits to host plants are greater under resource-limited conditions (Klironomos et al. 2001). Thus, I predict that mycorrhizas play an important role in resilience to drought in switchgrass and will increase in importance as a biofuel with climate change.

Fertilizer management may also impact soil microbes and C sequestration processes in biomass feedstock production, and these responses may be confounded by elevated temperature and drought. Nitrogen is typically the most limiting plant nutrient, and N fertilization of switchgrass grown for bioenergy is an important consideration in designing site management from the standpoint of maximizing N use and minimizing N loss to leaching (Nyakatawa et al. 2006; Garten et al. 2010). Certainly, like most crops, soil C storage beneath switchgrass, as well as aboveground biomass production can potentially be increased with applications of inorganic N fertilizer (Lee et al. 2007). However, previous studies assessing N fertilization and switchgrass aboveground biomass production have reported high annual, cultivar, and inter-site variability (Reynolds et al. 2000; Muir et al. 2001; Lee et al. 2007; Garten et al. 2010). Organic amendments, such as animal manure, contain nutrients in organic forms that must first be mineralized by soil microorganisms to be available for plant uptake. However, organic amendments typically also contain intra- and extra-cellular enzymes that aid in the decomposition of organic matter and stimulate microbial activity (Mohammadi et al. 2011). Alternatively, while inorganic fertilizers are readily available for plant use, ammonium and nitrate ions may leach into ground water and aquatic systems and have limited availability for plant uptake (Hallberg 1989). Understanding how different fertilization regimes affect microbial communities, including mycorrhizal associations, and thereby C sequestration processes, and how fertilizer amendments interact with climate change, could have important implications for efforts to co-optimize. Therefore, in my study, I investigate the influence of warmer and drier climatic conditions on aboveground production of switchgrass grown under various fertilizer regimes. Furthermore, I assess the role of climate and fertilizer management on belowground parameters such as soil organic carbon, root biomass production, and soil microbial communities.

Cellulose:lignin ratio of biofuel feedstock is also an important aspect in the selection of ideal materials for biofuel feedstock. Feedstock quality can be assessed by measuring percent cellulose, hemicellulose, lignin, ash, moisture content, N content and other minerals from plant tissue (Boateng et al. 2006). Lignin is the most recalcitrant component of the cell wall and can reduce the surface area available for enzyme activity, therefore Cellulose:lignin ratio is often used to project feedstock quality. Most studies to date have examined biomass yield, N application rates, and timing of N application (Muir et al. 2001; Christian et al. 2002; Thomason et al. 2004; McLaughlin and Kszos 2005; Parrish and Fike 2005; Guretzky et al. 2011), but few studies include cellulose:lignin ratio of switchgrass as an indicator of quality feedstock. Therefore, assessments of cellulose:lignin ratio of switchgrass grown under ambient and warmer/drier climatic conditions and with various fertilizer amendments were included. I hypothesize cellulose:lignin ratios will be high, with relatively low production of lignin, across all climates.

I hypothesize that switchgrass is a viable option for quality biofuel under warmer, drier climatic conditions. Specifically, I hypothesize that plant biomass will not decrease when grown under increased temperatures and decreased water availability, compared to biomass production when grown under ambient conditions. However, I hypothesize that AMF biomass and root colonization will increase with warmer and drier conditions, as the plants will increase their reliance on these fungi to forage for water. I hypothesize that under both climatic conditions, applications of organic manure will increase above-ground biomass production, compared to inorganic fertilizers or the non-fertilized control, due to positive effects of organic fertilizer on soil properties such as conservation of moisture and increased retention (slow-release) of nutrients.

MATERIALS AND METHODS

Soil Preparation:

Soil used for the greenhouse study was collected from Oklahoma State University Range Research Site near Stillwater, OK. Surface soil (0-15 cm) was sieved through a mesh (2 mm) to remove rocks and other plant debris. The initial soil properties were: pH of 7.8, 12.8 mg kg^{-1} NO_3-N, 1.25 mg kg^{-1} plant available (Mehlich 3) P and 144 mg kg^{-1} plant available K.

