MANAGING SWEET SORGHUM FOR OPTIMUM ETHANOL YIELD IN MISSOURI

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By

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The undersigned, appointed by the dean of the Graduate School,

have examined the Thesis entitled

MANAGING SWEET SORGHUM FOR OPTIMUM ETHANOL YIELD IN MISSOURI

Presented by Michael Joseph William Maw

A candidate for the degree of

Masters of Science in Crop, Soil, and Pest Management

And hereby certify that, in their opinion, it is worthy of acceptance.

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Time passes and eventually words fill each page of a thesis until at some point those words come to represent something greater than each word and sentence and paragraph and page can individually display. Eventually all of those parts make the thesis what it is, not a perfect title or data that stands out as revolutionary, but a thesis of some substantive value. This value is represented not only by an academic degree ascribed its author and designer, but as an idea, a suggestion, yea a prompting for future research.

These past three years research efforts have involved two aspects of learning. First, that of learning proper time and effort commitments for the thesis. In Spanish the common word for research is ‘investigacion’. The word suggests that we invest our time and efforts into the research such that through the re-doing of each step in the procedures we refine and hone our understanding of the natural world. Second, learning has come through repetition. It is interesting that in English our version of investigation becomes ‘to do something again’, or the ‘re’ antecedent coupled with the search. Researchers repeat, retry, re-grow, and reexamine the guiding principles and the thesis and its components. Do the data support the hypothesis such that through repeated research we might find some truth or glimpse of truth?

My parents, Jeanne Tyson Moore and Bryan Maw, have been huge supporters and have given the upbringing to pursue goals and desire good and true achievements.

My extended Christian brothers and sisters at Karis Community Church daily encourage me and provide a community of Believers to call me back to Truth such that I may approach each research day through that Biblical worldview and attempt to live out the Gospel.

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Thanks, God!!! May the words herein glorify your Name above all names!

Soli Deo Gloria

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LIST OF ABREVIATIONS

Item, in order of appearance	Abbreviation
Sweet Sorghum	SS
Grain Sorghum	GS
Dry Matter	DM
Stem Juice Yield	SJY
Fermentable Sugar Yield	FSY
Juice-derived Ethanol Yield	JEY
Lignocellulosic Ethanol Yield	LEY
Total Ethanol Yield	TEY
Nitrogen-Use Efficiency	NUE
Nitrogen Recovery Efficiency	NRE
Soil Organic Carbon	SOC
Particulate, Adsorbed, and Occluded Carbon	PAO-C

ABSTRACT

Sweet sorghum has the potential in Missouri for production as a biofuel feedstock, but little is known of the crop's yields and appropriate nitrogen management for optimizing ethanol yields. This thesis is a collection of three field studies examining the potential for sweet sorghum (*Sorghum bicolor* (L.) Moench) to be adopted as a biofuel feedstock for ethanol production in the Midwestern U.S. Limited research exists examining the optimum nitrogen fertilizer rate for maximum ethanol yields as well as sweet sorghum's adaptability to the lower Midwestern states. The first study included testing the effects of five N fertilizer rates (0, 56, 112, 168, 224 kg-N ha^{-1}) on the production of two sweet sorghum cultivars (Dale and Top 76-6) over three years in central Missouri. Yields measured included dry matter, stem juice, Brix, fermentable sugar, theoretical juice ethanol, theoretical lignocellulosic ethanol, and total theoretical ethanol. N fertilizer treatment mostly increased yields, as total dry matter yield averaged 16.8 Mg ha^{-1}, fermentable sugar yield averaged 1055 kg ha^{-1}, and total ethanol yield averaged 5828 L ha^{-1}. The optimal range for N fertilizer rates was between 112 and 168 kg N ha^{-1}. The second study included determining the above-ground plant N concentration, plant N content, N recovery efficiency, and physiological N-use efficiency of sorghum from the first study. Nitrogen treatment significantly affected plant N concentration and N content. Greater yields resulted in greater N recovery efficiency but did not always result in greater N-use efficiency. The optimum range for highest nitrogen recovery and use efficiencies was identified as 0-112 kg N ha^{-1}. The purpose of the third study was to better understand sweet sorghum's affect on soil organic carbon. This involved comparing the effects of an alternative sweet sorghum –soybean (*Glycine max* L.) rotation to a maize (*Zea mays* L.) –soybean rotation at three study sites in Missouri and Arkansas on yields, soil organic carbon, the labile soil carbon fraction and the physically-stabilized fraction. Sweet sorghum ethanol yields were greater than maize yields across sites, but the soil carbon similarly decreased regardless of crop and location.

Sweet sorghum is a high-yielding biomass feedstock that shows promise for production in Missouri, especially in marginal lands. With proper nitrogen fertilizer management sweet sorghum is shown to be an efficient plant for ethanol yield, but it may negatively affect soil organic carbon following land-use changes for biofuel production.

CHAPTER 1: INTRODUCTION AND BACKGROUND

Demand for alternative and sustainable fuels to supplement traditional fossil fuel has spurred the production of biofuel crops in the U.S., especially lignocellulosic feedstocks. Each year 15 billion gallons of renewable fuels are produced in the country, most of which is maize-derived ethanol (Salon, 2010). Before 1980 the U.S. produced almost no ethanol, but has become the world's second largest country in ethanol production behind Brazil (Gnansounou and Dauriat, 2005).

Maize ethanol is reaching its production limits, as approximately 20% of the national maize (*Zea mayz*) grain supply is used to fill the ethanol demand (Reinbott et al., 2009). As maize is a crop used for human food and livestock feed, other cellulosic feedstocks hold more promise for to supplying increasing ethanol demands. Most ethanol is sold as a 10:90 blend with gasoline at the gasoline stations. Gasoline provides 30% more energy than ethanol, although added ethanol allows the fuel to more cleanly combust and reduces greenhouse gas emissions. The blend wall for ethanol-gasoline mixtures has limited further ethanol production in the U.S., even as Brazil runs up to 25% ethanol in fuel mixes. Yet, continued pressure on emissions and fuel efficiency, as well as peak oil concerns, push for further developments from non-maize ethanol sources.

To achieve energy independence goals set by the U.S. government, biomass cropping systems will need to produce high yields with limited resources to become input-efficient. In order to accomplish this, low-input production practices need to be defined for the different systems that have been proposed. Nitrogen fertilization of biomass crops is often considered the greatest single expense incurred by producers, accounting for 15-35% of production costs (Amosson et al., 2011; Linton et al., 2011). As well, N-fertilizer production is based on fossil fuels and is a contributor to greenhouse gases, which may prevent biofuel feedstock production transitioning from a net fuel sink to net fuel source because of the high energy inputs required (Putnam et al., 1991).

Lignocellulosic ethanol, derived from plant material, could provide up to 60 billion gallons from 1.3 billion tons of leftover biomass, including maize stover and timber trimmings –ultimately replacing 30% of the U.S. transportation fuel (NASS, 2007). Lignocellulosic ethanol crops may be more protected from fluctuating food production and prices than dual-use crops (Gnansounou and Dauriat, 2005). Many lignocellulosic ethanol crops are being researched to fill the potential fuel demand, including sorghum (*Sorghum bicolor* (L.) Moench), switchgrass (*Panicum virgatum* L.), and miscanthus (*Miscanthus × giganteus*). The U.S. has the potential to produce around 1.3 billion dry matter tons yr^{-1} (Perlack et al., 2005) and sorghum may hold the best potential for filling this demand (Rooney et al., 2007).

Sweet Sorghum

Sorghum is a common annual C-4 grass crop grown for grain and forage throughout the South and Midwest U.S. because of its adaptability to production systems (Propheter et al., 2010a). Sweet sorghums and high biomass sorghums are two types that have gained renewed interest in biofuel systems for their large biomass yields and high stem sugar content. Traditionally, these sorghums have been mainly used for syrup and forage production (Almodares et al., 2007). Sweet sorghum was developed primarily for syrup production from the higher sugar content in the stalks. The first sweet sorghum cultivars were brought to the U.S. in the 1850s (Brandes, 1943). By 1930, sweet sorghum was grown in over 40 states (Cowgill, 1930). More cultivars were imported later and in 1941 a concerted effort was made to improve sugar yields from the cultivars. In the 1940s, with the demand for alcohol fuels, sweet sorghum ethanol was made in Louisiana for the war effort. Sweet sorghum is an efficient source of ethanol as its C4 photosynthesis pathways produce sucrose for energy storage, which is easily convertible to ethanol (Ali et al., 2007). Since then, sweet sorghum research has turned to developing the plant for emphasis in biomass, since it out-produces maize on average by almost 3 tons/ha dry matter (Hallam et al., 2001). Compared to maize, sorghum overall produces less grain, but more total biomass, resulting in potentially higher ethanol yields with cheaper costs (Hallam et al., 2001).

Sweet sorghum has several characteristics that make it more suitable for wide-spread production than other biofuel crops. Water-stressed growing environments improve sorghum's yield advantage as it is more drought-tolerant than other biomass crops and can remain dormant during these drought times until times of precipitation (Propheter et al., 2010a; Smith et al., 1987; Rooney et al., 2007). It scavenges soil nutrients well and has good N-use efficiency, which allows for reduced N fertilizer application (Gardner et al., 1994). Sweet sorghum may also be flood tolerant (Houx et al., 2013) and therefore, could be grown on marginal ground that has traditionally not been deemed suitable for row-crop production. Additionally, sorghum grows well across many U.S. latitudes and geographic locations (Smith, et al., 1987).

Sorghum is only the second grass species that has been genetically mapped and with only less than one percent increase in grain yields specifically in grain sorghum in the last 50 years, potential room exists for continued breeding to improve nutrient-use efficiency and crop yields (Schupska, 2009). The diverse germplasm of sorghum possesses great potential for improved performance through breeding and genomic investigation (Salon, 2010). New research should focus on increasing biomass and juice ethanol yield, increasing drought-tolerance, and optimization of sorghum plant composition to enhance ease of ethanol conversion (McCutchen, 2006). As of April 2011, no appreciable amount of sorghum biomass was sold or traded in the U.S. for bioenergy use (USDA Market News, April 18, 2011). Combining all the various sorghum types grown, it is estimated that around 7 million ha yr^{-1} of sorghum is produced in the U.S., although most is the grain type (Rooney, et al. 2007).

Several pathways exist for use of sweet sorghum in biofuel production (Tamang et al., 2011). The whole plant can be harvested for ethanol production. Biomass can be directly combusted (Cowgill, 1930) or lignocellulosic ethanol can be processed from the bagasse. Use of lignocellulosic ethanol could reduce greenhouse gas emissions by at least 60% because using whole crop material increases efficient energy use (Salon, 2010). According to Johnston et al. (2009), sorghum ranks second behind maize among 10 tested crops in ethanol conversion efficiency with 402 liters/ton. Unlike maize, sweet sorghum accumulates lignocellulosic biomass despite water stress because it produces less grain (Unger and Wiese, 1979). Sweet sorghum is a two-use crop for biofuel production, as the juice is squeezed from the stalks and then directly fermented into ethanol. The bagasse, or stems and leaves remaining after squeezing, can then be processed for cellulosic ethanol.

The U.S. Department of Energy's Biomass Program has funded cellulosic bio-refineries across the country, including several that are being built to process sorghum biomass on a commercial scale (U.S. DOE, 2010). One such ethanol refinery is ICM in Missouri that will handle 10 tons of dry matter per day. Sweet and high biomass sorghums have the potential to reach 20-40 Mg ha^{-1} with maximum production (Turhollow et al., 2010).

Sweet sorghum produced under high fertilization has the potential to yield 20-40 Mg ha^{-1} (Propheter et al., 2010a; Turhollow et al., 2010), but yields have been lower in Missouri because of soil conditions (Holou and Stevens et al., 2011). Putnam et al. (1991) showed that sweet sorghum in Minnesota generally produced higher amounts of DM and ethanol than maize alone. For example, the highest producing SS variety, Dale, respectively yielded 20 Mg and 3870 L/ha, while maize yielded 15 Mg dry matter and 2580 L/ha. Sweet sorghum M81-E has been shown to yield a higher green weight or total DM than the taller forage sorghum (Propheter et al., 2010a). According to Hallam et al. (2001), sweet sorghum yielded between 15.3-22.9 t dry matter ha^{-1} over five years in Iowa. Several researchers have demonstrated that delayed planting can significantly reduce sweet sorghum DM yields (Broadhead, 1969); Hipp et al., 1970; Houx and Fritschi, in press).

Nitrogen-Use and Management

Biofuel feedstock production faces an uphill battle for acceptance because the current production pathways are not as yet efficient or sustainable to match a growing global demand for lower-input ethanol systems. This limits the investment by farmers, the ethanol industry, and policy makers. Research continues to focus on designing alternative biofuel feedstock cropping systems that are both sustainable and profitable, more so than current maize-based systems (Dweikat et al., 2012).

Nitrogen is often the most limiting nutrient in row crop systems and may account for 15-35% of the production costs depending on application rate (Linton et al., 2011; Amosson et al., 2011). Nitrogen is a highly mobile nutrient and N fertilizer volatilizes, whereby 30-50% of applied-N may be lost from the rooting zone (Stevenson, 1985). Losses and low-use efficiencies of N fertilizer produced from fossil fuels by the Haber-Bosch process prevent biofuel cropping systems from increased sustainability and reduced crop production costs.

Two paths to increased sustainability are improving N uptake and increasing N-use efficiency, thereby reducing N loss by volatilization and leaching, thus reducing needed N inputs. This may be achieved by tightly suiting N fertilizer applications to crop requirements or enhancing the genetic ability for uptake and use (Dweikat et al., 2012; Fixen, 2007). Two efficiency markers become important in revealing sustainable N use. The first is N-recovery efficiency, which estimates the percentage of applied N that a plant accumulates (Nash and Johnson, 1967; Tamang et al., 2011; Wiedenfeld 1984). The second is physiological N-use efficiency, which indicates the yield per unit of plant N accumulation (Maranville et al., 1980; de Vries et al., 2010). A cropping system's N-use efficiency is directly related to increasing uptake efficiency from N fertilizer (Cassman et al., 2002). Optimizing crop uptake of applied N and the transfer of the plant N content into yield will help to match N fertilizer application to a crop and its environment and reduce the upfront N-fertilizer inputs as well as reduce back-end N-removal in dry matter.

Costs for producing one ton of sorghum biomass are lower than for perennial grasses, maize, and alfalfa (*Medicago sativa*) (Hallam, et al. 2001), and the energy required to convert sorghum juice to ethanol is lower than converting maize grain, because the maize grain must be preconditioned prior to digestion (Putnam et al., 1991). Sorghum is known for requiring less nitrogen than maize. The energy output/fossil energy input ratio is greater for sorghum than for several other biofuel crops, including maize (Almodares and Hadi, 2009). Wortmann et al. (2010) determined that SS when compared to maize and grain sorghum is 23% more energy-use efficient as the ratio of energy produced in ethanol per total energy inputs, including fuel and N fertilizer, and is greater than the other grain crops, following a seven site-year study in Nebraska. A study by Hallam et al. (2001) showed consistently higher biomass yields in SS at 140 kg N ha^{-1} than other perennial grasses and maize.

Nitrogen fertilizer rate studies for SS have been conducted in various countries around the world, including Iran (Almodares et al., 2007), Turkey (Ture et al., 1997), India (Kumar et al., 2011), Egypt (El-Latief, 2011), and Thailand (Pholsen and Sornsungneon, 2004). In the U.S., the optimum N fertilization rates for SS varies with soil test levels, soil pH, yield potential, precipitation, soil organic matter, and crop rotation (Stevens and Holou, 2011). Barbanti et al. (2006) recommends tightly-suiting fertilizer rates to crop needs in specific growth conditions. Sweet sorghum may fit into marginal cropland where drought hardiness and low-N requirements fit into a low-input, sustainable system, with the potential for consistent yields and profit as the biofuel-feedstock industry develops (Parish et al., 1985; Putnam, 1991; Propheter et al., 2010a; Stevens and Holou, 2011).

Sweet sorghum N fertilization has received much attention. Nitrogen fertilization of 44 kg ha^{-1} produced earlier maturity by up to two weeks (Cowgill, 1930). Tamang et al. (2011) reported that in northern Texas the optimum N requirements for ethanol production from SS were moderate, between 59-101 kg N ha^{-1}. At high levels of N (>200 kg N ha^{-1}), no difference was observed in yields although little difference was noted at low rates (<100N) as well (Almodares et al., 2007; Wortmann et al., 2010). Higher N fertilizer rates do decrease stem sugar quality as well as theoretical ethanol yield according to Wiedenfeld (1984), so that study recommended 112 kg N ha^{-1} for best management of yield and sugar quality. An additional way to supplement N fertilizer may be through growing SS after soybeans to reduce N need, reducing N response (Yamoah et al., 1998).

Only a handful of studies exist for the Midwest U.S. reporting different N treatment effects on SS (Holou and Stevens, 2011; Tamang, et al. 2011), especially Missouri. Putnam (1991) in Minnesota reported DM yields of 20-25 Mg ha^{-1} and total fermentable carbohydrate yields of 6700 kg ha^{-1} at a high N rate (179 kg N ha^{-1}), and in

Iowa, SS consistently produced high DM at 140 kg N ha^{-1} (Hallam et al., 2001). Holou and Stevens (2011), in Missouri, observed that SS roots develop more in loam soils than in clay or sand, allowing for faster nutrient and water uptake equaling higher yields. They recommend a minimum of 67 kg-N ha^{-1} for optimum juice, sugar, and biomass yield. Nitrogen rates as low as 56 kg ha^{-1} have been applied in Missouri to achieve nominal yields, suggesting that SS can be grown in low-input cropping systems (Houx et al., 2013). The differing responses documented in these studies suggest strong environmental influences on SS responses to N fertilization, and illustrate the need for optimization of N applications for local environments.

Producing SS specifically for lignocellulosic ethanol conversion of bagasse prompts two important considerations of N use. First, negative long-term soil implications of removing nutrients in the dry matter could lead to the depletion of soil nutrients, followed by decreased crop productivity, requiring increased fertilizer inputs to maintain production (Fixen 2007; Rooney et al., 2007; Helsel and Wedin, 1983). Holou and Stevens (unpublished) estimated that about 180 kg ha^{-1} N fertilizer must be applied each year to replace the amount removed when whole plants are harvested. Second, the bagasse N-content is important for the quality of ethanol conversion, whereby N fertilization may increase sucrose content and dry matter (Almodares et al., 2007; Stefaniak et al., 2012), but high bagasse N-content lowers energy conversion values (Helsel and Wedin, 1983).

To calculate the efficient use of applied fertilizer to added yields, the Nitrogen Fertilizer Use Efficiency by the difference method may be calculated. Applied N-use efficiency (NUE) by the difference method was presented by Guillard et al. (1995):

$$NUE = \frac{Yield\ at\ Nx - Yield\ at\ No}{N\ fertilizer\ at\ Nx}$$

where Nx stands for a particular N fertilizer level and N_0 stands for zero N fertilizer application.

Plant-available N does not only come through seasonal fertilizer application, but as well through the indigenous N pool in the soil (Cassman et al., 2002). Although the total N available for plant uptake in inorganic form supplied either by present N or added N fertilizer is only a small part of the total soil N pool. This equals approximately 2-6 percent of the 4000 kg N ha^{-1} present in the upper rooting zone common in the US corn belt (Cassman et al., 2002). Unfortunately, the slow mineralization rate of soil N pools necessitates timed applications of N fertilizer, but the management and environmental costs of excessive N-use limits any biofuel crop's sustainable edge for competitive development.