Switchgrass Collection and Establishment:

Switchgrass tillers (lowland cultivar: Alamo) were collected from the Cimarron Valley Research Station near Perkins, OK using a 12 cm diameter core to a depth of 10 cm. Aboveground biomass was clipped to 4 cm. Tillers, comprising individual clones, were transported to Oklahoma State University greenhouse facilities. Four clones were transplanted into each of 32 mesocosms (plastic containers 82 cm height x 50 cm width x 43 cm length) containing 32 kg (dry wt) of native prairie soil (described above). Plants were allowed to adapt to the greenhouse environment (18 - 22 ^{o}C with no supplemental lighting) for four weeks.

Treatment and Experimental Design:

Treatments consisted of a factorial combination of two climate conditions (ambient and increased temperature/decreased water) x four nutrient sources (N fertilizer, NPK fertilizer, poultry litter (PL) and control (C) with four replications for a total of 32 mesocosms. Mesocosms were arranged in a randomized complete block design.

Increased temperature was implemented by placing one-half of mesocosms in a greenhouse maintained with average ambient temperatures of 27 ^{o}C (typical daily high of 30^{o}C) and the other half in a greenhouse maintained 5-10 ^{o}C greater than ambient (typical daily high of 40^{o}C). Drought was implemented by maintaining a volumetric soil moisture content (VMC) at 20-25%, which is slightly above the permanent wilting point. For the well-watered treatment, VMC was maintained at 35-40% (field capacity). The VMC was monitored every other day using the Field Scout TDR. The TDR was calibrated for a clay soil.

All nutrient sources were applied one time based on N application rate of 150 kg ha^{-1}. The plants were maintained for 16 weeks at which time the majority of the switchgrass plants had senesced. At this time, aboveground biomass was harvested and dried at 60 ^{o}C for 72 hrs and weighed.

The main cell wall components (cellulose, hemicellulose and lignin content) were assessed on dried aboveground samples. Samples were ground and the acid detergent fiber (ADF), neutral detergent fiber (NDF) and acid detergent lignin (ADL) were determined. Acid and neutral solutions were used to determine ADF and NDF, respectively, using Ankom fiber analyzer. Acid detergent fiber and NDF are residues remaining after the extractions from these solutions and determined gravimetrically. Acid detergent lignin is the percent fiber remaining after ADF determination and further digestion with 72% sulfuric acid. Cellulose, hemicellulose and lignin content were calculated following Lemus et al. (2002):

Cellulose (%) = ADF (%) – ADL (%)

Hemicellulose (%) = NDF (%) – ADF (%)

Roots were washed free of soil, dried at 60 ^{o}C for 72 hrs, and weighed. Soil samples were collected from each mesocosm and partitioned for phospholipid fatty acid analysis and soil chemical analysis.

Soil NO_3-N was extracted using 1M KCl solution and was analyzed using the Lachat Quickchem 8000 Flow Injection Autoanalyzer (Kachurina et al. 2000). Plant available P and K were extracted with Mehlich 3 solution and quantified using the inductively coupled plasma emission spectroscopy (ICP) (Pittman et al. 2005). Soil pH was measured from a 1:1 soil/water ratio using a pH electrode (Asai et al. 2009).

Total lipids were extracted from soil samples based on the method of Bligh and Dyer (1959) with modifications by White et al. (1979) to determine the soil microbial biomass based on functional groups. Phospholipid fatty acid analysis was performed using Agilent 7890A gas chromatography with an Agilent 5975C series mass selective detector (GC-MS). Methyl nonadecanoate fatty acid (19:0) was used as the internal standard for all the samples. The following fatty acids were chosen to represent the gram positive bacteria: i15:0, a15:0, i16:0, i17:0, a17:0; for gram negative bacteria: 3-OH 14:0, 16:1_Δ9 (16:1ω7), 17:0_Δ9,10 (cy17:0), 2-OH 16:0, 19:0_Δ9,10 (cy19:0), 2-OH 12:0; for AM fungi: 16:1_Δ11(16:1ω5c), 20:1ω9, 22:1ω13; for saprophytic fungi: 18:1_ Δ9 (18:1ω9c), 18:2_Δ9,12 (18:2ω9) and for non-specific microbes 14:0, 15:0, 16:0, 17:0, 18:0, 20:0 (Zelles 1999; Wilkinson et al. 2002). The total microbial biomass or relative abundance of each functional group was expressed as nmol g^{-1} of soil.

Statistical Analysis

All data were analyzed using the MIXED procedure of SAS (SAS Institute, 2008). Climate, fertilizer and climate*fertilizer interaction were considered fixed effects and block as random effect. Dependent variables were checked for normality and homogeneity of variance using the UNIVARIATE procedure of SAS program. Mean comparisons were made using Fisher's protected LSD test (α=0.05).

RESULTS

Above- and Belowground Biomass Production, Root:Shoot Ratio

Aboveground biomass and relative growth rate were similar under both climatic conditions and there were no significant interactions between fertilizer and climate (Table 3.1).

Fertilizer did affect root biomass, as applications of nitrogen alone (urea) significantly decreased root biomass, compared to all other fertilizer treatments (Table 3.1). Applications of NPK increased root biomass by 57%, compared to the control, and by 59% compared to those applied with nitrogen alone. Applications of poultry litter resulted in root biomass similar to the NPK treatment.

Root:shoot ratio among nutrient sources and climate was not significantly different between treatments.

Cellulose, Hemicellulose and Lignin

Both climate and fertilizer source significantly affected cellulose concentration of switchgrass biomass. Cellulose content under warmer, drier conditions was significantly greater than ambient conditions. The plants amended with poultry litter had significantly more cellulose than plants receiving inorganic fertilizers (urea or NPK). However, hemicellulose and lignin content of switchgrass were not significantly influenced by fertilizer amendment or climate (Table 3.2). Cellulose:lignin ratio were slightly affected by the interaction of climate and fertilizer amendments. While most fertilizer treatments resulted in similar cellulose:lignin ratios when grown under warmer and drier conditions, the plants of the non-fertilized control had greater cellulose:lignin ratio than plants amended with poultry litter.

Soil Microbial Communities/Soil Biological Properties

The distinct microbial groups important to soil function and observed in this study were gram positive bacteria, gram negative bacteria, AM fungi, and saprophytic fungi. No significant effects were observed for any functional group in response to fertilizer treatment, and there were no significant interactions between fertilizers and climate condition (Table 3.3). Among these functional microbial groups, only AM fungi were affected by climate (Table 3.3). Arbuscular mycorrhizal fungal biomass in ambient condition was almost twice as large as that under drier and warmer conditions. The greater fungal biomass led to an overall greater fungi:bacteria ratio when plants were exposed to ambient climatic conditions, compared to the warmer and dried conditions (Table 3.3). These alterations in fungal:bacteria ratio was presumably in response to alterations in AMF, as AMF was the only functional group that significantly decreased when grown under warmer/drier conditions.

Soil Chemical Properties

Soil pH, SOM and soil K were not significantly different following fertilizer applications, alterations in climate, or fertilizer x climate interactions (Table 3.4). Soil NO_3-N and plant-available P were significantly affected by fertilizer, but there was no interaction with climate (Table 3.4). Application of inorganic fertilizers resulted in greater NO_3-N compared with the non-fertilized control or application of poultry litter. Plant available P was greater in soil following applications of inorganic NPK or poultry litter, compared to applications of N alone, or no fertilizer application. No significant differences in organic matter were observed in any fertilizer or climate treatments, and no fertilizer x climate interactions were observed (Table 3.4).