Single N fertilizer applications at the start of the growing season decrease potential crop uptake efficiency in proportion to N fertilizer rates; fertilizer is lost via denitrification, leaching, and volatilization before the plant can fully accumulate all available N (Cassman et al., 2002). This prompts a need to measure plant N concentrations and contents. Erickson et al. (2012) reported N concentrations ranging from 2.35-6.19 g N kg^{-1} for sweet sorghum grown in Florida after applying N in the range of 45-180 kg-N ha^{-1}. Wiedenfeld (1984) in Texas reported N removal of 48-140 kg ha^{-1}

across cultivars after applying and N treatments ranging from 0-224 kg N ha^{-1}, while a later study, Tamang et al. (2011), reported N removal means of 100 and 75 kg ha^{-1} in the two-year study under N rates 0-168 kg N ha^{-1} in Texas, as well. Other studies report much greater N contents. For instance, Propheter and Staggenborg (2010) reported N removal/accumulation of 172 kg ha^{-1} in 2007 and 160 kg ha^{-1} in 2008 when SS was fertilized with 180 kg N ha^{-1}. Han et al. (2011) documented N accumulations upwards of 339 kg N ha^{-1} when fertilizing SS with 120 kg of urea-N in three split applications. In general, significant increases in N accumulation with increasing amounts of fertilizer N are reported (Barbanti et al., 2006; Erickson et al., 2012; Wiedenfeld, 1984), with differences among cultivars observed in some instances (e.g. Wiedenfeld, 1984; Tamang et al., 2011). Nitrogen fertilization rate differences were observed by Wiedenfeld (1984) and Erickson et al. (2012), where N uptake amounts were 48-140 kg-N ha^{-1} and 80-166 kg-N ha^{-1}, respectively across all N rates. In contrast, Barbanti et al. (2006) in Italy and Holou and Stevens (unpublished), a Missouri study, reported no influence of N treatment on N removal.

Nitrogen recovery efficiency, (Nash and Johnson et al., 1967) is a relative measure for the efficient uptake of applied N fertilizer by SS. Nitrogen recovery efficiency (NRE) was calculated as:

$$\%NRE = \frac{(Plant\ N\ at\ Nx - Plant\ N\ at\ 0N)\ \times 100}{Nx}$$

where the N_x is the fertilizer application rate, 0N is the control plot with zero added-N, and Plant N is the above-ground plant N content in Kg ha^{-1}.

Nitrogen recovery efficiency (NRE) is the ratio of plant-N content over the applied-N, to indicate the efficiency of a plant to accumulate N fertilizer. In SS NRE ranges from 30-80% at low N rates and decreases at higher N rates (Stevens and Holou, 2011; Zegada-Lizarazu and Monti, 2012). Across N rates ranging from 0-168 kg-N ha^{-1}, SS NRE was found to be 22% in one Texas study (Tamang et al., 2011). This may be attributed to because sorghum's robust root system which effectively and timely exploits available soil N (Cassman et al., 2002). Wiedenfeld (1984) reported NRE of 9 to 37%, and presented that N rate can be a factor in N recovery, as increasing N rates decreased NRE. No N treatment response was reported by Tamang et al. (2011), although similar NREs were found, ranging from 14-34%.

Little is documented on SS N-use efficiencies across N rates. Geng et al. (1989) and Guillard et al. (1985) calculate N fertilizer-use efficiency as the ratio of yield over N fertilizer to estimate the optimum N application rate for maximum yield. Wiedenfeld (1984) observed that while theoretical ethanol yield increased with added plant-N content up to N application levels of 224 kg-N ha^{-1}, smaller increases in added yield per unit of N take-up resulted in a lower efficiency at the 224 kg-N ha^{-1} compared to 112 kg ha^{-1}. Few studies document NRE and NUE across several N rates applied to SS.

Nitrogen use efficiency (NUE), derived from Zweifel et al. (1987), is a ratio of yield over plant N content to identify effects of nitrogen recovery within each N treatment on yield. This is a different estimation of efficiency than the NUE by difference method previously presented herein. NUE was calculated as:

$$NUE = \frac{(Yield\ at\ Nx)}{Plant\ N\ at\ Nx}$$

where N_x is the fertilizer application rate and Plant N is the above-ground plant N content in Kg ha^{-1}.

In maize, genotypic differences in N-use efficiency at low N rates follow inconsistencies in use of accumulated N, but differences at high N rates correspond to variations in uptake efficiencies (Moll et al., 1982). Grain sorghum was found by Gardner et al. (1994) to have greater N-use efficiency at 0 kg N ha^{-1} rate compared to 40 kg N ha^{-1}, although biomass yields were 67-81% less. Zweifel et al. (1987) found NUE was 30% higher at zero-N added in grain sorghum. Little research has been conducted examining SS NUEs. Two studies estimate use efficiency based on N recovery. Wiedenfeld (1984) observed decreasing efficiency, or decreased biomass yield gains, with increased total N uptake. Erickson et al. (2013) showed that higher N concentrations in the above-ground biomass do not cause higher stem sugar contents, resulting in effective physiological NUE decreases.

Sugar and dry matter conversion to ethanol

Analyzing sugars or non-structural carbohydrates in the juice of sweet sorghum is central to understanding the potential for juice conversion to ethanol. Sweet sorghum is known to accumulate up to 25% digestible sugars for ethanol at maturity (Ritter et al., 2008; Propheter et al., 2010a). In this regard, three main sugars are measured: fructose, glucose, sucrose. These are the main sugars involved in the ethanol conversion. Glucose and fructose serve as the predominant reducing sugars while sucrose is the main disaccharide, making it the primary sugar of interest. Krishnaveni et al. (1984) showed that from dough stage in SS to dead ripe total sugar content increases while the reducing sugars, namely fructose and glucose, do not significantly increase, showing that increases in the main target sugar sucrose occur in later stages of a crop life. According to Wu et al. (2009), relative percentages of sugars were approximately 70% (sucrose), 20% (glucose), and 10% (fructose) in the M-81E sweet sorghum. Although, Prasad et al. (2007) indicated that sugar content and percentages greatly can differ among varieties.

Sweet sorghum can produce 20-30 t/ha of total sugars of which 40-45% are fermentable sugars and starch, more than equivalent to 200 bushels of maize per area (Murray et al., 2009; Wu et al., 2009). With total conversion of the sugars and starch to ethanol, this makes the potential ethanol production over 7000 L ha^{-1}, except normal pressing of stalks through roller presses only removes around 50-60% of total sugars (Cowgill, 1930; Weitzel et al., 1989; Wu et al., 2009). Other ways of removing juice may hold promise for removing high percentages of total sugars, including screw press and emulsification.

Most ethanol in the U.S. is produced from maize grain starch that is converted to glucose prior to fermentation for ethanol. Brazil has led the world for some time in the production of ethanol from sugar cane juice, which uses the sucrose for fermentation, eliminating converting starch to sugars. This is the same, simpler path for conversion of sweet sorghum juice to ethanol.

Brix is unit used for the percentage of soluble solids in the juice solution and is widely used to approximate sugar content (Murray et al., 2008). While not precise, using a Brix measurement to calculate fermentable sugar content correlates well to HPLC methods (Winstrom et al, 1984; Guigou et al., 2011). Brix is determined in the field with a handheld refractometer that measures the nonstructural carbohydrates, or sugars, suspended in the juice sample. Brix readings are multiplied by total estimated juice yields to calculate total FSY, while assuming sugars equal 75% of a Brix reading (Putnam et al., 1991; Wortmann et al., 2010

Ritter et al. (2008) showed that in sweet sorghum-grain sorghum crosses later harvests leads to higher sucrose content. Many sugar-related traits are highly correlated, including sucrose content, glucose content, fructose content, total sugar content, sucrose to sugar ratio, and Brix. Similar results were reported by Murray et al. (2008) and Krishnaveni et al. (1984). Many of these traits were positively related to plant height, while sucrose yield was significantly related to DM and Brix. Two early studies in Mississippi revealed that SS Brix readings in squeezed juice ranged from 15 to 18 (Ventre et al., 1937) and 15 to 22 (Ventre et al., 1948). Sweet sorghum cultivars Dale and Top 76-6 gave Brix readings of 16.07 and 12.67, respectively (Ali et al., 2007), perhaps showing that overall dissolved sugar levels have not changed in the last century. Broadhead (1969) reported that earlier plantings of SS are linked to higher Brix values at harvest, while Hipp et al. (1969) presented that a May drilling of sweet sorghum in Texas was linked to higher sugar yields than April or June plantings. In Missouri, Holou and Stevens (2011) measured Brix ranging from 14.2 to 18.9 units across years on a silt loam, as well supporting that Brix readings can be affected by annual differences in climate. Putnam et al. (1991) reported lower Brix readings in

Minnesota for Dale, 12.3-12.4%. The shorter growing season in Minnesota and early killing frost may have limited sugar increases.

Murray et al. (2008) examined latitudinal and juice extraction differences in sugar yields in Rio SS, a more photoperiod-sensitive cultivar, in Texas. They stated that total stem sugar yield by area was dependent on stem sugar concentration and stem juice yield per area, thus increasing sugar concentration would increase the crop's energy density and perhaps lower processing and transportation costs. They postulate that increasing stem sugar concentration as unlikely through breeding, but that focusing on increasing stem juice yield may be a better way to increase overall sugar production. Lingle (1987) found sink, but not source limitations in crops grown in non-limiting environments, concluding that grain is not a preferential carbohydrate sink in SS. This may apply to the comparison of different sorghum cultivars, especially photo-period sensitive to non-sensitive, increased energy density may come mostly by juice yield based on stem fresh weight and height and less as a function of seed production. To achieve high fermentation efficiency from sugars to ethanol, Wu et al. (2009) suggests that fermentable sugar contents in juices should not exceed 20% or the inhibition of yeast fermentation occurs.

Harvesting of sorghum must quickly be followed by juice extraction and processing, as sucrose degrades to fructose and glucose over time (Propheter et al., 2010a; Murray et al., 2008). These studies found that inconsistency in juice samples analyzed using HPLC can differ from Brix values if juice is allowed to degrade prior to analysis. To prevent degradation, the best practice is to store juice at near-freezing or below freezing temperatures soon after extraction prior to further laboratory analysis. Sugar loss after 15 days nears 50% at room temperature and only around 2% when stored consistently at or near freezing (Wu et al., 2009). Sucrose content dropped to zero at room temperature after 5 days, while no significant difference occurred in the refrigerated juice. Loss of sugars from un-squeezed stalks after 24 h may be as low as 1.5 percent if fresh stalks are stored before removing juice (Toledo, 2007).

Juice extraction efficiencies may vary based on the type and efficiency of a press (Holou and Stevens, 2011; Tamang et al. (2011). Holou and Stevens (2011) achieved extraction ratios of 63-75%, depending on year and location, ranging from 22,000-61,000 L ha^{-1}. In Texas, Tamang et al. (2011) collected 20,000-24,000 L ha^{-1}. Wortmann et al (2010) reported stem juice yield (SJY) of 17,000-22,000 L ha^{-1} in Nebraska under 0, 45, and 90 kg-N ha^{-1} applied. Nonetheless, the extraction efficiency of a small three roller sugarcane press may be realistic when designing scalable systems for local or on-farm processing.

Fermentable carbohydrate yields, or sugar yield, (FSY) varies in literature. Fermentable sugar yield is the total reducing sugars in the juice. Holou and Stevens (2011) found 2.2-9.9 Mg ha^{-1} FSY across seven N fertilizer application rates, showing increasing sugar with N rate in southeast Missouri. Erickson et al. (2012) reported FSYs of 4,800 kg ha^{-1} for sweet sorghum cv. 'M-81E' when grown in Florida with 180 kg N ha^{-1}. Several studies observed no N response (Erickson et al., 2012; Soileau and Bradford, 1985; Tamang et al., 2011; Wortmann et al., 2010), however, Soileau and Bradford (1985) reported 976-1791 kg ha^{-1} FSY with 0-180kg ha^{-1} N fertilizer in Alabama.

Apart from digesting the juice to test for ethanol yields, a common practice is used to calculate theoretical ethanol yields from juice via a standard conversion factor. Putnam et al. (1991) and Propheter et al. (2010a) assumed 1.76 kg of fermentable carbohydrates converted to 1 liter of ethanol. This assumes an 80% conversion of the sugars to ethanol with losses to incomplete extraction and inefficient fermentation (Smith et al., 1987). Putnam et al. (1991) simply used Brix values to calculate total fermentable carbohydrate yields by multiplying Brix readings by total estimated juice yields, using the 1.76 kg conversion rate. They reported that three public varieties grown in Minnesota, including Keller and Dale, yielded on average more ethanol than maize and other sorghums tested.

For SS produced over two years in Texas, Tamang et al. (2011) reported Juice-derived Ethanol Yields (JEY) ranging from 527-2047 L ha^{-1} across 5 N treatments (0 – 168 kg N ha^{-1}), while, in Nebraska, Wortmann et al. (2010) reported JEYs ranging from 967 to 3530 L ha^{-1} for 3 cultivars across seven site-years under N rates from 0-90 kg ha^{-1}. In both of these studies, JEY did not differ among N treatments. Much greater JEYs of 1597 to 8784 L ha^{-1} were previously reported by Smith et al. (1987) for eight irrigated sites in the continental U.S., and by Tew et al. (2008) for Dale (4390-4980 L ha^{-1}) and Topper 76-6 (4620-5780 L ha^{-1}), depending on harvest date in Louisiana grown using 112 kg N ha^{-1}.

For converting DM to expected ethanol yields, McAloon et al. (2000) reported conversion rates range from 280-370 L ethanol/ Mg, depending on the conversion process and DM composition. Propheter et al. (2010a) assumed 311 L ethanol/ Mg DM.

Sweet sorghum calculated ethanol yields are generally greater than other annual and perennial crops, including photoperiod-sensitive sorghums. Propheter et al. (2010a) reported that SS yielded the highest level of ethanol over a four year study in Kansas, while forage sorghums, photoperiod-sensitive sorghums, rotated maize, and continuous maize produced similar yields.

Few studies have estimated lignocellulosic ethanol yields (LEY) from SS bagasse, for example while Holou and Stevens (2011), Miller and Ottman, (2010) and Wortmann et al. (2010) did not estimate LEYs. However, Tew et al. (2008) used a conversion of 2.65 kg L^{-1} and reported LEY estimates of 5810 and 5000 L ha^{-1} for Dale and Top 76-6 SS, respectively, at 112 kg ha^{-1} N. In contrast, Tamang et al. (2011) calculated neutral detergent fiber digestibility for the sorghums to estimate LEY and reported estimates of 546 to 1485 L ha^{-1}. Actual LEY will differ with lignin content of a specific cultivar (Propheter et al., 2010) and may be affected by N management and year.

Total ethanol is a composite of the JEY and LEY estimates for a theoretical estimate of total ethanol yield from SS. In Kansas, yields averaged 9920 L ha^{-1} for cv. M-81E (Propheter et al., 2010). These concur with similar estimates by Tew et al. (2008) in Louisiana for Dale and Top 76-6. Propheter et al. (2010) estimated yields of 9656 and 10184 L ha^{-1} in 2007 and 2008, respectively, at 168N or 180N, which are similar to 2010 yields at 168N.

Soil organic carbon changes under sweet sorghum production

Current biofuel feedstock crop research primarily focuses on increasing above-ground biomass yields, while the below-ground effects of these energy cropping systems are largely unknown. Converting lands to sustainable biofuel cropping systems in the central U.S. may help to reduce atmospheric CO_2 by increasing soil organic carbon, SOC, using agronomic soils as potential carbon 'sinks' (Janzen, 2005). Decaying crop residues and roots may significantly contribute to soil organic carbon sequestration over time (Fernandez et al., 2002).

SOC pools have declined around the world with increased agricultural production and land-use change (Janzen 2005). The advent of biofuel feedstocks in the Central U.S. may produce large above-ground yields and concomitantly sequester more SOC through increased photosynthesis and root growth (Janzen, 2005; Meki et al., 2013). Marginal lands are a main focus for the production of biofuel feedstocks, where drought, flooding, soil conditions, or landscape position have prevented the land from being used for the conventional maize -soybean (*Glycine max*) rotation. Often perennial grasslands or pasture occupy marginal cropland and converting to annual biofuel crops, like maize and sorghum, may create a 'carbon debt', where the system becomes a source, and not sink, for atmospheric CO_2 (de Vries et al., 2010). In fact, the loss of sequestered C, enhanced erosion, and reduced water-use efficiency are early-emerging concerns for biofuel crops (Buxton et al., 1999; Dweikat et al., 2012). Tillage and crop changes are known to decrease SOC (Page et al., 2013).

Less SOC is sequestered in a maize-soy rotation than in continuous maize systems (Mirsky et al., 2005). Although this depressed sequestration may only hold true for short-term studies in shallow soil horizons. For example, Varvel (2006) found a steeper decline in SOC stocks in continuous maize compared to maize-soy rotations, especially at deep soil horizons, over 20 years.

Studying the impact of including or substituting alternative annual energy crops into the conventional maize-soy rotation may reveal SOC changes (Dweikat et al., 2012). In the Southern U.S., traditionally sorghum was a popular rotation crop with cotton (Cowgill, 1930) and shifting land-use from cotton to energy sorghum production increased SOC in one year, even at 100% dry matter removal in Texas (Cotton et al., 2013). If biofuel feedstocks are considered for total dry matter removal long-term changes in SOC may occur, stymieing any enlarging carbon sinks (Janzen, 2005; Wortmann et al., 2010; Meki et al., 2013).

Limited research exists identifying the effects of SS or SS-soy rotation systems on SOC pools in the U.S. Midwest. Several studies examine continuous grain sorghum (GS) and GS-soybean rotations and their effects on soil properties. Varvel (2006) reported no significant difference between maize-soybean and GS-soybean rotations in the first 10 years of a 20-year study, before SOC levels in GS entered a sharp decline. Holou and Stevens (2011) recommended a SS-soybean rotation for decreasing production costs by reducing N crop needs. Wright and Hons (2005b) found after 20 years soil under continuous GS had 15.3 Mg-C ha^{-1} in the top 150mm under conventional tillage. This concurs with Franzluebbers et al. (1995), who observed an 18% increase in mineralizable C, a more active C pool, in a GS-wheat-soybean rotation relative to a GS monoculture. They attributed this result to increased roots and residue from the intensive rotation system.

Recent studies examining the effects of increased atmospheric CO_2 using free-air CO_2 enrichment on sorghum growth indicate that higher photosynthetic rates resulted with concurrent increases in SOC sequestration in recalcitrant C pools in upper horizons, which indicates slowed SOC decay, less C decay occurs under drier soil conditions with increased CO_2 (Cheng et al., 2007; Prior et al., 2007).

Double cropping as a more intense system can add to SOC levels (Franzluebbers et al., 1995; Schomberg and Jones, 1999; Wright and Hons, 2005a). Schomberg and Jones (1999) reported that cropping management does affect SOC stocks and increased crop yields can negatively affect SOC levels. Another Texas study identified that a sorghum-wheat-soybean rotation increased SOC by the end the 20-year study, relative to a continuous GS (Wright and Hons, 2005a). Although this greater addition of SOC is likely because of the wheat, and not the soybean, as Wright and Hons (2005b) reported that continuous wheat monocultures had 46 and 58% greater SOC stocks than continuous GS or soybean monocultures, respectively, despite wheat producing half the grain yield of the other two crops.

Across depths, often short-term increases in SOC appear in the upper soil horizons, but stocks can be greater at lower depths (Wright and Hons, 2005a; 2005b; Franzluebbers 1995). In Nebraska, maize-soybean and sorghum-soybean rotations had higher SOC stocks deeper in the profile, although throughout all horizons SOC stocks increased the first 8 years before a change in tillage encouraged a steady 10-year decline in the top 300mm (Varvel, 2006). Dou et al. (2008) reported decreases in labile C fractions with depth.