DISCUSSION

Switchgrass has been proposed to be a model bioenergy crop for its adaptability to a wide range of environments and for its low fertilizer requirement. Because of this, it has been considered to plant this crop on marginal lands, thus reducing the competition of bioenergy crops with row crop agriculture or livestock production on prime agricultural land (Hartman et al. 2011). This greenhouse study was to provide empirical data to test the effectiveness of switchgrass for feedstock production when grown in low nutrient (marginal) soil under elevated temperatures and reduced soil moisture.

Production by first generation feedstocks like corn requires large quantities of nitrogen (N) and phosphorus (P) fertilizer for optimal yields. Biofuel feedstocks produced from annual food crops such as corn and soybean offer only marginal improvements in net energy production because their cultivation requires relatively high inputs of fossil fuels (Hill et al. 2006). Production of N fertilizer accounts for a major portion of agricultural fossil fuel use (Vitousek et al. 1997). Nitrogen is typically the most limiting plant nutrient, and N fertilization of switchgrass grown for bioenergy is an important consideration in designing site management from the standpoint of maximizing N use and minimizing N loss to leaching (Nyakatawa et al. 2006; Garten et al. 2010). Because switchgrass is a native grass species, it has been projected that successful growth for feedstock production may require less fertilizer input, compared to annual food crops.

Certainly, like most crops, aboveground biomass production can potentially be increased with applications of inorganic N fertilizer (Lee et al. 2007). However, biomass is not always increased in response to fertilization. In a review of 14 field trials comparing long term effects (20-120 years) of NPK fertilizer, no significant increases in production were observed on agricultural row crop production (Edmeades 2003). Similarly, previous studies assessing N fertilization and switchgrass aboveground biomass production have reported high annual, cultivar, and inter-site variability (Reynolds et al. 2000; Muir et al. 2001; Lee et al. 2007; Garten et al. 2010). Production costs and energy requirements for N fertilizer can be reduced through the use of organic amendments, such as manure or plant residues. In this study, the influence of organic and inorganic fertilizer on feedstock production was assessed. I hypothesized that organic fertilizer, in the form of poultry litter, would increase biomass production, compared to production of the non-fertilized control or plants receiving applications of inorganic N or NPK fertilizer. However, the data obtained do not support my hypothesis, as there was no influence of fertilizer on aboveground production. However, the data obtained do support using switchgrass as an effective feedstock crop; production was not significantly different between the non-fertilized control and any fertilizer treatment, even when grown in low nutrient (marginal) soil.

In this study, soil organic matter (SOM) was not significantly different between any fertilizer or climate treatments. Soil organic matter is generally linked to overall soil health, as increases in SOM generally lead to increases in cation exchange capacity, soil aggregate stability, and soil aeration. In addition, organic amendments are considered beneficial as they require decomposition to release nutrients for plant availability and are thus considered slow-release nutrient amendments, compared to chemical applications of nitrogen which are readily available for plant uptake but may be leached into adjacent ecosystems. Plant, animal, and microbial residues are substrates for the formation of SOM; therefore increases in plant litter and soil microbial activity is directly related to increases in SOM. Soil biota, especially microorganisms, contribute utmost to the formation of SOM from organic litter, and consequently microbial biomass is typically increased with increases in SOM (Birkofer et al. 2008, Thiele-Bruhn 2012). As I did not observe any treatment effects on plant biomass or total microbial biomass, it may be expected that SOM remain at similar levels across treatments, as well. However, typically, SOM is greater in soils receiving organic fertilizer (Birkofer et al. 2008, Thiele-Bruhn 2012). Increasing amounts of nitrogen (organic or inorganic) have also been shown to increase SOM (Halvorson and Reule 1999; Garten 2012), although this was not observed in my study. It is possible that the limited time (16 weeks) of the greenhouse study did not allow for substantial alterations in SOM formation or decomposition, regardless of treatment. While even a single application of organic fertilizer has been shown to significantly increase soil microbial communities and SOC (Hammesfahr et al. 2011), SOM formation and decomposition is directly affected by multiple interacting factors (e.g. soil texture, soil temperature and moisture, microbial composition, plant quality and quantity). Therefore, assessing implications of fertilizer amendments on SOM formation is difficult.

In agreement with previous predictions, such as those by Parrish and Fike (2005) and Garten et al. (2010), and in support of my first hypothesis, the data indicate aboveground biomass production was not reduced in response to increasing temperatures and drought. This study provides empirical data to support previous suggestions, based largely on extrapolations of models and field observations (e.g. McLaughlin and Kszos 2005), that switchgrass can tolerate a wide range of environmental conditions, without a loss in feedstock production. Furthermore, this study showed no climate x fertilizer interaction, indicating switchgrass was not influenced by fertilization when grown under well-watered, ambient temperatures or elevated temperatures and drought conditions.

For feedstock production, it is not only important to consider overall production, but tissue components of cellulose, hemicelluloses, and lignin are also important considerations. It is preferred to have a feedstock with relatively greater cellulose content, compared to lignin, for efficient conversion to ethanol. At this time, cellulose is the only polysaccharide that has been used for commercial ethanol production, as it is currently the only one for which there are commercially available deconstructing enzyme mixtures (Sticklen, 2008). Like cellulose, hemicellulose can also be converted into fermentable sugars by enzymatic hydrolysis for the production of cellulosic ethanol (Sticklen, 2008). Hence, the greater the percentage of cellulose to lignin ratio reflects greater efficiency for lignocellulosic biomass to be converted to ethanol. This study showed both fertilizer and climate influenced the percentage of cellulose in switchgrass shoots. When grown under ambient conditions, plants grown with applications of urea produced shoots with relatively greater cellulose content, compared to those grown with NPK. However, while these values are significant, there were small differences in the percentage of cellulose, lignin, or hemicellulose between treatments. When grown under higher temperatures and drought conditions, the non-fertilized control produced greater cellulose content than the urea treatment, but again, these values are, overall, not substantial. Furthermore, neither lignin, hemicelluloses, nor cellulose:lignin were affected by fertilizer treatment or climate. In summary, there were few alterations in cellulose content in response to fertilizer or climate. These data were notable and reassuring in that switchgrass produced similar biomass, with similar cellulose content, when grown under low-input management (no additional fertilizer), compared to growth following either inorganic or organic fertilizer amendments. Furthermore, these results held true under warmer and drier conditions. This may be valuable information for feedstock producers.

Nutrient sources did significantly affect belowground biomass, with similar responses between both climates. There was greater root production following applications of inorganic NPK fertilizer, compared to urea (N alone) or the non-fertilized control, and applications of organic fertilizer (poultry litter) increased root production compared to applications of urea. Investment into root biomass may have large implications on SOM and soil organic carbon storage, and may also have implications on production of shoot biomass in subsequent years.

Microbes such as gram positive, gram negative bacteria, AM and saprophytic fungi are distinct microbial functional groups that are vital to sustainable soil and plant function and health (Anderson 2003). The potential exists for changes in the composition of these groups of the soil microbial community in response to changes in climate, such as rising temperatures and reduced precipitation (e.g. Pendall et al. 2004; Compant et al. 2010). In fact, climate is considered to be a major driver influencing microbial communities, including fungi (Robinson, 2001; Lopez-Gutierrez et al. 2008), and specifically AM fungi (Helgason and Fitter 2009; Antunes et al. 2011; Hawkes et al., 2011). Arbuscular mycorrhizal fungi can increase drought tolerance by producing hyphae with access to small soil pores, expanding belowground water uptake and increasing rates of water movement from soil into host plants (Ruiz-Lozano et al. 1995; Augé 2001) or by increasing nutrient acquisition transferred from the soil to the plant (Ruiz-Lozano and Azcón 1996; Johnson et al. 1997; Wu and Xia 2006). However, at very low soil water, fungal hyphae in soil may stop growing or contract if the hyphae cannot maintain structural integrity. Mycorrhizal hyphae in soils and roots have been shown to increase, decrease, or remain unchanged in response to drought, and appear to be context-dependent depending on host, soil, and an array of environmental factors (Miller et al. 1995; Denef et al. 2001; Lutgen et al. 2003; Staddon et al. 2003; Clark et al. 2009; Querejeta et al. 2009; Hawkes et al. 2011).   In this study, AMF was the only soil microbial group that was significantly affected by increased temperatures and drought; AMF biomass was reduced when plants were grown under warmer, drier conditions, compared to fungal biomass produced when plants were grown under ambient conditions. Overall fungal production (combined totals of AM and saprophytic fungi) was also greater under ambient conditions, and this led to a greater fungi:bacteria ratio. Therefore, the data collected do not support my previous hypothesis that AMF biomass production increase in response to elevated temperatures and increased drought.