When studies examine smaller, more labile, pools of SOC, short-term changes may be used to estimate similar sensitivity to changing levels in the total SOC. Dou et al. (2008) reported strong positive correlations between SOC and soil microbial biomass C, mineralizable C, particulate organic matter C, dissolved organic C, and hydrolyzable C after 20 years. Yet, SOC appeared to be more sensitive to management changes than any of the labile C pools. These varying SOC fractions have different rates of decay, prompting further examination of these fractions for affecting short-term increases (Shang and Tiessen, 2000).

Few studies have been conducted examining SS cropping effects on SOC and more labile C pools. In Spain, Fernandez et al. (2003) found up to 48% of ^{14}C tracer added to SS in pots translocated to below-ground biomass and 9 percent in soil, of which 88% of that remained after 14 months of crop harvest, adding to increased long-term SOC pools. Smith et al. (1987) found that SS grown in a tall latitudinal range of states from Michigan to Maryland to Mississippi may not significantly differ in many yield characteristics. While SS effects on SOC across latitudes are unknown, SOC and labile-C fractions are known to be lower in the warmer Southern U.S. where C sequestration is lower because of more rapid decomposition rates and increased temperature (Gosling et al., 2013; Wright and Hons, 2005a). Gosling et al. (2013) composited over 150 separate soil organic matter (SOM) studies, finding that moving from temperate to warm climates resulted in nearly a 60% overall decline in total SOM. Future climate change with accompanying warmer temperatures and increased C decay harkens specific land management by soil, latitude, and climate (Janzen, 2005).

Labile, or light or active, fractions of the SOC are more sensitive and may show short-term changes in whole-soil carbon stocks (Janzen et al., 1992; Gosling et al., 2013). Despite Dou et al. (2008) suggesting that SOC is a strong indicator of management changes, Chan et al. (2001) proposed adopting preferential soil procedures to separate labile fractions to gain clearer pictures of the SOC effects between different cropping strategies. A known test that is quick and relatively safe for testing labile-C is potassium permanganate oxidation (Blair et al., 1995; Weil et al., 2003). Developed by Blair et al. (1995), the procedure originally called for reacting a 0.333M $KMnO_4$ solution with soil to oxidize the labile C fraction of SOC. This high concentration of $KMnO_4$ was found to not accurately differentiate the actual labile-C pool (Weil et al., 2003; Tirol-Padre and Ladha, 2004), so the method was later modified by Weil et al. (2003) to use 0.02M $KMnO_4$ for a smaller fraction of the SOC to be reacted for measurement. This correlated well with total SOC and other known labile C portions, including Particulate Organic Matter (POM) and the Walkley-Black method for separating labile C (Mirsky et al., 2005; Chan et al., 2000).

The procedure for determining Labile-C includes reacting 20.0 mL of 0.02M $KMnO_4$ with 2.5 g of soil. Briefly, soil and permanganate solution are added to 50-ml screw-top plastic centrifuge tubes and placed on a mechanical horizontal shaker for 15 min at 200 rpm followed by separation in a table-top centrifuge for 5 min at 3000 rpm. Supernatants are then diluted with deionized water and light absorption of the diluted solution is measured at 550nm in a spectrophotometer to detect the oxidation of the $KMnO_4$.

Labile-C concentration calculations use the following equation presented in Weil et al. (2003):

$$LabileC\ (mg\ kg^{-1}) = [0.02\ mol\ L^{-1} - (a + bz)] \times (9000\ mg\ C\ mol^{-1}) \times \left(\frac{0.02\ L\ solution}{0.0025\ kg\ soil}\right)$$

where 0.02 mol/L is the concentration of the $KMnO_4$ solution before reaction, *a* is the intercept of the standard curve, *b* is the slope of the standard curve, *z* is the absorbance value of the diluted supernatant, 9000 mg C mol^{-1} is the assumed mass of C oxidized by 1 mol of MnO_4 reducing from Mn^{7+} to Mn^{4+}, 0.021 is the volume of KMnO solution reacted, and 0.0025 kg is the mass of soil used.

Since the basic labile-C procedure using $KMnO_4$ oxidation was developed by Blair et al. (1995), and refined and simplified by Weil et al. (2003), the limited labile-C research over the past few decades has focused on tillage, fertilization, and further defining the procedure’s suitable use in cropping systems management (Weil et al., 2003; Tirol-Padre and Ladha, 2004). These studies found that labile-C fractions are decent predictors of SOC changes, although Dou et al. (2008) found that several active fractions were similar in sensitivity to SOC.

A more stable SOC fraction than labile-C, the particulate, adsorbed, and occluded carbon (PAO-C) fraction may be quickly separated through the method recently developed by Veum et al. (2011). This rapid slacking test isolates the physically-stabilized SOC fraction containing free light OM, intra-aggregate particulate OM, and mineral-associated OM (Veum et al., 2013). PAO-C was found to be an indicator of early SOC changes in agroforestry and vegetative buffer strips and contains higher concentrations of organic C than SOC (Veum et al., 2011; 2013). This newer fractionation method has not been used to predict early SOC changes in biofuel cropping systems.

For the PAO-C method, from each sample 100g of soil is slaked in DI water over a 23 µm sieve, then wet-sieved four times at 30 sec per time to separate sand and clay portions. The soil residue on the sieve is then back-washed, oven-dried, weighed, and ground. This is the PAO fraction. Combustion on ignition determines the SOC in the PAO-C fraction.

The portion of SOC in the PAO-C fraction of the soil, on a sand-free basis, is calculated according to Veum et al. (2011):

$$PAOC\ (mg\ kg^{-1}) = \frac{Occluded\ SOC}{PAO\ residue} \times \frac{PAO\ residue}{Whole\ soil\ (-sand)}$$

where Occluded SOC in mg kg^{-1} is the C measured in the PAO residue after combustion, PAO residue (kg) is the remaining material after the wet slaking procedure, and Whole soil (kg) is the soil mass with the sand content removed.

Many labile-C studies examine multiple factors such as tillage, crop, and fertilizer (Cotton et al., 2013; Franzluebbers et al., 1995; Page et al., 2013; Varvel et al., 2006). Mirsky et al. (2005) suggested more experiments are needed that isolate individual management treatments, such as crop rotation, to better understand labile-C changes with management.

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CHAPTER 2: NITROGEN YIELD RESPONSE IN SWEET SORGHUM PRODUCED FOR BIOFUEL FEEDSTOCKS IN MISSOURI

Abstract

Increasing demand for high-yielding biofuel feedstocks elicits the need for the alternative ethanol industry to fully understand sweet sorghum (*Sorghum bicolor* (L.) Moench) yield response to varying N fertilization rates in the Midwest U.S. has arisen. The objectives of this study were to determine the optimum N fertilization levels for the production of two sweet sorghum cultivars (Dale and Top 76-6) over three years in central Missouri. The effects of 5 rates of N fertilizer (0, 56, 112, 168, 224 kg-N ha^{-1}) were tested on dry matter yields, stem juice yields, Brix, fermentable sugar yield, theoretical juice ethanol yield, theoretical lignocellulosic ethanol yield, and total theoretical ethanol yield. N rate was found to be significant across most years and yield parameter. Total dry matter yields averaged 16.8 Mg ha^{-1}, juice yields averaged 9113 L ha^{-1}, and fermentable sugar yields averaged 1055 kg ha^{-1} across years and cultivars. Brix generally did not differ with N treatment and the cultivars performed similarly for most yield parameters. Total ethanol yields averaged 5828 L ha^{-1} and were highest between 112 and 168 kg-N ha^{-1}, indicating that producing sweet sorghum in Missouri may reach optimum yields within that fertilization range. Annual precipitation and temperature differences under dryland conditions greatly influenced dry matter, stem juice, and sugar yields, thereby affecting theoretical ethanol yields, such that years with decreased rainfall and lower temperatures coincided with decreased yields. Sweet sorghum is

adapted to Missouri climate and produces optimum yields at low to moderate levels of N fertilizer.

Introduction

To achieve energy independence goals set by the U.S. government, biomass cropping systems will need to be input-efficient. In order to accomplish this, low-input production practices need to be defined for the different systems that have been proposed. Nitrogen fertilization of biomass crops is often considered the greatest single expense incurred by producers, accounting for 15-35% of production costs (Amosson et al., 2011; Linton et al., 2011). Nitrogen fertilizer production is based on fossil fuels and is a contributor to greenhouse gases, which may prevent biofuel feedstock production transitioning from a fuel sink to fuel source (Putnam et al., 1991).

Sweet sorghum (*Sorghum bicolor* (L.) Moench) is a C4 annual grass crop that is known for high water-use efficiency and high N-use efficiency (Gardner et al., 1994). Sweet sorghum is known to be drought tolerant (Propheter et al., 2010a) and may also be flood tolerant (Houx et al., 2013) and therefore, could be grown on marginal ground that is not suitable for maize or soybean production. Sweet sorghum with high fertilization has the potential to yield 20-40 Mg DM ha^{-1} (Propheter et al., 2010a; Turhollow et al., 2010), but lower yields have been observed in Missouri (Holou and Stevens et al., 2011). Nitrogen rates as low as 56 kg ha^{-1} have been applied in Missouri to achieve nominal yields, suggesting that it can be grown in low-input cropping systems (Houx et al., 2013).

Sweet sorghum N fertilization requirements have received attention from numerous researchers. For instance, Wiedenfeld (1984) observed that stem sugar quality and theoretical juice ethanol yield of SS grown in Texas was maximized at 112 kg N ha^{-1} and that these traits decreased at higher N rates. In a later Texas study, Tamang et al. (2011) found optimum N fertilizer application for ethanol production to be between 59-101 kg N ha^{-1}. Similarly, for SS grown in southeast Missouri, Holou and Stevens (2011) found optimum juice, sugar, and dry matter (DM) yields with applications of 67 kg N ha^{-1}. In contrast, Almodares et al (2007) found DM and stem sugar increases at N rates up to 200 kg ha^{-1}. Similarly, Putnam (1991) reported DM yields of 20-25 Mg ha^{-1} and total fermentable carbohydrate yields of 6700 kg ha^{-1} at a high N rate, 179 kg ha^{-1}-N in Minnesota. In the Midwest, sweet sorghum response to N fertilization is equally varied. In Iowa, sweet sorghum consistently produced high DM at 140 kg ha^{-1} N (Hallam et al., 2001), while, in Nebraska no significant effect of N treatments ranging from 0 to 90 kg N ha^{-1} were observed (Wortmann et al., 2010). Clearly, the response to N fertilization differed considerably among these studies conducted in different environments across the US corn belt.

The differing responses documented in these studies suggest strong environmental influences on sweet sorghum responses to N fertilization, and illustrate the need for optimization to local environments. Further, understanding the relationship between varying N-fertilization rates and sorghum yields for ethanol production is an important step towards greater fertilizer-use efficiency, minimizing inputs in biomass cropping systems, and sustainable sweet sorghum production. While there are extensive data on yields for several biofuel crops, including SS, there are few studies in literature on yield response to different N rates in the Midwest for SS. The objectives of this study were to determine the influence of five N fertilization rates on sweet sorghum DM production, stem juice yield (SJY) and brix, fermentable sugar yield (FSY), and theoretical ethanol yield.

Materials and Methods

This study was conducted at the Bradford Research Center (BRC) in central Missouri (38’ 53’’ N; 92’ 12’’ W) on a Mexico Silt Loam (fine, smectitic, mesic, Vertic Epiaqualf) soil (USDA-NRCS, 2012) in 2009-2011. Thirty year (1983 to 2012) average temperature and annual precipitation at BRC are 12.8 °C and 1145 mm, respectively.

The experiment was arranged as a strip plot design with four replications. Nitrogen treatments (0, 56, 112, 168, and 224 kg ha^{-1} N) were the main plots arranged in randomized complete blocks and SS cultivars were subplots. In 2010 and 2011, the previous years’ remaining SS crop litter was removed prior to spring planting. Sweet sorghum (SS) cultivars ‘Dale’ and ‘Topper 76-6’ were sown in 0.76 m rows in late May of 2009-2011 following disked tillage.. A spring hailstorm damaged the crop in 2011, prompting a late June replant and shorter growing season. Main plots were 12 rows wide and 15 m long and subplots were six rows wide and 15 m long.

Nitrogen was broadcast applied each year as SuperU urea (Koch Agriservices), a coated, pelletized urea for delayed N release. The 56 and 112 kg N ha^{-1} treatments were applied as single applications at planting and the 168 and 224 kg N ha^{-1} treatments were split-applied with 112 kg ha^{-1} applied at planting and the remaining N applied approximately two weeks post-emergence. Hereafter the N treatments will be referred to as 0N, 56N, 112N, 168N, and 224N.

At physiological maturity or first killing frost (whichever came first), plants from a 2-m long section of row were cut to a stubble height of 0.05m and fresh weights of SS samples were recorded. In 2009, subsamples were squeezed with a small hand-powered roller press, and in 2010 and 2011, stem sample from the 2-m row section were crushed in a three-roller sugarcane press. Stem juice was collected, strained through cheesecloth, and volume and weight were recorded. Juice sugars were determined by Brix immediately after extraction with an r^2mini handheld refractometer (Reichert Technologies, Inc., Buffalo, NY) on duplicate 1.0 mL aliquots taken from the stem juice after thorough mixing. The average of both readings was used for calculations of Brix percentages. Fresh weight of the remaining bagasse was determined, and a bagasse subsample was weighed and dried at 55 °C until weights stabilized to determine bagasse dry matter yield.

While not precise, using Brix to calculate fermentable sugar content has been shown to correlate well to HPLC methods (Winstrom et al, 1984; Guigou et al., 2011). Commonconversion calculations were used to estimate fermentable sugar yield (FSY), theoretical juice ethanol yield (JEY), and theoretical bagasse ethanol yield (LEY). Brix readings were multiplied by total estimated juice yields to calculate total FSY, while assuming sugars equal 75% of Brix (Putnam et al., 1991; Wortmann et al., 2010). Theoretical juice ethanol yield (JEY) was calculated as 1.76 kg sugars L^{-1} stem juice using an 80% conversion efficiency (Putnam et al., 1991; Smith et al., 1987). To calculate total theoretical lignocellulosic ethanol yields (LEY), a conversion of 311 L Mg^{-1} DM was used according to Propheter et al. (2010a).

Applied nitrogen use efficiency (NUE) was determined as per Guillard et al. (1995):

$$NUE = \frac{Yield\ at\ Nx - Yield\ at\ No}{N\ fertilizer\ at\ Nx}$$

where Nx stands for a certain N fertilizer level and N_0 stands for zero N fertilizer applied.

Statistical Analysis

Analysis of variance was performed with PROC MIXED (SAS Institute, 2009) with replication, replication x N rate, replication x cultivar, and replication x cultivar x N rate as random effects. Dependent variables included total DM, SJY, FSY, juice-derived ethanol yield, cellulosic ethanol yield, and total ethanol yield. Treatment means were compared using t-tests provided by the ADJUST=Tukey option in SAS using an alpha value of 0.05. PROC CORR was used to correlate the various yield variables.

Results and Discussion

Weather

Environmental conditions were variable during the study period (Table 4.1). The 2009 growing season was cooler with higher than normal rainfall. In 2010, precipitation and temperature were close to normal for the region. Conditions in 2011 were the driest of the three study years with less than half the precipitation measured in 2009.

Dry Matter Yield

Sweet sorghum DM yield differed among years (P<0.0001) and N rates (P=0.0007), and a significant year x N rate interaction (P=0.0181) was observed. Dry matter yields in 2010 were more than 30 percent greater than those in 2009 and 2011 (Table 4.2). However, no differences in DM yield were observed between Dale and Topper 76-6 (Table 4.2), both cultivars responding similarly to N treatment.

When analyzed by cultivar, DM yields were similar for all N treatments and in both cultivars in 2009 and 2011. However, when averaged across both cultivars, DM yields in 2009 were significantly greater for the 168N (14.4 Mg ha^{-1}) than the 0 N (10.2 Mg ha^{-1}) treatment. In 2010, mean DM yields were 44 and 35 percent greater than in 2009 and 2011, respectively, and yields of the 168N treatment were significantly greater than those of the 0N treatment for both cultivars.

The lack of DM yield response to N treatment in 2009 and 2011 may be attributed to several reasons when compared to crop year 2010. In 2009, cool temperatures during June—August and high rainfall during seedling emergence and early growth possibly depressed sorghum growth and increased N leaching losses. In 2011, a hailstorm damaged emerging seedlings, necessitating replanting in early July. Since fertilizer N had already been applied to the first planting, no N was applied at replant. Thus, some of the applied N was likely lost to volatilization and/or leaching and was unavailable for plant uptake. As well, several researchers have demonstrated that delayed planting can significantly reduce sweet sorghum DM yields (Broadhead, 1969; Hipp et al., 1970; Houx and Fritschi, in press). The limited growing season associated with delayed planting may also have limited the extent to which differences in N availability were able to influence the growth of sweet sorghum.

While the greatest DM yields achieved in the 168N treatment in 2010 (27.8 Mg kg^{-1}) were less than the 32.6 Mg ha^{-1} reported by Propheter et al. (2010) in Kansas at the same 168N rate, the DM yields achieved in this study were comparable to those reported by others. For instance, Erickson et al. (2012) reported an average DM yield of 17.7 Mg ha^{-1} for one study year at two sites in Florida to examine the response of DM to N fertilization rates from 45-180 kg-N ha^{-1}. In that study, they found no yield increases with increasing N applications. The lower DM yields in 2011 were associated with reduced precipitation, and are similar to yields of 13.6 Mg ha^{-1} and 12.6 Mg ha^{-1} reported by Tamang et al. (2011) and Parrish et al. (1985) under similar conditions with similar precipitation. Under similar management conditions in Minnesota, Putnam et al. (1991) measured DM yields similar to the 27.4 Mg ha^{-1} at 112N in 2010 for Dale. With 3.6-11.9 Mg ha^{-1}, the dry matter yields observed by Holou and Stevens (2011) in southeast Missouri were generally below those observed in this study in central Missouri.

Juice Yield

Juice yields ranged from 3,676-17,113 L ha^{-1} across all years and treatments (Table 4.3). When analyzed across all years, significant differences in juice yields were observed among years ($P<0.0001$), N rates ($P=0.002$), and for year x N rate interactions ($P=0.0277$). Juice yields were similar in 2009 and 2010, and almost doubled in 2011, when the greatest juice yields were similar to the lowest yields in 2009 and 2010 (Table 4.3). In 2011, juice yields of Top 76-6 were significantly greater than those of Dale, 16% more across N treatments than Dale. In 2009 and 2010, juice yields followed similar trends and overall means were not different between cultivars. Juice yields of Top 76-6 increased significantly with additional N in 2009 and 2010, but for Dale the N treatment effect on juice yield was only significant in 2010. In 2009 and 2010, the greatest yields were for the 168N treatments, but 168N treatments were not significantly different from the 56N treatment in 2010 and the 112N treatment in 2009 (Top 76-6). The lower juice yields in 2011 may be attributed to below-normal precipitation during early growth and anthesis. Juice yields in 2009 were similar to 2010, even though 2009 DM yields were significantly lower than 2010 DM yields. Again, this may have been a result of greater in-season precipitation in 2009 than 2010. Stem juice yields moderately correlated with DM across N rates and years (R^2=0.429) although the highest correlation appeared at the 112N rate (R^2=0.507). The weakest relationship was in 2011, where climate and a shorter season cut SJY in half, while DM only decreased 35 percent.