Previous studies have reported that while fungi are generally highly resistant to water stress and can grow at relatively low water content (Harris, 1981; Schimel et al., 1989), fungi are susceptible to extremely low soil water and increased stress (Williams, 2007). During times of high stress, plants may divert resources away from AMF, resulting in a reduction in mycorrhizal hyphal production (Eom et al. 1999; Entry et al. 2002; Bell et al. 2009; Olsson et al. 2010; Owens et al. 2012). Indeed, the beneficial effects of AM symbionts can become detrimental to plant growth under extreme drought, as low water availability can inhibit the flow of phosphorus from AMF to plants (Al-Karaki and Al-Raddad 1997). In this study, while the temperatures were maintained above-average (typical daily high temperature of 40 ^{o}C) and plants experienced drought conditions (maintained at slightly above the permanent wilting point) for 16 weeks, it is possible these conditions did not result in high stress for these C_4 plants. Plants with C_4 physiology have high water use efficiency and stomatal conductance, and may not rely heavily on fungal symbionts for water acquisition (Edwards et al. 2010). In this scenario, the AM fungal biomass was reduced with no negative consequence to the host plant, as the plant was not highly dependent on the fungi for water or nutrient uptake. Indeed, similar aboveground biomass was produced whether plants were grown under ambient conditions or in higher temperatures and drought.   However, switchgrass has been shown to be highly mycotrophic in low nutrient prairie soil (Wilson and Hartnett 1997), and reliance on the symbiosis would not be expected to be reduced under periods of stress, such as increased temperature or drought. Therefore, I present an alternative hypothesis, that the switchgrass plants grown in this study did not perceive stress under any of the climatic conditions. Similar biomass production across treatments could also be indicative of a lack of induced stress at these temperature and water treatments. Plants often respond to drought by avoiding water deficits through reduced evapotranspiration by increasing root-to-shoot ratios (Chaves et al. 2003). The lack of alterations in root:shoot ratios following higher temperature and drought conditions may further indicate the lack of perceived stress under these conditions.

Drought conditions have been shown to alter soil microbial community composition through differential drought tolerance among functional groups of microorganisms. For example, more drought-tolerant microorganisms such as fungi would be expected to increase in relative abundance, while abundance of gram-negative bacteria usually decline in response to drought. These changes lead to an increased ratio of fungi to bacteria under dry conditions, as measured by an increased fungal:bacteria ratio (Drenovsky et al. 2004, Gray et al. 2011). However, in this study, the fungal:bacterial ratio decreased in response to soil drying, as a result of additive declines of AM and saprophytic hyphal production.

Furthermore, the degree of physiological stress of a system can be estimated using the bacterial stress index (BSI), based on relative abundance of gram-negative bacteria (BSI = cy17:0 + cy19:0/ 16:1ω7c + 18:1ω7c) for each sample (Bossio et al. 1998; Gray et al. 2011). The BSI was not significantly different between the soil communities of either of the environmental conditions manipulated, and in fact these ratios tended to be higher from soils maintained under ambient climate, indicating drought and high temperatures did not induce stress in the soil microbial communities in this study.