Juice extraction efficiencies may vary based on the type and efficiency of a press (Holou and Stevens, 2011; Tamang et al. (2011). Stem juice yields in our study were less than half the juice yields presented from studies where an 80% juice extraction efficiency used by many to estimate juice yields (Clegg et al., 1996; Wortmann et al., 2010) regardless of N treatment or cultivar, indicating that more efficient juice extraction methods would have increased yields. Holou and Stevens (2011) achieved extraction ratios of 63-75%, depending on year and location, with yields much higher than we collected, ranging from 22-61,000 L ha^{-1}. In Texas, Tamang et al. (2011) collected 20-24,000 L ha^{-1}. Nonetheless, the extraction efficiency of the three roller press used in the last two years of this study may be realistic when designing scalable

Table 2.1. Mean monthly precipitation (mm) and temperature (°C) during the growing season of sweet sorghum for study years 2009-2011 near Columbia, MO.

	2009		2010		2011	
Month	Temperature	Precipitation	Temperature	Precipitation	Temperature	Precipitation
May	17.7	128.78	17.5	107.70	16.4	130.05
June	23.2	142.51	24.5	83.82	23.9	77.22
July	22.5	128.06	25.4	203.96	27.6	59.44
August	21.8	102.61	25.1	105.16	24.6	60.71
September	18.5	74.67	19.4	175.77	17.4	45.72
October	9.9	249.16	14.4	10.67	13.8	25.91
Average/ Total	18.9	829.75	21.1	687.07	20.7	405.13

Table 2.2. Total dry matter yields of sweet sorghum by cultivar and N fertilizer rate in study years 2009-2011 near Columbia, MO.

	2009			2010			2011		
	Cultivar			Cultivar			Cultivar		
N Rate	Dale	Top 76-6	Mean	Dale	Top 76-6	Mean	Dale	Top 76-6	Mean
	---------- Mg ha-1 ----------								
0	9.7a	10.8a	10.2b	14.6b	16.1b	15.4b	13.6a	12.9a	13.3a
56	12.4a	11.2a	11.8ab	21.8ab	22.0ab	21.9ab	14.0a	14.9a	14.4a
112	12.6a	14.9a	13.8ab	23.9ab	27.1ab	25.5a	15.0a	16.1a	15.6a
168	12.3a	16.5a	14.4a	27.4a	28.2a	27.8a	16.9a	16.3a	16.6a
224	12.3a	14.2a	13.2ab	23.3ab	23.9ab	23.6a	15.1a	14.6a	14.8a
Mean	11.8A	13.5A	**12.7C**	22.2A	23.4A	**22.8A**	14.9A	14.9A	**14.9B**
N rate	0.108			0.0018			0.1356		
Cultivar	0.0592			0.3929			0.9923		
Cultivar x N	0.4031			0.9644			0.7327		

Means in a column with the same lowercase letter are not significantly different at P = 0.05

Means in a row with the same uppercase letter are not significantly different at P = 0.05

Table 2.3. Stem Juice yield of sweet sorghum by cultivar and N fertilizer rate in study years 2009-2011 near Columbia, MO.

	2009			2010			2011		
	Cultivar			Cultivar			Cultivar		
N Rate	Dale	Top 76-6	Means	Dale	Top 76-6	Means	Dale	Top 76-6	Means
				---------- L ha-1 ----------					
0	6371a	5981b	6176b	6020b	6612b	6316b	3676a	4507a	4091b
56	9817a	6660b	8239b	6694ab	8635ab	7664ab	4260a	5510a	4885ab
112	13389a	11341ab	12365ab	8289ab	14803ab	11546ab	5321a	6500a	5910ab
168	16010a	16507a	16259a	11826a	17113a	14469a	5935a	6934a	6434a
224	13082a	15511a	14297a	9441a	13240ab	11340ab	6408a	6991a	6699a
Means	11734A	11200A	**11467A**	8454A	12080A	**10267A**	5120B	6088A	**5604B**
N rate	0.0806			0.0226			0.003		
Cultivar	0.6996			0.0615			0.0333		
Cultivar x N	0.6569			0.3668			0.9869		

Means in a column with the same lowercase letter are not significantly different at P = 0.05

Means in a row with the same uppercase letter are not significantly different at P = 0.05

systems for local or on-farm processing. In contrast, comparable to 2010 juice yields, Wortmann et al (2010) reported SJY of 17-22,000 L ha^{-1} in Nebraska under 0, 45, and 90 kg-N ha^{-1} applied.

Brix

Brix percentages varied by year and cultivar (P<0.0001), and there was a significant year x cultivar interaction (P=0.016), but no differences by N treatment (P=0.5538). Across years and cultivar, Brix readings ranged from 13.1-17.2 (Table 4.4). Among all the N treatments, there were no differences in Brix across all years indicating that Brix is independent of differing N applications. The strong year x cultivar interaction (P=0.0011) revealed that across all years Top 76-6 had the highest juice sugar percentages over Dale within each year. As well, Top 76-6 was similar in 2009 and 2010, reflecting the SJY similarities. Dale was more variable between 2009 and 2010, but revealed similarities in 2010-2011 which reveals differences in the SS sugar production, not appearing in the DM or JEY. Among years, 2010 had the highest Brix, which was 4 percent greater than 2009 and 11% greater than 2011, all different by year (P<0.0001).

In 2009, while no N treatment effect was observed, Dale (14.3) consistently had lower Brix than Top 76-6 (16.5) across all N fertilizer applications. This was seen as well in 2010 and 2011, indicating that Top 76-6 had higher juice sugar concentrations across any environmental effects. The higher 2010 Brix agrees with the higher DM and SJY in 2010 over other years. Although the higher Brix in 2009 over 2011 indicates that the shorter 2011 growing season may have prevented the stem sugar concentration from reaching the levels recorded in the two previous years.

Brix readings are similar to many other studies (Audilakshmi et al., 2010; Soileau and Bradford, 1985; Holou and Stevens, 2011). In Missouri, Holou and Stevens (2011) measured Brix ranging from 14.2 to 18.9 across years on a silt loam, as well supporting that Brix can be affected by annual differences in climate. Putnam et al. (1991) reported lower Brix readings in Minnesota for Dale, 12.3-12.4%. The shorter growing season in Minnesota and early killing frost may have limited Brix increase, as supported by Ventre et al. (1948), which found that Brix increased with later stages of maturity. Differences measured between Dale and Top 76-6 in our study reinforces previous studies identifying Brix differences across panels of SS varieties, including Tamang et al. (2011) and Almodares and Hadi (2009), which measured juice Brix in 36 released lines and hybrids. For N treatment, Tamang et al. (2011) identified decreases in Brix at 134N in one year and Wiedenfeld et al. (1984) identified decreases in Brix at 224N. These two study results differed from our measurements, which is not unusual; Soileau and Bradford (1985) reported no differences from 0-180 kg ha^{-1} N fertilizer.

Fermentable Sugar Yield

Fermentable sugar yields were calculated based on Brix determinations from the extracted juice. Overall analyses of FSY revealed significant year ($P<0.0001$), N treatment ($P<0.0001$), year x N treatment ($P=0.0376$) and year x cultivar effects ($P=0.0164$). Across years and N rates, FSY ranged from 393-2060 kg ha^{-1} (Table 4.5), with the greatest FSYs measured in 2009 and 2010. Fermentable sugar yields were similar in 2009 and 2010, and were more than twice those of 2011, a response which, as supported by a strong correlation (R^2=0.9864; across N rates and years), was consistent with juice yield trends.

In all three years, N treatment significantly influenced FSY of Topper 76-6 (Table 2.5). However, for Dale, FSY differences between N treatments were only significant in 2009. In 2009, FSY increased with increasing N rate from 910 kg ha^{-1} at 0N to 2007 kg ha^{-1} at 224N for Topper 76-6 and from 716 kg ha^{-1} to 1753 kg ha^{-1} for Dale. The 168N treatment with the greatest FSYs was only significantly different from the FSYs of the 0N treatments but not the other fertilized treatments, except for Topper 76-6 in 2009 when FSYs in 168N and 224N were greater than the 56N treatment. FSY had weak correlation to DM at high N rates across the three years, but generally showed a strong positive relationship at low N rates (0-112N), even though FSY takes into account stem juice yields and Brix.

FSY in 2009 was similar to that in 2010 despite lower DM yield in 2009 and a different stem juice extraction method, because measured Brix values were high with increased sugar concentrations following above-average precipitation, despite lesser DM yields. Less FSY in 2011 is mostly attributable to lower SJY as stem sugar levels were only marginally lower. Why 2011 FSY did not match the higher 2009 yields despite closer, but still different, DM is likely attributable to above-average rainfall in 2009 that allowed for increased above and below-ground plant growth and N uptake. This is consistent with reports that moderate drought stress, as seen in 2011, can impact FSY at anthesis and encourage earlier stalk sugar accumulation (Massacci et al., 1996; Miller and Ottman, 2010), but does not impact final sugar yields. Tamang et al. (2011) reported

Table 2.4. Brix percentages in sweet sorghum by cultivar and N fertilizer rate in 2009-2011 near Columbia, MO.

	2009			2010			2011		
	Cultivar			Cultivar			Cultivar		
N Rate	Dale	Top 76-6	Means	Dale	Top 76-6	Means	Dale	Top 76-6	Means
	%								
0	13.1a	15.9a	14.5a	14.9a	16.1a	15.5a	14.2a	15.7a	15.0a
56	14.5a	16.9a	15.7a	15.7a	16.4a	16.0a	14.6a	14.6a	14.6a
112	14.5a	16.6a	15.6a	15.6a	17.0a	16.3a	13.5a	14.7a	14.1a
168	14.7a	16.3a	15.5a	15.1a	16.6a	15.8a	13.7a	14.3a	14.0a
224	14.6a	16.8a	15.7a	15.3a	17.2a	16.3a	13.7a	14.2a	14.0a
Means	14.3Acd	16.5Ba	**15.4B**	15.3Bb	16.6Aa	**16.0A**	13.9Bd	14.7Abc	**14.3C**
N rate	0.1563			0.2657			0.0605		
Cultivar	<0.0001			<0.0001			0.0034		
Cultivar x N	0.5837			0.6361			0.3475		

Means in a column with the same lowercase letter are not significantly different at P = 0.05

Means in a row with the same uppercase letter are not significantly different at P = 0.05

Table 2.5. Fermentable sugar yield in sweet sorghum by cultivar and N fertilizer rate in 2009-2011 near Columbia, MO.

	2009			2010			2011		
	Cultivar			Cultivar			Cultivar		
N Rate	Dale	Top 76-6	Means	Dale	Top 76-6	Means	Dale	Top 76-6	Means
	---------- kg ha-1 ----------								
0	716b	910b	813b	674a	799b	736b	393a	415b	404b
56	1006ab	863b	934b	780a	1057ab	919ab	467a	638ab	552ab
112	1417ab	1214ab	1316ab	978a	1880a	1429ab	543a	665ab	604a
168	1716a	1903a	1810a	1325a	2060a	1693a	617a	723a	670a
224	1753a	2007a	1880a	1064a	1713ab	1388ab	607a	730a	668a
Means	1322A	1380A	**1351A**	964B	1502A	**1233A**	523A	634A	**580B**
N rate	0.0002			0.0117			0.0015		
Cultivar	0.768			0.0012			0.2189		
Cultivar x N	0.7174			0.3344			0.7382		

Means in a column with the same lowercase letter are not significantly different at P = 0.05

Means in a row with the same uppercase letter are not significantly different at P = 0.05

mean FSY in northern Texas of 1.6 and 2,2 Mg ha^{-1} in 2008 and 2009, respectively, regardless of N rate, suggesting that their reduced rainfall, may explain reduced FSY in dryer years. These values are similar to those observed in this study for 2009 and 2010 FSY, but not for 2011 when FSYs were significantly lower, likely as a result of the reduced water availability. This is also supported by Holou and Stevens, (2011) who found a significant relationship between FSY and stem moisture content.

Fermentable sugar yields in this study were lower than most reported in the literature because of the previously mentioned 80% juice extraction assumption used by others (Wortmann et al., 2010; Tew et al., 2008). Holou and Stevens (2011) found 2.2-9.9 Mg ha^{-1} FSY across seven N rates in a loam soil, showing increasing sugar with N rate in southeast Missouri. Erickson et al. (2012) reported FSYs of 4,800 kg ha^{-1} for sweet sorghum cv. 'M-81E' when grown in Florida with 180 kg N ha^{-1}, more than double those observed in this study. Several studies observed no N response (Erickson et al., 2012; Soileau and Bradford, 1985; Tamang et al., 2011; Wortmann et al., 2010), which differs from results presented here. However, Soileau and Bradford (1985) reported 976-1791 kg ha^{-1} FSY with 0-180kg ha^{-1} N fertilizer in Alabama, matching well with FSYs reported in this study. In 2010, FSY and Brix values were higher in Top 76-6 than in Dale, which supports that they belong to two difference sorghum genetic groups (Murray et al., 2009). Similar environmental stress in 2009 and 2011 resulted in similar theoretical sugar yields, reflecting data presented by Day et al. (1995).

Ethanol Yield from Sweet Sorghum Juice

Analysis of ethanol yield from juice (JEY) revealed significant differences among years ($P<0.0001$), N rate ($P<0.0001$), year x N rate interactions ($P=0.003$). In 2009 and 2010 JEYs were similar while 2011 yields were half those in the previous years (Table 2.6). In 2010, Top 76-6 average JEY was 36 percent greater than that of Dale, but, no cultivar differences were observed in 2009 and 2011. While JEYs increased with additional N in Top 76-6 in all three years, the N treatment effect was only significant in 2009 for Dale. In 2009, JEYs of the 168N treatment were greater than those of the 0N and 56N treatments in both cultivars. In 2010 and 2011, JEYs of Top 76-6 were greater at 112N than at 0N. Additional N beyond either the 168N treatment (2009) or the 112N treatment (2010, 2011) did not increase estimated JEY.

Theoretical SS JEYs reported in the literature span a broad range within studies and across studies. Similar theoretical ethanol yields were reported in studies. For example, Tamang et al. (2011) reported JEYs ranging from 527-2047 L ha^{-1} across 5 N treatments (0 – 168 kg N ha^{-1}) for sweet sorghum produced over two years in Texas, while, in Nebraska, Wortmann et al. (2010) reported JEYs ranging from 967 to 3530 L ha^{-1} for 3 cultivars across seven site-years under N rates from 0-90 kg ha^{-1}. In both of these studies, JEY did not differ among N treatments. Juice ethanol yields documented for SS grown in Arizona were two to three times greater (2310-3192 L ha^{-1}) than those observed in our study (Miller and Ottman, 2010). Further, imposition of drought stress did not negatively affect JEYs in their study, contrasting with the 2011 results from our study. Much greater JEYs of 1597 to 8784 L ha^{-1} were previously reported by Smith et al. (1987) for eight irrigated sites in the continental U.S., and by Tew et al. (2008) for Dale

Table 2.6. Juice ethanol yields from sweet sorghum by cultivar and N fertilizer rate in 2009-2011 near Columbia, MO.

	2009			2010			2011		
	Cultivar			Cultivar			Cultivar		
N Rate	Dale	Top 76-6	Means	Dale	Top 76-6	Means	Dale	Top 76-6	Means
	---------- L ha-1 ----------								
0	457b	468b	457b	383a	454b	418b	228a	250b	239c
56	524b	512b	525b	443a	601ab	522ab	365a	303ab	284bc
112	703ab	641ab	703ab	556a	1068a	812ab	313a	416a	364ab
168	983a	1096a	983a	753a	1170a	962a	355a	412a	384a
224	994a	1077a	995a	604a	973ab	789ab	350a	433a	391a
Means	732A	759A	**733A**	548B	853A	**701A**	363A	302A	**332B**
N rate	0.0021			0.0117			0.0002		
Cultivar	0.2157			0.0012			0.108		
Cultivar x N	0.6565			0.3344			0.6809		

Means in a column with the same lowercase letter are not significantly different at P = 0.05

Means in a row with the same uppercase letter are not significantly different at P = 0.05

(4390-4980 L ha^{-1}) and Topper 76-6 (4620-5780 L ha^{-1}), depending on harvest date in Louisiana grown under 112N. These differences likely exist because higher stem juice extraction was calculated in these specific studies.

Lignocellulosic Ethanol Yield

Lignocellulosic ethanol yield (LEY) was estimated based on bagasse DM yields and ranged from 2623 to 8774 L ha^{-1} across the three years, N treatments and cultivars. Lignocellulosic ethanol yields varied by year ($P<0.0001$) and a year x N treatment ($P=0.0549$) interaction was observed, but no differences in mean LEY were found between cultivars in any of the three years. Theoretical LEYs in 2010 were greater than those in 2011, which were greater than those in 2009 (Table 2.7). Nitrogen treatments did not affect LEYs in 2009 or 2011, but, in 2010, LEYs for Dale were greater at 168N than at 0N and for Topper 76-6 were greater at 112N than 0N.

Few studies have estimated lignocellulosic ethanol yields from SS bagasse. Holou and Stevens (2011), Miller and Ottman, (2010) and Wortmann et al. (2010) did not estimate LEYs, while Propheter et al. (2010) applied a conversion put forth by McAloon et al. (2000) for maize biomass to SS, which we adopted for this study. Tew et al. (2008) used a conversion of 2.65 kg L^{-1} and reported LEY estimates of 5810 and 5000 L ha^{-1} for Dale and Top 76-6, respectively, at 112 kg ha^{-1} N. These LEY estimates are within the range observed in our study. In contrast, Tamang et al. (2011) calculated neutral detergent fiber digestibility for the sorghums to estimate LEY and reported estimates of 546 to 1485 L ha^{-1}, 3-4 times lower than those found here. Actual LEYs will differ with lignin content of specific cultivar (Propheter et al., 2010) and may thus also be affected by N management and year.

Total Ethanol Yield

Lignocellulosic ethanol estimates made up the largest portion of potential ethanol yields for the three years. Ethanol yields ranged from 3486-10183 L ha^{-1} for the three years. Total ethanol yields were significant according to year ($P<0.0001$), but not by N rate or year x N rate interaction. Estimates in 2009 and 2011 are similar while 2010 yields were highest (Table 2.8). In 2009 and 2011 no N treatment effects appeared, dominated by the lack of N rate effects to DM and LEY. As well, no cultivar differences arose in any study year. The second study year, 2010, was the only year with N rate effects observed, as total theoretical ethanol yields increased at or above 112N.

SS total ethanol yields reflected those from Kansas, where yields averaged 9920 L ha^{-1} for cv. M-81E at similar N rates (Propheter et al., 2010). These results concur with similar estimates by Tew et al. (2008) in Louisiana for Dale and Top 76-6. Propheter et al. (2010) estimated yields of 9656 and 10184 L ha^{-1} in 2007 and 2008, respectively, at 168N or 180N, which are similar to 2010 yields at 168N. While Tamang et al. (2011) yields were much less than any we estimated (1301-3127 L ha^{-1}). Estimates for 2009 and 2011 are below any reported in literature, indicating a strong environmental impact on SS yields, where cooler spring temperatures and reduced rainfall correspond with decreased yields.

Table 2.7. Lignocellulosic ethanol yields of sweet sorghum by cultivar and N fertilizer rate in 2009-2011 near Columbia, MO.

	2009			2010			2011		
	Cultivar			Cultivar			Cultivar		
N Rate	Dale	Top 76-6	Means	Dale	Top 76-6	Means	Dale	Top 76-6	Means
					L ha-1				
0	3074a	2491a	2827a	4635b	5018b	4777b	4094a	4041a	4068a
56	3861a	3070a	3401a	6772ab	6835ab	6803ab	4358a	4621a	4490a
112	3873a	3891a	3860a	7429ab	8423a	7926a	4677a	4987a	4832a
168	3817a	5211a	4504a	8525a	8774a	8650a	5257a	4930a	5094a
224	3818a	4402a	4110a	7257ab	7417ab	7337a	4687a	4544a	4615a
Means	3646A	3835A	**3740B**	6904A	7293A	**7099A**	4615A	4625A	**4620C**
N rate	0.1635			0.0039			0.1327		
Cultivar	0.6057			0.3875			0.9632		
Cultivar x N	0.4492			0.9617			0.6833		

Means in a column with the same lowercase letter are not significantly different at P = 0.05

Means in a row with the same uppercase letter are not significantly different at P = 0.05

Table 2.8. Total ethanol yields of sweet sorghum by cultivar and N fertilizer rate in 2009-2011 near Columbia, MO.

	2009			2010			2011		
	Cultivar			Cultivar			Cultivar		
N Rate	Dale	Top 76-6	Means	Dale	Top 76-6	Means	Dale	Top 76-6	Means
	---------- L ha-1 ----------								
0	3438a	3842a	3733a	4918b	5472b	5195b	4316a	4392a	4354a
56	4406a	4013a	4165a	7215ab	7436ab	7326ab	4623a	4964a	4793a
112	4698a	4941a	4782a	7985ab	9490a	8738a	5013a	5412a	5213a
168	4896a	6272a	5549a	9278a	10183a	9731a	5570a	5456a	5513a
224	4758a	5542a	5150a	7862ab	8391ab	8126a	5073a	5044a	5058a
Means	4420A	4931A	**4676B**	7452A	8194A	**7823A**	4919A	5054A	**4986B**
N rate	0.2222			0.0035			0.1247		
Cultivar	0.2714			0.1645			0.5991		
Cultivar x N	0.7586			0.9375			0.8774		

Means in a column with the same lowercase letter are not significantly different at P = 0.05

Means in a row with the same uppercase letter are not significantly different at P = 0.05

Fertilizer Nitrogen-Use Efficiency

Using the NUE equation presented by Guillard et al. (1995), the fertilizer NUE was calculated for each cultivar and N treatment. Overall, the greatest NUEs were achieved in the 56N and 112N treatments, and JEYs, LEYs, or TEYs of the 168N and 224N treatments did not significantly differ from the 112N treatment for either cultivar in any of the three years. Therefore, from an agronomic perspective, optimal N fertilization under the conditions of this study was in the range of 56-112 kg N ha^{-1}. These results match well with other studies where N-fertilizer applications between 59-112 kg ha^{-1} N in were suggested based on research conducted in Texas (Tamang et al., 2011; Wiedenfield 1984), and 67 kg-N ha^{-1} based on experiments conducted in southeastern Missouri (Holou and Stevens, 2011).

Conclusions

Sweet sorghum responses to N-fertilizer applications were limited in all three years. Moderate amounts of N fertilizer of 56 or 112 kg ha^{-1} generally resulted in ethanol yields that were not different from treatments that received 168 or 224 kg-N ha^{-1}. No significant ethanol yield increases or yield reductions were observed as a result of high N applications (168N and 224N). Thus, optimal N fertilization in this region is likely between 56 and 112 kg N ha^{-1}. Distinct precipitation amounts and distribution as well as temperatures resulted in different yields among years. Juice and FSYs of the SS did not correlate well with DM yields in 2009, indicating that reduced DM associated with cooler growing season temperatures and adequate precipitation did not strongly affect juice and sugar yields. Conversely, average temperatures and drought conditions prevailed in 2011 and impacted DM as well as juice and sugar yields, lowering ethanol yields. Theoretical ethanol yields from bagasse were on average 89% greater than from stem juice, and JEY contributed nine percent to the total estimated ethanol yield which averaged 6259 L ha^{-1} for the 112N treatment across both cultivars and the three years. Given the relatively modest requirements for N and relative maintenance of total ethanol yields in a drought-afflicted year, results from this study are consistent with the often suggested suitability of SS for marginal lands.

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CHAPTER 3: NITROGEN FERTILIZER RECOVERY AND USE-EFFICIENCIES IN SWEET SORGHUM GROWN FOR BIOFUEL IN MISSOURI

Abstract

Well-developed nitrogen management is essential to improving the sustainability of biofuel feedstocks for ethanol production. This comes in part by optimizing the applied-N recovery of the crop and translating that into improved ethanol yields. This three-year study (2009-2011) in central Missouri, U.S., included the production of two sweet sorghum (*Sorghum bicolor* (L.) Moench) cultivars (Dale and Top 76-6) under five nitrogen treatment rates (0, 56, 112, 168, 224 kg ha^{-1}) to examine the above-ground plant nitrogen concentration, plant nitrogen content, nitrogen recovery efficiency, and physiological nitrogen-use efficiency. Plant nitrogen concentrations differed by nitrogen treatment in two of the three years and were highest with lower dry matter yields. Total nitrogen removed in the dry matter differed by nitrogen treatment and cultivar among years and was weakly related to plant nitrogen concentrations. Greater ethanol yields resulted in improved nitrogen recovery efficiency but did not always result in greater nitroegen-use efficiency. The optimum nitrogen range for the highest recovery and use efficiencies was identified as 0-112 kg N ha^{-1}.

Introduction

Biofuel feedstock production faces an uphill battle for acceptance because the current production pathways are not as yet efficient or sustainable to match a growing global demand for lower-input ethanol systems. This limits the investment by farmers, the ethanol industry, and policy makers. Research continues to focus on designing alternative biofuel feedstock cropping systems that are both sustainable and profitable, more so than current maize-based systems (Dweikat et al., 2012).

Nitrogen is often the most limiting nutrient in row crop systems and may account for 15-35% of the production costs depending on application rate (Linton et al., 2011; Amosson et al., 2011). Nitrogen is highly mobile and can easily volatilize whereby 30-50% of applied-N may be lost from the rooting zone (Stevenson 1985). Losses and low-use efficiencies of N fertilizer produced from fossil fuels by the Haber-Bosch process prevent biofuel cropping systems from increased sustainability and reduced crop production costs.

Two keys to increased sustainability are improving N uptake and increasing N-use efficiency through breeding and selection, thereby reducing N loss through volatilization and leaching, hence reducing needed N inputs into the crop production system. This may be achieved by matching N fertilizer applications to crop requirements or enhancing the genetic ability for uptake and use (Dweikat et al., 2012; Fixen 2007). Two efficiency markers become important in revealing sustainable N use. The first marker is nitrogen recovery efficiency, which estimates the percentage of applied N that a plant accumulates (Nash and Johnson, 1967; Wiedenfeld 1984; Tamang et al., 2011). The second is physiological N-use efficiency, which estimates the ethanol yield benefits of added plant N accumulation (Maranville et al., 1980; de Vries et al., 2010). A cropping system's N-use efficiency is directly related to increasing uptake efficiency from N fertilizer (Cassman et al., 2002). Optimizing crop uptake of applied N and the transfer of plant N content into ethanol yield will help to better suit N fertilizer application to a crop and its environment and reduce the upfront N-fertilizer inputs as well as reduce back-end N-removal in dry matter.

Sweet sorghum (*Sorghum bicolor* (L.) Moench) is a promising C-4 annual plant traditionally grown for syrup or forage. It is growing in popularity for producingsugary stem juice and the remaining bagasse after juice extraction that can be converted to ethanol (Cowgill 1930; Putnam et al., 1991; Tamang et al., 2011). Most of the U.S.-produced ethanol is maize-based, supported by a fully-developed maize production system (Putnam et al., 1991; Smith et al., 1987; Dweikat et al., 2012). Similar to maize, SS can potentially be produced at any latitude throughout the contiguous U.S. and may fit into marginal cropland where drought hardiness and low-N requirements fit into a low-input, sustainable system, with the potential for consistent yields and profit as the biofuel-feedstock industry develops (Parish et al., 1985; Putnam, 1991; Propheter et al., 2010a; Stevens and Holou, 2011). Wortmann et al. (2010) determined from a seven site-year study in Nebraska that SS, when compared to maize and grain sorghum, is 23% more energy-use efficient as the ratio of energy produced in ethanol per total energy inputs including fuel and N fertilizer is greater than the other grain crops.

Producing SS specifically for lignocellulosic ethanol conversion of bagasse prompts two important considerations of N use. First, negative long-term soil implications of removing nutrients with the dry matter could lead to the depletion of soil nutrients, followed by decreased crop productivity, requiring increased fertilizer inputs to maintain production (Fixen 2007; Rooney et al., 2007; Helsel and Wedin, 1983). Holou and Stevens (unpublsihed) estimated that about 180 kg ha^{-1} N fertilizer must be applied each year to replace the amount removed when whole plants are harvested. Second, the bagasse N-content is important to the quality of ethanol conversion, since N fertilization increases sucrose content and dry matter (Almodares et al., 2007; Stefaniak et al., 2012), but high bagasse N-content lowers energy conversion values (Helsel and Wedin, 1983).

Single N fertilizer applications at the start of the growing season decreases potential crop uptake efficiency in proportion to N fertilizer rates, because fertilizer is lost to denitrification, leaching, and volatilization before the plant can fully take up all available N (Cassman et al., 2002). Nitrogen uptake efficiency, or N-recovery, in SS ranges from 30-80% at low N rates and decreases at higher N rates (Stevens and Holou, 2011; Zegada-Lizarazu and Monti, 2012). Across N rates, ranging from 0-168 kg-N ha^{-1}, SS NRE was found to be 22% in one Texas study (Tamang et al., 2011).

Plant N content in SS is lower than in maize (Wortmann et al., 2010), but may remove 48-140 kg N ha^{-1}, increasing with N fertilizer rate (Wiedenfeld 1984; Tamang et al., 2011). In a recent Missouri study SS removed 179-355 kg-N ha^{-1}, and N treatment had no affect (Holou and Stevens, unpublished). Little is documented on SS N-use efficiencies across N rates. Geng et al. (1989) and Guillard et al. (1985) calculate N fertilizer-use efficiency as the ratio of yield over N fertilizer to estimate the optimum N application rate for maximum yield. Calculating plant physiological NUE as a ratio of yield over plant-N content may be used to delineate the yield benefits with changes in N uptake (Zweifel et al., 1987). Wiedenfeld (1984) observed that while theoretical ethanol yield increased with added plant N content up to 224 kg-N ha^{-1}, less added yield per unit of N take-up resulted in a lower efficiency at the 224 kg-N ha^{-1} compared to 112 kg ha^{-1}.

Few published studies document NRE and NUE across several N rates in SS. The objectives of this study were to: 1) measure N concentration and whole-plant total N removal across N treatments, and 2) determine N recovery efficiency and N-use efficiency associated with each N treatment. Therefore, we hypothesize that increasing applications of N fertilizer will result in increased N removal and decreased efficiencies of N uptake and N-use.

Materials and Methods

This three-year study was conducted in 2009-2011 at the Bradford Research Center (BRC) in central Missouri (38’ 53” N; 92’ 12” W) on a Mexico Silt Loam (fine, smectitic, mesic, Vertic Epiaqualfs) (USDA-NRCS, 2012). Three-year means for temperature and precipitation was 12.8 °C and 1145 mm, respectively and were recorded from a weather station and provided through the Missouri Agricultural Weather Database.

This experiment was designed to highlight the effects of 5 N rates on two sweet sorghum cultivars (cv. Dale and Top 76-6, Mississippi State University) and wasarranged as a strip plot design with four replications. The five N rates (0, 56, 112, 168, and 224 kg-N ha^{-1}) were the main plots of a randomized complete block design with sweet sorghum cultivar (cv. Dale and cv. Top 76-6, Mississippi State University) as subplots. Each main plot of N treatment measured 15 m x 15 m and each cultivar subplots measured 4.56 m x 15 m.

Each spring prior to planting of the sweet sorghum cultivars, plots were tilled by disking. In the second and third year of the study, the previous year's crop stubble was removed from the soil surface to a clean seed bed. Sweet sorghum cultivars were planted into 0.76 m row-spacing in early-June of each year. For the N fertilizer treatments each year, pelletized urea-N was broadcast applied as SuperU urea (Koch Agriservices), a delayed-release formula for extended N availability. All the N treatment rates were hand-spread at planting as single applications, except for the 168 and 224 kg-N ha^{-1}, which were split-applied with 112 kg-N ha^{-1} at planting and the remaining N fertilizer applied two weeks after emergence.

For plant harvest, a 2.0m row of sweet sorghum plants were cut from each plot at 0.05m above the soil surface at physiological maturity or after the first killing frost, whichever came first. Samples were bundled and the fresh bundle weight was immediately recorded. The bundles were then crushed through a three-roller sugarcane press to extract the stem juice, which was collected, strained through cheesecloth, and weighed to calculate stem juice yield. Then from the remaining bagasse, or sorghum stems after juice extraction, a subsample was taken and dried in a forced-air dryer at 60°C until dry. Final dry weights and stem juice yield were used to extrapolate for total dry matter yields, Brix, fermentable sugar yield, theoretical juice-derived ethanol yields, and lignocellulosic ethanol yields. Methods, calculations, and yield results are presented in the previous chapter.

Dry SS samples were ground through a 2-mm screen using a Wiley Mill (company information) and then ground through a cyclone mill to pass a 1-mm screen. Subsamples of evenly-mixed ground materials were then analyzed using a LECO Auto Analyzer to determine N concentration. Total Nitrogen (TN) removal rates for each plot were calculated as the product of DM yield and N concentration. Nutrient concentrations were not calculated for juice samples as previous studies have shown minimal N content compared to total N removal rates (Propheter and Staggenborg, 2010; Tamang et al., 2011).

Nitrogen recovery efficiency (NRE), from Nash and Johnson et al. (1967), is a relative measure for the efficient uptake of applied N fertilizer by the sorghums. Nitrogen recovery efficiency (NRE) was calculated as:

$$\%NRE = \frac{(Plant\ N\ at\ Nx - Plant\ N\ at\ 0N)\ \times 100}{Nx}$$

where the N_x is the fertilizer application rate, 0N is the control plot with zero added-N, and Plant N is the above-ground plant N content in Kg ha^{-1}.

Nitrogen use efficiency (NUE), derived from Zweifel et al. (1987), is a ratio of yield over plant N content to identify effects of N recovery within each N treatment on yield. NUE was calculated as:

$$\text{NUE} = \text{Yield at } N_x \,/\, \text{Plant N at } N_x$$

where N_x is the fertilizer application rate and Plant N is the above-ground plant N content in Kg ha^{-1}.

Statistical Analysis

Analysis of variance was performed using the PROC MIXED procedure through SAS (SAS Institute, 2009) software with replication, replication x N rate, replication x cultivar, and replication x cultivar x N rate as random. Dependent variables included nitrogen concentration, total nitrogen content, nitrogen recovery efficiency, and nitrogen use efficiency were analyzed within and among year. Treatment means were compared using t-tests provided by the ADJUST=Tukey option in SAS at alpha value of 0.05.

Results and Discussion

Nitrogen Concentration

Nitrogen concentrations in SS DM differed across the three years ($P<0.0001$) and there was a N treatment affect ($P=0.0006$), as averages for 2009 (4.20 g kg^{-1}) and 2010 (3.95 g kg^{-1}) were similar to each other, while 2011 concentrations (5.01 g kg^{-1}) were greater (Table 3.1). Nitrogen concentrations in the two cultivars did not differ within any of the three years. In 2009, N concentrations ranged from 3.69-4.76 g $kg^{-1}$for the two cultivars, but differences in N concentrations were not significant among N treatments. However, in 2010 and 2011, N concentrations increased with increasing amounts of N application in 2010 for Top 76-6 and in 2011 for both Dale and Top 76-6. The strongest responses to N application were observed in 2011 when concentrations in the 224N treatment reached more than 6.0 g kg^{-1} while they were less than 4.0 g kg^{-1} in the 0N treatment.

Higher N concentrations in 2011 are difficult to explain, because crop dry matter yields are lower in 2011 than 2010. The decreased dry matter yields in 2011 are attributable to a shorter season with the late replant so that the SS accumulated the available N in the rhizosphere but could not translate that into yields prior to the killing frost, thereby concentrating the plant N. These results are largely consistent with those of other researchers (Barbanti et al., 2006; Beyaert and Roy, 2005; Erickson et al., 2012; Propheter and Staggenborg, 2010). Erickson et al. (2012) reported N concentrations ranging from 2.35-6.19 g N kg^{-1} for SS grown in Florida and fertilized with N in the range of 45-180 kg-N ha^{-1}. The linear increase in N concentrations with increasing amounts of N fertilizer is similar, albeit more pronounced, to the responses observed in this study for 2010 and 2011, but not in 2009 when N concentration was not influenced by N treatment. The more pronounced N response presented by Erickson et al. (2012) may be attributable to a higher rate of N concentration in N treatments with the second split application of fertilizer farther into the crop's growth (two weeks). Propheter and Staggenborg (2010) in Kansas had N concentrations matching or exceeding our study's concentrations under N rates of 168-180 kg-N ha^{-1} resulting in 13.7 and 17.0 g kg^{-1} across two years.

Nitrogen Uptake

Total N uptake was calculated for aboveground tissue based on total dry matter (DM) yield (not shown) and the N concentration of that tissue and ranged from 28.3 to 110.5 kg ha^{-1} across all years and both cultivars. The N content was significantly different by year ($P<0.0001$), N treatment ($P=0.0002$), year x N interaction ($P=0.0307$), cultivar ($P=0.0372$), and displayed a year x cultivar interaction ($P=0.0419$). The average N content in the aboveground DM for the three years was 48.8, 83.9, and 75.5 kg N

Table 3.1. Nitrogen concentrations in sweet sorghum for three years 2009-2011 near Columbia, MO.

	2009			**2010**			**2011**		
	Cultivar			**Cultivar**			**Cultivar**		
N Rate	**Dale**	**Top 76-6**	**Means**	**Dale**	**Top 76-6**	**Means**	**Dale**	**Top 76-6**	**Means**
	----- g kg^{-1} -----								
0	3.78a	3.83a	3.80a	3.08a	3.53b	3.30b	3.85b	3.93b	3.89c
56	4.35a	4.20a	4.27a	3.29a	3.43b	3.36ab	4.30b	4.23b	4.28bc
112	4.61a	4.37a	4.49a	4.13a	4.50ab	4.31ab	5.37ab	5.31ab	5.34ab
168	4.28a	4.15a	4.21a	3.55a	5.15a	4.35ab	5.58a	5.28ab	5.43ab
224	3.69a	4.76a	4.22a	4.49a	4.35ab	4.42a	6.14a	6.07a	6.11a
Means	4.14A	4.26A	**4.20B**	3.71A	4.19A	**3.95B**	5.05A	4.97A	**5.01A**
N rate	0.7278			0.0076			0.0008		
Cultivar	0.5913			0.052			0.5781		
N rate x Cultivar	0.1714			0.1751			0.9325		

Means in a column with the same lowercase letter are not significantly different at P = 0.05

Means in a row with the same uppercase letter are not significantly different at P = 0.05

Table 3.2. Total nitrogen removed in sweet sorghum for three years 2009-2011 near Columbia, MO.