An alternative, or additive, possibility for the lower AM biomass under ambient conditions, compared to elevated temperatures and drought, is that the AM fungi shifted in community composition, with an environmental sorting of AM fungal taxa based on differential AM fungal adaptations to environmental conditions, leading to changes in AM fungal community composition. It has been shown that AM fungi may be more responsive to environmental conditions rather than their host plant (Worchel et al. 2013) and AM fungal isolates may produce fewer spores, arbuscules, or extra-radical hyphae, compared to other isolates (Antunes et al. 2010). Host plants shape distinctive AM fungal communities even when inoculated with the same AM fungal species (Bever et al. 1996; Uibopuu et al. 2009) and these altered communities have been shown to differentially impact growth of host plants (Bever 2002). As plants can allocate preferentially to the most beneficial fungal partner (Bever et al. 2009; Kiers et al. 2011), it is possible that switchgrass plants may alter AM fungal communities to promote their own success, selecting for fungal communities that are most beneficial when growing under ambient conditions as compared to conditions of drought and increased temperatures.

One component of our understanding of sustainable feedstock production is providing empirical data to support the predictive assessments that switchgrass is a ‘model’ crop for the bioenergy industry throughout the southeastern and south central USA. This study clearly indicates switchgrass can be a productive bioenergy crop under extreme climate conditions, and is able to produce high quality biomass (based on cellulose:lignin ratios) under an array of fertilizer amendments. Importantly, switchgrass biomass quality and quantity were similar in treatments receiving no fertilizer applications, compared to either organic or inorganic fertilizations. Possibly due, at least in part, to their strong symbiotic relationship with mycorrhizal fungi, this study supports that switchgrass can produce high-yielding feedstocks with little or no fertilizer or irrigation.

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Table 3.1. The effects of climate (ambient vs warmer and drier) and fertilizer on switchgrass root biomass, and root:shoot ratio.

	Shoot Biomass (g)	Root biomass (g)	Root:Shoot Ratio
Climate	ns	ns	ns
Fertilizer	ns	*	ns
Climate*Fertilizer	ns	ns	ns
Climate			
Ambient	25.41	29.98	1.18
Warmer drier	25	31.50	1.26
Fertilizer			
Control	25.51	24.23 b	0.95
Nitrogen	20.13	23.95 c	1.19
NPK	29.91	37.99 a	1.27
Poultry litter	25.57	37.59 a	1.47

* indicate level of significance at 0.05 probability and ns = non-significant. Means followed by different letters within each column are significant at alpha = 0.05.

Table 3.2. The effects of climate (ambient vs warmer, drier) and fertilizer on the cellulose, hemicellulose, lignin content (%) and cellulose:lignin of switchgrass.

	Cellulose	Hemicellulose (%)	Lignin	Cellulose:Lignin Ratio
Climate	*	ns	ns	ns
Fertilizer	*	ns	ns	ns
Climate*Fertilizer	*	ns	ns	*
Climate				
Ambient	30.48 b	25.88	5.61	5.54
Warmer, drier	31.67 a	25.81	5.84	5.55
Fertilizer				
Control	32.45 a	25.61	5.64	5.84
Nitrogen	29.96 b	25.86	5.57	5.66
NPK	30.51 b	25.38	5.86	5.23
Poultry litter	31.37 a	25.92	5.82	5.45

* indicate level of significance at 0.05 probability and ns = non-significant. Means followed by different letters within each column are significant at alpha = 0.05.