	2009			**2010**			**2011**		
	Cultivar			**Cultivar**			**Cultivar**		
N Rate	**Dale**	**Top 76-6**	**Means**	**Dale**	**Top 76-6**	**Means**	**Dale**	**Top 76-6**	**Means**
	---------- kg ha $^{-1}$ ----------								
0	37.3a	34.0c	35.6b	45.4b	57.3b	51.4b	52.7c	47.8b	50.2c
56	52.1a	28.3bc	40.2ab	63.5ab	74.7ab	69.1ab	60.2bc	63.8ab	62.0bc
112	48.7a	51.8abc	50.2ab	98.2a	104.3a	101.3a	82.2abc	88.7a	85.4ab
168	52.5a	72.0a	62.3a	97.6a	110.5a	104.1a	94.7a	83.9ab	89.3ab
224	43.8a	67.3a	55.5ab	83.8ab	103.2a	93.5a	91.4ab	89.2a	90.3a
Means	46.9A	50.7A	**48.8C**	77.7B	90.0A	**83.9A**	76.2A	74.7A	**75.5B**

	2009	2010	2011
N rate	P = 0.0502	P = 0.0042	P = 0.0014
Cultivar	P = 0.5103	P = 0.0164	P = 0.6856
N rate x Cultivar	P = 0.0486	P = 0.8991	P = 0.4497

Means in a column with the same lowercase letter are not significantly different at P = 0.05

Means in a row with the same uppercase letter are not significantly different at P = 0.05

ha^{-1} in 2009, 2010, and 2011, respectively (Table 3.2). Nitrogen contents in aboveground biomass were greater in 2010, than in 2011, which in turn were greater than in 2009. Nitrogen content increased with increasing N fertilizer application in every year for Topper 76-6 and in 2010 and 2011 for Dale. The N content of the two cultivars was not different in 2009 and 2011, but, on average across treatments, Top 76-6 accumulated 14% more N than Dale in 2010. The reasons underlying the lack of N response by Dale in 2009 are unclear.

In 2010, plant N accumulation was almost 40 % greater than in 2009, largely corresponding to increased DM yields which were 44% greater in 2010 than 2009. N removal means in 2010 ranged from 45.4-110.5 kg ha^{-1}, with no increase in N uptake above 112N, while in 2009 differences occurred at 168N. This indicates that in years with increased precipitation like in 2009, a likely result will be reduced N uptake, which in turn equals reduced yields, perhaps through increased leaching of N from the rhizosphere. Nitrogen content in 2011 is less than 2010 values, despite the highest N concentrations being in 2011, indicating that increased concentrations are balanced by reduced yields so that dry matter may be the determining factor in N uptake and removal (Barbanti et al., 2006). As well, phosphorus and potassium availability may affect plant N uptake.

Sweet sorghum N accumulation in aboveground dry matter ranges widely among studies (Erickson et al., 2012; Propheter and Staggenborg, 2010; Tamang et al., 2011; Wiedenfeld 1984). Many studies report similar or slightly higher N contents compared to our study, including Tamang et al. (2011), Wiedenfeld (1984), and Erickson et al.

(2012). Wiedenfeld (1984) reported N removal of 48-140 kg ha^{-1} across cultivar and N treatments ranging from 0-224N, and Tamang et al. (2011), reported means of 100 and 75 kg ha^{-1} in the two-year study under N rates 0-168N.

Other reports show greater N contents than in ourthis study. For instance, Propheter and Staggenborg (2010) reported N accumulations of 172 kg ha^{-1} in 2007 and 160 kg ha^{-1} in 2008 when fertilized with 180 kg N ha^{-1}, and Han et al. (2011) documented N accumulations upwards of 339 kg N ha^{-1} when fertilizing SS with 120 kg urea-N in three split applications. Holou and Stevens (unpublished) concurred with higher N uptake between 179 and 355 kg ha^{-1} across N fertilization of 0-134 kg N ha^{-1}. In general, significant increases in N accumulation with increasing amounts of fertilizer N are reported (Barbanti et al., 2006; Wiedenfeld, 1984; Erickson et al., 2012), with differences among cultivars observed in some instances (e.g. Wiedenfeld, 1984; Tamang et al., 2011). Nitrogen rate differences were observed by Wiedenfeld (1984) and Erickson et al. (2012), where total N uptake amounts were 48.4-139.9 kg-N ha^{-1} and 80-166 kg-N ha^{-1}, respectively across all N rates. According to Wiedenfeld (1984), SS cv. Rio accumulated increasing N up to the highest N fertilizer rate, 224N, while cv. MN 1500 did not remove more N above 112N, which differs from our results where no differences above 112N were observed across cultivars. In contrast, Barbanti et al. (2006) and Holou and Stevens (unpublished) reported no influence of N treatment.

Variations in N accumulation from year to year are also commonly observed (Barbanti et al., 2006; Holou and Stevens, unpublished; Tamang et al., 2011). In a two-year study that included two SS cultivars, Tamang et al. (2011) observed large differences in N removal between years (78-129 kg N ha^{-1} vs. 45-99 kg N ha^{-1}) and found that the cultivars performed similarly overall.

Relationships of N concentration and N removal with dry matter, sugar, and ethanol yields

Correlation analyses were conducted to examine the relationships of N concentration, N removal, dry matter yield, stem juice yield, fermentable sugar yield, theoretical juice-derived ethanol, theoretical lignocellulosic ethanol, and total ethanol. Nitrogen concentration and N removal were correlated to each other (R^2=0.4678) among years and N treatment.

The strongest correlation observed between the N concentration and N removal occurred in the 0N rate, or the zero- added N fertilizer treatment (R^2=0.5553). Nitrogen concentration did not correlate to any yield parameter (R^2<0.0172), although plant N removal correlated well to DM (R^2=0.7482), LEY (R^2=0.7723), and TEY (R^2=0.7361). This was true within each N treatment, except for at 224N (R^2<0.586). When conducted by year, correlation analyses revealed that in 2009, N removal and N concentration had a weaker relationship than among the three years (R^2=0.3737). N removal in 2009 strongly correlated to all other yield factors (R^2>0.6872), despite having weak correlations across the three years with SJY, FSY, and JEY. Similar correlation values were present in 2010, except that any strong relationship between N removal and the yield factors mostly diminished at 224N (R^2<0.279). Another key difference in 2010 compared to 2009 was that N concentration remained highly correlated to N removal across most N rates (R^2=0.6835). These relationships evidence significant dependence in

N response between all factors, except for N concentration, which never appears to have any strong relationship to the other measured SS factors. N concentration in 2011 strongly correlated to total N removed (R^2=0.8607), while DM has a fairly weak correlation to N concentration, but highly significant relationship to total N. As well, N concentration correlated (R^2=> 0.60) well with SJY, FSY, and JEY, markedly different from the previous two years.

Nitrogen Recovery Efficiency

Nitrogen recovery efficiency (NRE) provides an estimate of the fertilizer N uptake by the SS plants. Nitrogen recovery efficiency differed by year (P<0.0001), but no N treatment of cultivar differences were calculated. No significant differences by N rate occurred within any year, although across years, overall means regardless of N rate were 13%, 37%, and 23% recovery in 2009, 2010, and 2011, respectively (Table 3.3). The lack of N treatment and cultivar differences perhaps indicates that a surplus of N was available for uptake and growth. Despite depressed yields in 2011, SS took-up 13-36% of applied N fertilizer.

The lack of N rate response on NRE was surprising considering the differences that were seen by N rate in many of the measured SS yields, which differs from the results of Wiedenfeld (1984) who reported similar efficiencies of 9 to 37%, but suggested that N rate can be a factor in N recovery, as increasing N rates decreased NRE. No N treatment response was reported by Tamang et al. (2011), and with similar NREs ranging from 14-34%, with an average recovery efficiency of 22%. Much higher NREs of 35-93% were reported by Beyaert and Roy (2005) for cumulative harvests of multi-cut sorghum in Canada, although their harvesting scheme alters the direct comparison with our study. Our results may indicate, as reported by Erickson et al. (2012), that available, residual soil-N supplies large quantities of the plant N content, regardless of applied fertilizer.

Nitrogen Use Efficiency

Nitrogen use efficiencies were calculated for dry matter yield, fermentable sugar yield, and total theoretical ethanol yield. Overall NUE DM ranged from 163.4-355.0 kg kg^{-1} and statistical analyses across all three years revealed a significant year effect with the greatest NUE DM in 2010, followed by 2009, and then 2011 (Table 3.4). No N treatment x cultivar interactions were observed in any of the three years and cultivars were only different in 2010 (Table 3.4). However, NUE DM was significantly influenced by N treatment in two (2010, 2011) out of the three years. The only cultivar difference was found in 2010 when Dale NUE DM was greater across N rates (290.7 kg kg^{-1}) than that of Top 76-6 (247.2 kg kg^{-1}).

In 2010 and 2011 when NUE DM was strongly influenced by N treatment, the greatest NUEs were observed in the 0N and 56N treatments, but few (Dale) or no significant differences (Topper 76-6) differences were found between NUE at 56N and NUEs at 112N, 168N and 224N. This indicates that added N fertilizer may not in turn equal more efficient uptake and efficient use by the plant.

A common method for calculating use efficiency in literature is by fertilizer NUE which is the ratio of yield over applied N fertilizer, but that may assume that all applied N is accumulated by the plant. Geng et al. (1989) reported the fertilizer NUE of SS grown

Table 3.3. Nitrogen Recovery Efficiency of sweet sorghum in 2009-2011 near Columbia, MO.

	2009			**2010**			**2011**		
	Cultivar			**Cultivar**			**Cultivar**		
N Rate	**Dale**	**Top 76-6**	**Means**	**Dale**	**Top 76-6**	**Means**	**Dale**	**Top 76-6**	**Means**
	%								
56	28a	*	*	41a	31a	36a	13a	29a	21a
112	10ab	16a	13a	44a	58a	51a	26a	36a	31a
168	9ab	23a	16a	27a	51a	39a	25a	21a	23a
224	3b	15a	9a	28a	20a	24a	17a	18a	18a
Means	12A	18A*	**13C***	35A	40A	**37A**	20A	26A	**23B**
N rate	0.2448			0.3707			0.7409		
Cultivar	0.1602			0.6177			0.5833		
N rate x Cultivar	0.7538			0.5936			0.4408		

Means in a column with the same lowercase letter are not significantly different at P = 0.05

Means in a row with the same uppercase letter are not significantly different at P = 0.05

* Missing values prevented calculation of NRE

Table 3.4. Nitrogen Use Efficiency for dry matter yield of sweet sorghum in 2009-2011 near Columbia, MO.

	2009			**2010**			**2011**		
	Cultivar			**Cultivar**			**Cultivar**		
N Rate	**Dale**	**Top 76-6**	**Means**	**Dale**	**Top 76-6**	**Means**	**Dale**	**Top 76-6**	**Means**
	---------- kg kg $^{-1}$ ----------								
0	247.2a	208.2a	227.7a	324.7ab	286.2a	305.4A	267.4a	260.3a	263.9a
56	232.1a	234.8a	233.5a	355.0a	296.6a	325.8A	232.4ab	236.7ab	234.6ab
112	225.2a	237.3a	231.2a	250.9b	225.6a	238.2B	190.0b	190.5ab	190.3bc
168	238.3a	237.0a	237.6a	282.1ab	196.4a	239.2B	181.1b	190.0ab	185.6bc
224	290.5a	211.9a	251.2a	240.9b	231.4a	236.1B	165.9b	163.4b	164.6c
Means	246.6A	225.8A	**236.7B**	290.7A	247.2B	**269.0A**	207.4A	208.2A	**207.8C**
N rate	0.9329			0.0002			0.0004		
Cultivar	0.2655			0.0249			0.9061		
N rate x Cultivar	0.1535			0.4140			0.9595		

Means in a column with the same lowercase letter are not significantly different at P = 0.05

Means in a row with the same uppercase letter are not significantly different at P = 0.05

Table 3.5. Nitrogen Use Efficiency for fermentable sugar yield in sweet sorghum in 2009-2011 near Columbia, MO.

	2009			**2010**			**2011**		
	Cultivar			**Cultivar**			**Cultivar**		
N Rate	**Dale**	**Top 76-6**	**Means**	**Dale**	**Top 76-6**	**Means**	**Dale**	**Top 76-6**	**Means**
	---------- kg kg $^{-1}$ ----------								
0	17.6a	22.9a	20.2a	15.5a	13.8a	14.6a	8.30a	11.03a	9.66a
56	21.2a	13.2a	17.2a	11.7a	14.4a	13.0a	7.87a	8.25a	8.06a
112	23.9a	32.5a	28.2a	10.1a	15.9a	13.0a	7.24a	8.90a	8.05a
168	28.6a	26.3a	27.4a	14.1a	18.1a	16.1a	5.76a	8.09a	6.92a
224	37.8a	31.0a	34.4a	11.2a	16.6a	13.9a	7.53a	9.82a	8.67a
Means	25.8A	25.2A	**24.6A**	12.5B	15.8A	**14.1B**	7.34B	9.21A	**8.3C**
N rate	0.0658			0.6877			0.2334		
Cultivar	0.8610			0.0416			0.0041		
N rate x Cultivar	0.4795			0.5308			0.6932		

Means in a column with the same lowercase letter are not significantly different at P = 0.05

Means in a row with the same uppercase letter are not significantly different at P = 0.05

Table 3.6. Nitrogen Use Efficiency for total ethanol from sweet sorghum in 2009-2011 near Columbia, MO.

	2009			**2010**			**2011**		
	Cultivar			**Cultivar**			**Cultivar**		
N Rate	**Dale**	**Top 76-6**	**Means**	**Dale**	**Top 76-6**	**Means**	**Dale**	**Top 76-6**	**Means**
	---------- $L\ kg^{-1}$ ----------								
0	85.2a	75.5a	80.4a	109.7ab	96.9a	103.3ab	87.9a	87.2a	87.5a
56	83.2a	80.4a	81.8a	117.1a	100.4a	108.7a	76.7ab	79.1ab	77.9ab
112	83.6a	92.3a	88.0a	83.8ab	79.2a	81.5b	63.2b	64.3ab	63.7bc
168	90.3a	88.2a	89.3a	95.8ab	73.3a	84.6b	59.6b	63.7ab	61.6bc
224	102.9a	83.5a	93.2a	81.3b	81.4a	81.3b	55.9b	56.4b	56.1c
Means	89.1A	84.0A	**87.0A**	97.5A	86.2A	**92.0A**	68.7A	70.1A	**69.4B**
N rate	0.779			0.0014			0.0004		
Cultivar	0.4372			0.0667			0.5353		
N rate x Cultivar	0.5448			0.5696			0.9731		

Means in a column with the same lowercase letter are not significantly different at P = 0.05

Means in a row with the same uppercase letter are not significantly different at P = 0.05

in California with 102 kg N ha^{-1} was 295 kg kg^{-1}, which matches our physiological NUE based on accumulated N.

Nitrogen-use efficiency for FSY estimates the amount of fermentable sugar that is produced per unit of N content. Fermentable sugar yield NUEs ranged from 5.76-37.8 kg kg^{-1} across years, cultivars and N treatments (Table 3.5). Among the three years, FSY NUE was greatest in 2009, followed by 2010 and 2011, at 24.6, 14.1, and 8.3 kg kg^{-1}. Nitrogen treatment did not influence FSY NUE in any of the three years, but, a significant cultivar effect was observed in 2010 and 2011, when Top 76-6 had greater FSY NUE than Dale. During the following years, steadily decreasing NUE was seen across all N rates, perhaps suggesting that while higher plant N concentrations were observed specifically in 2011, more inefficiency was seen because of a delayed growing season and limited precipitation than other years. This more concentrated N with decreased DM yields in 2011 did not result in concomitant increases in stem sugar contents (Erickson et al., 2012). We are not aware of any other SS studies that report NUE by FSY. Of important note is the fact that there were N treatment effects in DM NUE, but not in the FSY NUE. This observation suggests that while physiological NUE may decrease in DM yields with increasing N fertilizer application, the SS’s ability to produce sugars is unaffected, thereby the overall NUE for the plant is buffered. This perhaps indicates that SS is already an efficient plant in creating sugars, a primary source of SS ethanol.

Nitrogen-use efficiencies for the production of total ethanol, or the composite of JEY and LEY, reveals significant year differences ($P<0.0001$), N treatment effects in two of the three years ($P=0.0118$), and a year x N rate interaction ($P=0.0009$). Among years, 2009 and 2010 NUEs were similar and greater than NUE in 2011, of 87.0, 92.0, and 69.4 L kg^{-1}, respectively (Table 3.6). This reflects NUEs identified for DM and FSY, markedly identifying the drought stress effects of 2011 on ethanol yield. Within years, 2009 had no N treatment significance, likely because increased precipitation diluted any N rate effects seen in the following years. Study years 2010 and 2011 revealed decreases in the volume of ethanol yield with increasing N rates, as 56N and 0N corresponded to the highest NUEs in 2010 and 2011, respectively. No cultivar differences were identified in any year and Dale and Top 76-6 NUEs were similar throughout the N treatments. These trends match with previous studies on grain sorghum (Gardner et al., 1994; Zweifel et al., 1987), that NUE decreases with increasing plant N concentration, although similar calculations do not appear for SS in literature.

Conclusion

In general, greater SS N concentrations were associated with lower DM yields, but greater N removal amounts and thus N removal was associated with high DM in years with optimal growing conditions. Greater overall yields resulted in greater NRE but did not always result in greater NUE. Nitrogen-use efficiency of fermentable sugar yield exhibited a stronger correlation to increased precipitation than N treatment and was considerably reduced in a year with below-normal precipitation and a shorter growing season. Total ethanol yield NUE, on the other hand, was highly responsive to N treatment effects, where the ethanol yield decreased with increasing N fertilizer rate. Overall, the best NREs and NUEs were achieved in the low N treatments (0-112 kg ha^{-1} N fertilizer).

Using a simple N budget for crop management is not recommended for optimum production in the long-term, as matching crop yields to crop N use is perhaps more beneficial. Continued efforts are needed to improve NRE and NUE of SS through plant breeding and crop management which, in turn, will improve sustainability and the competitiveness of SS as a low-input biofuel feedstock.

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CHAPTER 4: SHORT-TERM SOIL CARBON CHANGES IN SWEET SORGHUM-SOY AND MAIZE-SOY ROTATIONS ACROSS CENTRAL U.S. LATITUDES

Abstract

Emerging biofuel feedstock systems require better understandings of their affects on soil organic carbon (SOC) in soils. The first two years of sweet sorghum (*Sorghum bicolor* (L.) Moench) –soybean (*Glycine max* L.) and maize (*Zea mays* L.) –soybean rotations at three locations in Missouri and Arkansas were compared for yields, SOC, a labile SOC fraction by potassium permanganate oxidation (Labile-C), and a physically-stabilized fraction of SOC known as the particulate, adsorbed, and occluded carbon (PAO-C). Ethanol yield differences were identified between the two cropping systems, but no differences between sweet sorghum and maize rotations were found within locations for soil carbon pools. The northernmost study site maintained greater SOC and labile soil carbon fractions, which corresponded to greater crop dry matter yields. Soil carbon pools decreased over the two years, but the SOC losses were similar across the three sites, suggesting that the latitudinal gradient studied did not affect the crop-soil interaction.

Introduction

Current biofuel feedstock research primarily focuses on increasing above-ground biomass yields, while the below-ground effects of these energy cropping systems are largely unknown. Converting lands to sustainable biofuel cropping systems in the

Central U.S. may help to reduce atmospheric CO_2 by increasing soil organic carbon (SOC), using agronomic soils as potential carbon 'sinks' (Janzen 2005). Decaying crop residues and roots from these crops may significantly contribute to soil organic carbon sequestration over time (Fernandez et al., 2002).

Soil organic C pools have declined around the world with increased agricultural tillage and land-use change (Janzen 2005). The advent of biofuel feedstocks in the Central U.S. may produce large above-ground yields and concomitantly sequester more SOC through increased photosynthesis and root growth (Janzen, 2005; Meki et al., 2013). Marginal lands are a main focus for the production of biofuel feedstocks, where drought, flooding, soil conditions, or landscape position have limited production of maize (*Zea mays* L.) and soybean (*Glycine max* L.). Often perennial grasslands or pastures occupy marginal croplands and conversion to annual biofuel crops, like maize and sorghum (*Sorghum bicolor* (L.) Moench), may create a 'carbon debt', where the systems become sources, and not sinks, for atmospheric CO_2 (de Vries et al., 2010). In fact, loss of sequestered C, enhanced erosion, and reduced water-use efficiency are early-emerging concerns for biofuel crops (Buxton et al., 1999; Dweikat et al., 2012), mainly a result of tillage and plant species changes which can cause SOC losses (Page et al., 2013).