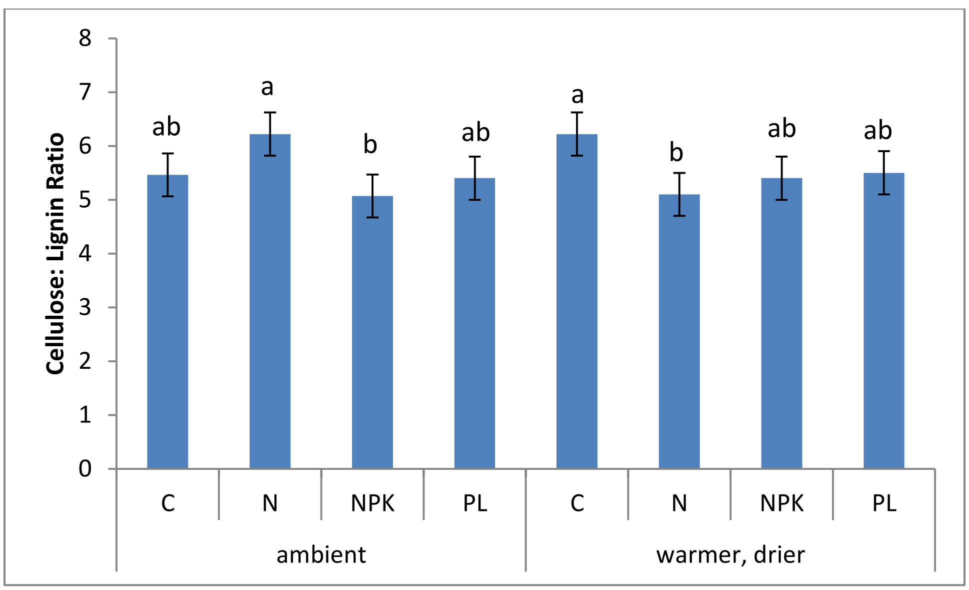

Figure 3.1. The effects of climate (warmer, drier vs ambient) and fertilizer (C=control/non-fertilized, N = nitrogen, NPK = nitrogen, phosphorus and potassium applied, PL = poultry litter) on the cellulose:lignin of switchgrass. Different letters above each column are significant at alpha=0.05.

Table 3.3. The effects of climate (ambient vs warmer, drier) and fertilizer on soil biological properties - microbial biomass using PLFA and expressed as nmol g^{-1} of soil.

	Gram Positive Bacteria	Gram Negative Bacteria	AMF	Saprophytic Fungi	Total Microbial Biomass	Fungi: Bacteria Ratio	Gn B Stress
Climate	ns	ns	*	ns	ns	*	ns
Fertilizer	ns	ns	ns	ns	ns	ns	ns
Climate*Fertilizer	ns	ns	ns	ns	ns	ns	ns
Climate							
Ambient	15	1.17	0.7a	0.75	18	0.09 a	0.28
Warmer, drier	15	0.49	0.4b	0.55	16	0.06 b	0.09
Fertilizer							
Control	15	0.47	0.55	0.57	16	0.07	0.07
Nitrogen	14	0.56	0.39	0.55	16	0.07	0.06
NPK	16	0.76	0.65	0.78	18	0.09	0.08
Poultry litter	15	1.51	0.56	0.68	17	0.08	0.53

* indicate level of significance at 0.05 probability, and ns = non-significant. Means followed by different letters within each column are significant at alpha = 0.05.

Table 3.4. The effects of climate (ambient vs warmer, drier) and fertilizer on soil chemical properties.

	Soil pH	NO_3-N	P mg kg^{-1}	K	SOM (%)
Climate	ns	ns	ns	ns	ns
Fertilizer	ns	*	*	ns	ns
Climate*Fertilizer	ns	ns	ns	ns	ns
Climate					
Ambient	7.9	8	0.57	159	0.53
Warmer, drier	7.9	10	0.65	158	0.52
Fertilizer					
Control	7.9	2 c	0.31b	160	0.56
Nitrogen	7.8	21 a	0.25 b	154	0.49
NPK	7.8	11 a	1.00 a	156	0.53
` Poultry litter	7.9	1 c	0.89 a	162	0.52

* indicate level of significance at 0.05 probability and ns = non-significant. Different letters within each column and variable indicate means are significantly different at alpha = 0.05.

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