Sweet sorghum (SS) is a C4 annual proposed for biofuel feedstock and is an alternative to maize for marginal lands where nutrients and/or water availability may limit maize and soybean yields. Sweet sorghum is known for production of large biomass yields, sugary stems, and drought hardiness (Parrish et al., 1985; Putnam et al., 1991; Massacci et al., 1996). Sweet sorghum stem juice can be converted to ethanol and the SS dry matter (DM), like maize DM, has been proposed as feedstock for lignocellulosic ethanol production (Tamang et al., 2011). Sweet sorghum yields more total fermentable carbohydrates, or sugars, for ethanol conversion than maize (Parish et al., 1985; Propheter et al., 2010; Putnam et al., 1991). Parish et al. (1985) reported SS sugar yields of 5.8 Mg ha^{-1} and maize grain yields of 4.8 Mg ha^{-1} in Virginia, both of which are converted to ethanol. Three-times the biomass yields of maize were collected in SS plots by Propheter et al. (2010), and Putnam et al. (1991) found SS to have higher ethanol yields than maize under drought conditions. Sweet sorghum is adapted to a broad range of environments. For instance, Smith et al. (1987) found that SS grown in a wide range from Michigan to Maryland to Mississippi produces similar yields regardless of latitude.

Limited research on the influence of SS-soy rotations on SOC pools exist. Substituting SS in place of maize may reveal SOC changes different from those observed under long-term maize systems (Mirsky et al., 2008; Dweikat et al., 2012). In the Southern U.S., traditionally sorghum was a popular rotation crop with cotton (Cowgill 1930) and shifting land-use from cotton to energy sorghum production increased SOC in one year, even with 100% dry matter removal in one Texas study (Cotton et al., 2013). If biofuel feedstocks are considered for total dry matter removal, long-term changes in SOC may occur, perhaps stymieing enlargement of soil carbon sinks (Janzen, 2005; Wortmann et al., 2010; Meki et al., 2013).

Labile, also known as light or active, fractions of the SOC are more sensitive and may show short-term changes in whole-soil carbon stocks (Janzen et al., 1992; Gosling et al., 2013). Despite Dou et al. (2008) suggesting that SOC is a strong indicator of management changes, Chan et al. (2001) proposed adopting soil analysis procedures to separate labile fractions to gain clearer pictures of the influence of different cropping strategies on SOC. Many labile-C studies (Cotton et al., 2013; Franzluebbers et al., 1995; Page et al., 2013; Varvel et al., 2006) examine multiple factors such as tillage, crop, irrigation, or fertilizer that confound the elucidation of crop management effects; Mirsky et al. (2005) suggested more experiments are needed that isolate individual management treatments, such as crop rotation, to better understand changes in labile-C with management.

To date, no information on the effects of SS cultivation on soil labile carbon fractions appears to have been published. While SS effects on SOC across latitudes are unknown, pools of SOC and labile-C fractions are known to be smaller in the warmer Southern U.S. than Northern U.S. as C sequestration is reduced because higher temperatures cause greater decomposition rates (Gosling et al., 2013; Wright and Hons, 2005a). Gosling et al. (2013) examined more than 150 separate SOM studies and found that moving from temperate to warm climates resulted in nearly 60% decline in total SOM.

Two more C fractions of that have been shown to predict SOC changes are the labile-C and particulate, adsorbed, and occluded carbon (PAO-C) pools. The labile-C fraction is elucidated by the potassium permanganate oxidation method presented by

Blair et al. (1995), but later adapted by Weil et al. (2003) into a quick, relatively safe method that correlates well to total SOC and other known labile portions, including particulate organic matter (Mirsky et al., 2005), and the Walkley-Black method (Chan et al., 2000).

Analyis of an intermediate fraction for SOC was recently developed by Veum et al. (2011) to measure the particulate, adsorbed, and occluded carbon (PAO-C), a more stable SOC fraction than labile-C. This rapid slacking test isolates the physically-stabilized SOC fraction containing free light OM, intra-aggregate particulate OM, and mineral-associated OM (Veum et al., 2013). PAO-C was found to be an indicator of early SOC changes in agroforestry and grass vegetative buffer strips and contains higher concentrations of organic C than the total SOC pool (Veum et al., 2011; 2013). This newer fractionation method has not yet been used to predict early SOC changes in biofuel systems.

In this study, two-year rotations of SS-soybean and maize-soybean at three sites across a latitude gradient in the central U.S were compared. The objectives of the study were: 1) to determine yields of SS-soybean and maize-soybean systems for biofuel feedstock production across a latitudinal gradient, 2) to evaluate short-term effects of SS and maize-based rotations on SOC, labile-C, and PAO-C pools, and 3) to determine if labile-C and PAO-C fractions are reliable short-term predictors of SOC changes across soil depths in these systems. We hypothesized that SOC and labile-C would decrease within the establishment years across all locations, although soil C levels would decrease less in the SS-soybean system compared to the maize-soybean system because of increased above- and below-ground biomass growth.

Materials and Methods

Site description and crop management

Research was conducted at three dryland sites across a latitudinal gradient and frost zones in Missouri and Arkansas in 2010 and 2011. The sites were: University of Missouri (MU) Horticulture and Agroforestry Research Center, New Franklin, MO (39’ 02” N, 92’ 76” W); MU Southwest Research Center, Mount Vernon, MO (37’ 08” N, 93’ 86” W); USDA-ARS Dale Bumpers Small Farm Research Center, Booneville, MO (35’ 07” N, 93’ 98” W). The soils studies included a Sibley silt loam with loess parent material (fine-silty, mixed superactive, mesic Typic Argiudolls) at New Franklin, a Dapue silt loam with alluvium parent material (fine-silty, mixed, active, mesic Fluvertic Hapludolls) at Mount Vernon with a mean pH of 6.6, and a Leadvale silt loam with loamy pedisediment parent material (fine-silty, siliceous, semiactive, thermic Typic Fragiudults) at Booneville with a 4.4 mean pH. All three sites are considered marginal for row crop production because of slope, landscape position, and soil properties, or combinations thereof.

The research at the three sites was initiated in May 2010, and the experiments at the three sites were similarly arranged in randomized complete block designs with four replications per site. Two biofuel crop production systems were designed to compare the SS-soybean rotation to the maize-soybean rotation prevalent in the U.S. for biofuel feedstock production. The potential alternative SS-soybean crop rotation included SS cv. M-81E (MSU Cares). For the maize-soy and the SS-soy rotations each main plot was divided into two 30 m by 60 m subplots with maize or SS planted in one half and soybean in the other half in 2010. In 2011, the maize and SS were rotated with soy within respective main plots such that both C4 annuals were planted in the 2010 soybean plots and soybean was planted into the first-year maize and SS stubble.

Prior to experiment initiation in 2010, all three sites were grown under perennial grasses, predominantly tall fescue (*Festuca arundinacea*). The Mount Vernon site was the only one that had recently had annual forages planted on the plot area. Crops were planted in late May of each year on 0.76 m row spacing. Prior to spring 2011 tillage, 2010 crop dry matter was mowed and either raked or bailed from plots to simulate total biomass removal for lignocellulosic ethanol production. Single applications of urea were broadcast at planting, with maize and SS receiving 160 and 80 kg ha^{-1} N fertilizer, respectively. Pre-and post-emergent herbicide applications of glyphosate was used for the maize and soybean plots as needed, and a pre-emergent herbicide application of S-Metolachlor was applied on the SS plots at planting for weed control.

In 2010 and 2011, SS DM, SJY, FSY, JEY, LEY, and TEY and maize DM, grain yield, theoretical grain-derived ethanol, maize LEY, and maize TEY were determined at all three sites following the first killing frost. No maize grain yields were obtained in 2011 as a result of severe drought during maize anthesis. Soybean grain yields were not determined in this study.

Maize grain yield was determined based on ears harvested from a 10 m row section. Ears were husked and dried in a forced-air dryer at 60°C. Grain was then separated from cobs, weighed, and grain yield and theoretical ethanol yield from grain was calculated a whole-crop basis. Ethanol conversion was based on Putnam et al. (1991), where 417 L ethanol is produced per Mg of grain.

In each SS plot 0.5-m sections from two adjacent rows were randomly selected and cut by hand at 0.01m above the ground. The whole SS samples were weighed for a fresh weight and then squeezed through a three-roller sorghum press to extract stem juice. After juice extraction, a subsample of bagasse was collected, weighed, and dried similar to maize dry matter, following which dry matter yield (DM), stem juice yield (SJY), fermentable sugar yield (FSY), and theoretical ethanol yields were calculated, as per equations given in Chapter 2.

Soil Sampling and Soil Analyses

Bulk density (BD) was determined at each site by removing 50mm diameter soil cores from three depth increments consisting of 0-50mm, 50-100mm, and 100-200mm from one pit from near the edge of each replication in the fall of 2010. Soil cores were dried and BD was calculated on a mass per volume basis and applied to each respective site and replication and used for calculation of the three SOC pools.

To examine biofuel cropping rotation effects on SOC pools, three soil cores to a depth of 0.6 m were removed from the inter-row of each maize and SS plot after harvest in Dec. 2010. Each soil core was split into soil depth increments of 0-50 mm, 50-100 mm, and 100-200 mm and then composited by depth. Soil samples were air-dried, ground, and sieved through a 2 mm screen to remove visible roots and rocks. In Dec. 2011, soil cores were again taken in maize and SS plots, following the same procedure as in 2010. Soil organic carbon analyses were limited to the chosen soil horizons to elucidate more dynamic SOC changes in the upper rooting zone.

To potentially capture any short-term SOC changes, three C pools were chosen, including SOC, labile-C fraction by potassium permanganate oxidation (labile-C) and the particulate, adsorbed, and occluded (PAO-C) portion. For SOC, each of the three sampling increments was analyzed by combustion on ignition (Ellert et al., 2001) on a LECO combustion analyzer.

Labile-C was determined by the potassium permanganate oxidation method according to Weil et al. (2003), where 20.0 mL of 0.02M $KMnO_4$ was reacted with 2.5 g of soil. Briefly, soil and permanganate solution were added to 50-ml screw-top plastic centrifuge tubes and placed on a mechanical horizontal shaker for 15 min at 200 rpm followed by separation in a table-top centrifuge for 5 min at 3000 rpm. Supernatants were diluted with deionized water and light absorption of the diluted solution was recorded at 550nm using a spectrophotometer.

Labile-C concentrations were then calculated using the equation presented in Weil et al. (2003):

$$LabileC\ (mg\ kg^{-1}) = [0.02\ mol\ L^{-1} - (a + bz)] \times (9000\ mg\ C\ mol^{-1}) \times \left(\frac{0.02\ L\ solution}{0.0025\ kg\ soil}\right)$$

where 0.02 mol/L is the concentration of the $KMnO_4$ solution before reaction, *a* is the intercept of the standard curve, *b* is the slope of the standard curve, *z* is the absorbance value of the diluted supernatant, 9000 mg C/mol is the assumed mass of C oxidized by 1 mol of MnO_4 reducing from Mn^{7+} to Mn^{4+}, 0.021L is the volume of $KMnO_4$ solution reacted, and 0.0025 kg is the reacted soil mass.

The PAO-C fraction was determined by the wet slaking test developed by Veum et al. (2011). From each sample 100g of soil was slaked in DI water over a 23 µm sieve, then wet-sieved four times at 30 sec per time to separate sand and clay portions. The soil residue on the sieve was back-washed, oven-dried, weighed, and ground. This is the PAO fraction. Soil organic carbon in the PAO-C fraction was determined by combustion on ignition as for whole soil SOC.

The portion of SOC in the PAO-C fraction of the soil, on a sand-free basis, was calculated according to Veum et al. (2011):

$$PAOC\ (mg\ kg^{-1}) = \frac{Occluded\ SOC}{PAO\ residue} \times \frac{PAO\ residue}{Whole\ soil\ (-sand)}$$

where Occluded SOC in mg kg^{-1} is the C measured in the PAO residue after combustion, PAO residue (kg) is the material remaining after the wet slaking procedure, and Whole soil (kg) is the soil mass with the sand content removed.

Statistical Analysis

Analysis of variance was performed using the PROC MIXED procedure of the SAS (SAS Institute, 2009) software with year, location, and crop as random effects and soil layer as fixed effect. Dependent variables included dry matter, stem juice yield, fermentable sugar yield, theoretical ethanol yield, concentration of SOC, labile-C concentration, PAO-C concentration, SOC stocks, labile-C stocks, and PAO-C stocks, change in SOC, and change in labile-C. Treatment means were compared using t-tests provided by the ADJUST=Tukey option in SAS at an alpha value of 0.05. Correlations for the soil and yield parameters were calculated with PROC CORR.

Results and Discussion

The establishment year, 2010, had observationally normal temperature and precipitation for the climate across locations, while 2011 had reduced precipitation and above-average temperatures at all sites, although no weather data were recorded for any site. Limited in-season precipitation in 2011 reduced crop growth and final yields across all sites, especially at Booneville, where the severe extended drought caused crop failure such that no final yields of maize and SS were determined. While SS yields were determined at New Franklin and Mount Vernon in 2011, due to the limited precipitation maize grain production were severely limited and grain yields were not determined. The environmental conditions during the two growing season provided exceptional circumstances for observing dryland growing conditions on marginal lands through the Central U.S.

Biomass and Ethanol Yields

Yields across crop and location were examined for 2010, however the second crop year, 2011, did not have any recorded maize yields, although SS was harvested and yields were determined at the New Franklin and Mt. Vernon sites. Drought prevented maize grain yields at the Booneville location both years. Three yields were compared by crop and location, including DM, lignocellulosic ethanol yield, and total ethanol yield. DM yields were greatest at New Franklin (14.0 Mg ha^{-1}), averaged across crops followed by Mount Vernon and Booneville, of 3.54 and 1.33 Mg ha^{-1}, respectively (Table 4.1). Maize DM was significantly lower than SS DM across locations, but at the most southerly location Booneville, the DM yields were similar between maize and SS, but with reduced yields. New Franklin SS DM was 77% greater than maize yields, showing SS's deferential partitioning to biomass over grain production more than maize.

Theoretical lignocellulosic ethanol yields (LEY) estimate the potential ethanol yields by converting DM to ethanol. Lignocellulosic ethanol yields followed DM, with 75% greater yields at New Franklin than Mount Vernon, which was the second-highest yielding site (Table 4.1). Total ethanol yields were calculated by summing lignocellulosic ethanol yields for each crop with the respective estimated ethanol yields of maize grain or SS juice. Total SS ethanol production is again highest at New Franklin with 7831 L ha^{-1}, but all SS and maize estimates were similar at Booneville and Mount Vernon, ranging 106-2456 L ha^{-1}.

In general, yields of SS were lower than those reported in published studies from the Central U.S. (Holou and Stevens, 2011; Wortmann et al., 2010; Propheter et al., 2010). M-81E yields in Kansas were 28.6-32.6 Mg ha^{-1} (Propheter et al., 2010), such that only New Franklin DM was close to average yields for other studies. Interestingly, with small sample sizes and large error terms, the Brix, SJY, FSY, and SS juice-derived ethanol did not differ among locations. Brix values were in line with the only other Missouri study (Holou and Stevens, 2011). Sweet sorghum DM, SJY, FSY, juice ethanol, lignocellulosic ethanol, and total ethanol yields were at minimum 60% less than other reported values (Holou and Stevens, 2011; Wortmann et al., 2010). Holou and Stevens identified differences in yields with soil type and Wortmann et al. (2010) found differences by location, although Buxton et al. (1999) did not.

Table 4.1. Dry matter yields (DM), Brix, SS stem juice yield (SJY), maize grain yield, SS fermentable sugar yield (FSY), maize grain ethanol (MEY), SS juice-derived ethanol (JEY), lignocellulosic ethanol (LEY), and total ethanol (TEY) in Maize and Sweet Sorghum across the three study sites in 2010: Booneville, AR, Mount Vernon, MO, and New Franklin, MO.

Crop	DM	Brix	SS SJY	Maize grain	SS FSY	MEY	SS JEY	LEY	TEY
	- Mg ha^{-1} -	----% -----	-- L ha^{-1} --	-------- Kg ha^{-1} ----------		------------------------- L ha^{-1} ---------------------------			
Booneville, AR									
Maize	0.340bc	-	-	0b	-	0	-	106bc	106c
Sweet Sorghum	2.32bc	13.7a	4866a	-	511a	-	229a	722bc	1012c
Means	1.33B	-	-	-	-	-	-	414B	1511B
Mount Vernon, MO									
Maize	0.244c	-	-	1982b	-	739b	-	76c	815c
Sweet Sorghum	6.83b	14.5a	5318a	-	581a	-	330a	2125b	2456bc
Means	3.54B	-	-	-	-	-	-	1101B	1635B
New Franklin, MO									
Maize	5.25b	-	-	7067a	-	2633a	-	1634b	4267b
Sweet Sorghum	22.7a	14.4a	12193a	-	1338a	-	760a	7071a	7831a
Means	14.0A	-	-	-	-	-	-	4352A	6049A

Means in a column with the same lowercase letter are not significantly different at P=0.05

Means in a columns with the same uppercase letter are not significantly different at P=0.05

All maize yields were less than SS at New Franklin and Mount Vernon likely because late-season sustained rainfall allowed for SS's continued growth, outcompeting maize for drought adaptability. Maize yields were well less than other regional studies (Propheter et al., 2010), but maize grain yields were similar at New Franklin to yields reported by Wortmann et al (2010) of 7.36-8.38 Mg ha^{-1}.

Soil Organic Carbon

Soil organic carbon concentrations and stocks were calculated for both study years, 2010 and 2011, and were separated by soil depth, 0-50mm, 50-100mm, and 100-200mm. No SOC differences by crop were observed between maize and SS at any study site or year (Tables 4.2-4.4). Therefore, crops values were pooled and the three sites were compared by soil depths for differences across years (Tables 4.5). While differences did occur with site x year interactions, these were not considered because SOC levels in the second study year were assumed to be related to and based-upon the first year's SOC levels. This was the case for all soil parameters considered in this study.

In the top soil layer sampled, SOC concentrations in 2010, the first year of rotation establishment, revealed differences between sites. New Franklin contained 33% and 44% more SOC g kg^{-1} than Mount Vernon and Booneville, respectively (Table 4.5). Overall means across sites were 18.6 g kg^{-1}. This was significantly greater than in 2011, which experienced a decrease in SOC concentration to 15.1 g kg^{-1}, a seven percent decrease. Decreases were not significant at all locations, but surprisingly the decrease between years was similar among sites, even though New Franklin SOC concentrations decreased the most (-4.41 g kg^{-1}), the trend was consistent within all the sites, with an average loss of 2.93 g kg^{-1}. In the second soil layer (50-100mm), SOC concentration decreased the most at New Franklin where a 28% drop occurred. The deepest soil layer (100-200mm) experienced similar decreases in SOC concentration of 17% across years. Amounts of SOC decreased at similar increments, regardless of different beginning SOC levels in the top and lowest soil layers. This was not the case in the 50-100mm depth, where Mount Vernon lost less SOC than New Franklin, an 88% greater loss. The consistent loss of SOC may be explained by the transition of all sites from long-term pasture and forage systems to row crop production, which encouraged increased SOC decay through initial tillage and forage removal prior to crop establishment in 2010 and decreased organic matter additions with complete removal of crop dry matter prior to 2011 plantings. As well, reduced or negligible 2011 yields may have been a strong contributing factor to the reduced soil C.

Several studies examine continuous grain sorghum and grain sorghum-soy rotations and their effects on soil properties, including studies in Texas and Nebraska. Varvel (2006) reported no significant difference in SOC between maize-soy and sorghum-soy rotations in the first 10 years of a 20-year study. Holou and Stevens (2011) recommended a SS-soy rotation for decreasing production costs by reducing crop N needs. Wright and Hons (2005b) found that, after 20 years continuous production, grain sorghum had 15.3 Mg-C ha^{-1} in the top 150mm under conventional tillage, which is less than our results for SOC in the top 200mm. This concurs with Franzluebbers et al. (1995), which observed an 18% increase in mineralizable C, a more active C pool, in a sorghum-wheat-soy rotation compared to a sorghum monoculture. They attributed this

Table 4.2. Soil organic carbon (SOC) concentrations, total SOC, Labile-C (C_L) concentrations, total C_L , particulate, adsorbed, and occluded soil organic carbon (PAO-C) concentrations, and total PAO-C in Maize and Sweet Sorghum (SS) across three soil layer depths near New Franklin, MO.

	2010				**2011**						**Change, Δ2010-2011**			
					Layer 1, 0-50 mm depth									
Crop	**[SOC]**	**[C_L]**	**SOC**	**C_L**	**[SOC]**	**[C_L]**	**[PAO-C]**	**SOC**	**C_L**	**PAO-C**	**[SOC]**	**[C_L]**	**SOC**	**C_L**
	-------- g kg $^{-1}$ ------		------- Mg ha^{-1} -----		------------------- g kg $^{-1}$ -----------------			---------------- Mg ha^{-1} --------------			---------- g kg $^{-1}$ -----		------- Mg ha^{-1} -------	
Maize	24.5	2.04	8.85	0.708	19.1	1.70	17.4	6.13	0.544	5.58	-4.29	-0.189	-1.37	-0.061
SS	25.7	2.19	8.43	0.702	19.6	1.81	17.0	7.05	0.582	5.69	-4.54	-0.378	-1.38	-0.121
Means	25.1	2.12	8.64	0.705	19.3	1.76	17.2	6.59	0.563	5.63	-4.41	-0.284	-1.38	-0.091
					Layer 2, 50-100 mm depth									
Crop	**[SOC]**	**[C_L]**	**SOC**	**C_L**	**[SOC]**	**[C_L]**	**[PAO-C]**	**SOC**	**C_L**	**PAO-C**	**[SOC]**	**[C_L]**	**SOC**	**C_L**
	-------g kg $^{-1}$ --------		------ Mg ha^{-1} ------		----------------- g kg $^{-1}$ -------------------			-------------- Mg ha^{-1} ----------------			------- g kg $^{-1}$ ---------		------- Mg ha^{-1} -------	
Maize	18.9	1.51	7.47	0.595	13.3	1.05	8.90	5.27	0.414	3.52	-5.58	-0.263	-1.42	-0.104
SS	17.4	1.28	6.86	0.432	12.7	1.03	10.5	5.00	0.407	4.15	-4.71	-0.108	-1.86	-0.098
Means	18.1	1.39	7.17	0.513	13.0	1.04	9.70	5.13	0.410	3.83	-5.15	-0.186	-1.78	-0.101
					Layer 3, 100-200 mm depth									
Crop	**[SOC]**	**[C_L]**	**SOC**	**C_L**	**[SOC]**	**[C_L]**	**[PAO-C]**	**SOC**	**C_L**	**PAO-C**	**[SOC]**	**[C_L]**	**SOC**	**C_L**
	------- g kg $^{-1}$ -------		------ Mg ha^{-1} ------		------------------ g kg $^{-1}$ -------------------			------------- Mg ha^{-1} -----------------			-------- g kg $^{-1}$ --------		------ Mg ha^{-1} -------	
Maize	10.9	0.772	9.90	0.701	10.1	0.649	7.43	8.66	0.589	6.75	-1.01	-0.123	-1.24	-0.112
SS	11.4	0.864	10.3	0.663	8.8	0.598	6.05	8.01	0.543	5.49	-2.55	-0.121	-2.31	-0.110
Means	11.1	0.818	10.1	0.682	9.5	0.623	6.74	8.34	0.566	6.12	-1.78	-0.122	-1.78	-0.111

*, **, *** indicate significance at P<0.05, 0.01, 0.001, respectively

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Table 4.3. Means of soil organic carbon (SOC) concentrations, total SOC, Labile-C (C_L) concentrations, total C_L , particulate, adsorbed, and occluded soil organic carbon (PAO-C) concentrations, and total PAO-C in Maize and Sweet Sorghum (SS) across three soil layer depths near Mount Vernon, MO.

	2010				**2011**						**Change, Δ2010-2011**			
	Layer 1, 0-50 mm depth													
Crop	**[SOC]**	**[C_L]**	**SOC**	**C_L**	**[SOC]**	**[C_L]**	**[PAO-C]**	**SOC**	**C_L**	**PAO-C**	**[SOC]**	**[C_L]**	**SOC**	**C_L**
	---- g kg $^{-1}$ ----		--- Mg ha^{-1} ---		----------- g kg $^{-1}$ ------------			--------- Mg ha^{-1} ------------			----- g kg $^{-1}$ ----		---- Mg ha^{-1} ----	
Maize	18.1	1.33	11.6	0.852	15.2	0.973	12.5	11.6	0.567	7.98	-2.85	-0.356	-1.57	-0.210
SS	15.3	1.27	10.5	0.816	13.7	0.782	10.6	10.5	0.501	6.78	-2.16	-0.491	-1.74	-0.314
Means	16.7	1.30	11.0	0.834	14.5	0.878	11.5	11.0	0.534	7.38	-2.50	-0.423	-1.65	-0.262
	Layer 2, 50-100 mm depth													
Crop	**[SOC]**	**[C_L]**	**SOC**	**C_L**	**[SOC]**	**[C_L]**	**[PAO-C]**	**SOC**	**C_L**	**PAO-C**	**[SOC]**	**[C_L]**	**SOC**	**C_L**
	---- g kg $^{-1}$ ----		--- Mg ha^{-1} ---		----------- g kg $^{-1}$ ------------			--------- Mg ha^{-1} ------------			----- g kg $^{-1}$ ----		---- Mg ha^{-1} ----	
Maize	10.6	0.887	6.81	0.568	11.4	0.653	9.33	6.81	0.418	5.98	-0.1	-0.284	-0.064	-0.206
SS	11.6	0.979	7.40	0.627	11.0	0.609	7.85	7.39	0.462	5.03	-0.507	-0.427	-0.325	-0.165
Means	11.1	0.805	7.11	0.598	11.2	0.631	8.59	7.11	0.44	5.50	-0.304	-0.356	-1.51	-0.186
	Layer 3, 100-200 mm depth													
Crop	**[SOC]**	**[C_L]**	**SOC**	**C_L**	**[SOC]**	**[C_L]**	**[PAO-C]**	**SOC**	**C_L**	**PAO-C**	**[SOC]**	**[C_L]**	**SOC**	**C_L**
	---- g kg $^{-1}$ ----		--- Mg ha^{-1} ---		---------- g kg $^{-1}$ -------------			--------- Mg ha^{-1} ------------			----- g kg $^{-1}$ ----		---- Mg ha^{-1} ----	
Maize	9.51	0.773	12.4	0.996	7.82	0.347	5.68	12.4	0.481	7.88	-1.28	-0.368	-1.77	-0.510
SS	10.4	0.837	12.9	1.06	8.39	0.505	6.53	12.9	0.700	9.05	-0.907	-0.234	-1.26	-0.324
Means	9.96	0.805	12.6	1.03	8.11	0.426	6.11	12.6	0.590	8.47	-1.09	-0.301	-1.51	-0.417

*, **, *** indicate significance at P<0.05, 0.01, 0.001, respectively

to increased roots and residue from the intensive system. Their study differs from our study as we did not include wheat in the rotation, and soybean residue was not measured to elucidate the additions from each portion of the rotation.

Consistent with results presented here, across depths, short-term increases in SOC often appear in the upper soil horizons, but stocks can be greater at lower depths (Wright and Hons, 2005a; 2005b; Franzluebbers 1995). In Nebraska, maize-soybean and GS-soybean rotations had increasing levels of SOC with increasing soil depth, although all horizons SOC stocks increased the first 8 years before a change in tillage encouraged a steady 10-year decline in the top 300mm (Varvel, 2006).

As well, the conversion of land-use from pasture systems to annual row crop production reduced SOC stocks as expected. Page et al. (2013) reported a loss of 3.4 Mg ha^{-1} yr^{-1} after tilling pasture for row cropping, which supports soils with large SOC stocks may continue to experience carbon decay if row crops do not maintain sufficient C sequestration, especially in warmer climates with higher rates of decay and marginal soils with poorer growing conditions.

Labile Carbon

Labile-C was determined to identify short-term crop management effects on SOC by chemically separating the most active C fraction in the soils. For each study site, labile-C levels showed no crop response (Tables 4.2-4.4), except for Booneville in 2010, where soils under SS had a slightly greater labile-C fraction than in maize plots (Table 4.4). No other crop effects were determined at other sites across both years, so crops were pooled to examine differences between sites across years.

Clear differences in labile-C between soil depths were elucidated, with similar trends found across sites. Among the three sites pooled, Labile-C concentrations decreased 0.296 g kg^{-1} in 0-50mm, which was greater than that the 0.223 and 0.178 g kg^{-1} at 50-100mm and 100-200mm, respectively (Table 4.5). Booneville generally had the lowest Labile-C concentrations, and New Franklin and Mount Vernon were not different. The greatest differences delineations within location appeared in the top soil sample layer. Through soil depths, the changes between the three sites were mostly consistent, where Labile-C amounts at Mount Vernon changed the least of the sites overall, although all sites had similar losses in the 50-100mm soil sample layer. Decreases in Labile-C stocks were greatest in the deepest soil layer, 33% more than 0-50mm layer, and 58% more than 50-100mm layer. In 2010, Labile-C fractions were greater at Mount Vernon than the other sites, but in 2011 the differences were nonexistent, suggesting that the land-use change imposed similar changes to this active fraction. Changes in Labile-C concentrations and stocks from 2010 to 2011 reveal reduced active C fractions in the soil. This follows the losses in SOC stocks, but at greater percentages, where SOC decreased 15% in the 0-50mm soil layer but 23% was lost in the Labile-C fraction. This reflects the more active and short-term properties of Labile-C. SOC and Labile-C losses were 11% and 21%, respectively, at 50-100mm, and 15% and 35%, respectively, at 100-200mm. This may be attributed to increased soil microbiological decay of the active C portions through the tilled depths with reduced C additions in 2011. While our results reveal similar trends in SOC and Labile-C stock changes, the greatest relative change occurred in the labile fraction. Culman et al. (2013) reported similar trends under maize and, as well, consistent with our findings that management affects the Labile-C pool.

Lower Labile-C concentrations in a maize-soy rotation than continuous maize systems were reported by Mirsky et al. (2008). They reported no difference in the labile fraction after the second year, as well, which supports our findings that Labile-C pools appear to reach similar levels across location in the second year. Unfortunately, Mirsky et al. (2008) do not clearly differentiate treatment effects of crop rotation and fertility in maize systems, even as their Labile-C levels were similar to our observations.

Particulate, Adsorbed, Occluded Carbon

PAO-C fractions of the SOC were measured in the second year of the rotation and changed by soil depth, but not by crop, matching trends observed for SOC and labile (Table 4.5). Distinct differences between locations appeared. PAO-C concentrations were highest at New Franklin, 33% and 50% greater than at Mount Vernon and Booneville, respectively in the top soil layer. The PAO-C concentrations were greatest in the upper horizon. In the 50-100mm and 100-200mm soil layers no differences were observed between locations, indicating that the while New Franklin PAO-C concentrations in the top horizon were more available for decay than the two lower soil depths, PAO-C is dependent upon a respective location’s soil characteristics and land-use. PAO-C fractions were generally largest at Mount Vernon. Since PAO-C fractions were not examined in 2010, changes in this medium-activity fraction cannot be calculated. Yet, it appeared that PAO-C fractions followed similar trends for location differences as observed in SOC and Labile-C.

Table 4.4. Means of soil organic carbon (SOC) concentrations, total SOC, Labile-C (C_L) concentrations, total C_L , particulate, adsorbed, and occluded soil organic carbon (PAO-C) concentrations, and total PAO-C in Maize and Sweet Sorghum (SS) across three soil layer depths near Booneville, AR

	2010				**2011**						**Change, Δ2010-2011**			
	Layer 1, 0-50 mm depth													
Crop	**[SOC]**	**[C_L]***	**SOC**	**C_L**	**[SOC]**	**[C_L]**	**[PAO-C]**	**SOC**	**C_L**	**PAO-C**	**[SOC]**	**[C_L]**	**SOC**	**C_L**
	------- g kg $^{-1}$ ----		----- Mg ha $^{-1}$ ---		------------------ g kg $^{-1}$ ------------			---------------- Mg ha $^{-1}$ -----------			-------- g kg $^{-1}$ -----		------- Mg ha $^{-1}$ ----	
Maize	14.0	0.813	8.40	0.488	10.1	0.602	8.55	6.08	0.361	5.13	-1.78	-0.193	-0.71	-0.081
SS	14.1	0.895	8.45	0.537	12.8	0.728	8.40	7.69	0.437	5.04	-1.95	-0.167	-1.57	-0.100
Means	14.0	0.854	8.43	0.513	11.5	0.665	8.47	6.89	0.399	5.08	-1.86	-0.180	-1.14	-0.091
	Layer 2, 50-100 mm depth													
Crop	**[SOC]**	**[C_L]**	**SOC**	**C_L**	**[SOC]**	**[C_L]**	**[PAO-C]**	**SOC**	**C_L**	**PAO-C**	**[SOC]**	**[C_L]**	**SOC**	**C_L**
	------ g kg $^{-1}$ -----		----- Mg ha $^{-1}$ ----		---------------- g kg $^{-1}$ --------------			--------------- Mg ha $^{-1}$ ------------			-------- g kg $^{-1}$ -----		------ Mg ha $^{-1}$ -----	
Maize	12.9	0.704	7.72	0.423	11.6	0.653	9.16	6.98	0.392	5.49	-2.2	-0.121	-0.508	-0.030
SS	13.1	0.807	7.86	0.484	12.0	0.670	10.4	7.17	0.401	6.21	-1.14	-0.138	-0.687	-0.083
Means	13.0	0.789	7.79	0.453	11.8	0.661	9.76	7.08	0.397	5.85	-1.67	-0.130	-0.598	-0.057
	Layer 3, 100-200 mm depth													
Crop	**[SOC]**	**[C_L]**	**SOC**	**C_L**	**[SOC]**	**[C_L]**	**[PAO-C]**	**SOC**	**C_L**	**PAO-C**	**[SOC]**	**[C_L]**	**SOC**	**C_L**
	--- g kg $^{-1}$ ----		--- Mg ha $^{-1}$ ---		------------------ g kg $^{-1}$ -----------			----------- Mg ha $^{-1}$ ----------			----- g kg $^{-1}$ ---		---- Mg ha $^{-1}$ ---	
Maize	7.08	0.447	10.0	0.631	6.16	0.381	5.84	8.72	0.538	8.25	-1.30	-0.118	-1.83	-0.269
SS	8.26	0.490	11.7	0.693	6.49	0.367	4.98	9.18	0.519	7.04	-2.42	-0.101	-2.10	-0.310
Means	7.67	0.468	10.8	0.662	6.33	0.374	5.41	8.95	0.528	7.65	-1.86	-0.110	-1.97	-0.290

*, **, *** indicate significance at $P<0.05$, 0.01, 0.001, respectively

Table 4.5. Means of soil organic carbon (SOC) concentrations, total SOC, Labile-C (C_L) concentrations, total C_L , particulate, adsorbed, and occluded soil organic carbon (PAO-C) concentrations, and total PAO-C across three soil horizons at New Franklin, MO (NF), Mount Vernon, MO (MV), and Booneville, AR (BV)

	2010				**2011**						**Change, Δ2010-2011**			
	Layer 1, 0-50 mm depth													
Location	**[SOC]**	**[C_L]**	**SOC**	**C_L**	**[SOC]**	**[C_L]**	**[PAO-C]**	**SOC**	**C_L**	**PAO-C**	**[SOC]**	**[C_L]**	**SOC**	**C_L**
	---- $g\ kg^{-1}$ ----		--- $Mg\ ha^{-1}$ -----		--------- $g\ kg^{-1}$ --------------			----------- $Mg\ ha^{-1}$ ---------			------$g\ kg^{-1}$ ----		---- $Mg\ ha^{-1}$ ----	
NF	25.1a	2.12a	8.04b	0.678b	22.2a	1.76a	17.2a	6.21b	0.563a	5.52ab	-4.41a	-0.284a	-1.38a	-0.091a
MV	16.7b	1.30b	10.7a	0.834a	15.6ab	0.878b	11.5b	9.27a	0.562a	7.38a	-2.50a	-0.424a	-1.65a	-0.232b
BV	14.0c	0.854c	8.43ab	0.512c	12.8b	0.665b	8.47b	8.07b	0.399b	5.08b	-1.86a	-0.180a	-1.14a	-0.091a
Means	18.6	1.42	9.05	0.675	15.1	1.10	12.4	7.46	0.508	5.99	-2.93	-0.296	-1.39	-0.148
	Layer 2, 50-100 mm depth													
Location	**[SOC]**	**[C_L]**	**SOC**	**C_L**	**[SOC]**	**[C_L]**	**[PAO-C]**	**SOC**	**C_L**	**PAO-C**	**[SOC]**	**[C_L]**	**SOC**	**C_L**
	----- $g\ kg^{-1}$ -----		----$Mg\ ha^{-1}$ ----		---------- $g\ kg^{-1}$ ------------			----------- $Mg\ ha^{-1}$ ---------			----- $g\ kg^{-1}$ -----		---- $Mg\ ha^{-1}$ ----	
NF	18.1a	1.30a	7.17a	0.513ab	13.0a	1.04a	9.70a	5.13b	0.411a	3.83b	-5.15b	-0.185ab	-1.64b	-0.101a
MV	11.1b	0.933b	7.11a	0.598a	11.2a	0.631b	8.59a	7.56a	0.404a	5.50a	-0.304a	-0.356b	-0.195a	-0.186a
BV	13.0b	0.756b	7.79a	0.453b	11.8a	0.661b	9.76a	7.08a	0.397a	5.85a	-1.67ab	-0.123a	-0.60ab	-0.056a
Means	14.1	0.996	7.35	0.521	12.0	0.777	9.35	6.59	0.404	5.06	-2.37	-0.223	-0.811	-0.114
	Layer 3, 100-200 mm depth													
Location	**[SOC]**	**[C_L]**	**SOC**	**C_L**	**[SOC]**	**[C_L]**	**[PAO-C]**	**SOC**	**C_L**	**PAO-C**	**[SOC]**	**[C_L]**	**SOC**	**C_L**
	----- $g\ kg^{-1}$ -----		--- $Mg\ ha^{-1}$ ----		---------- $g\ kg^{-1}$ ------------			---------- $Mg\ ha^{-1}$ -----------			----- $g\ kg^{-1}$ ----		---- $Mg\ ha^{-1}$ ----	
NF	11.1a	0.751a	10.1a	0.682b	9.48a	0.623a	6.74a	8.61a	0.566a	6.12b	-1.78a	-0.122a	-1.78a	-0.111a
MV	9.13a	0.742a	12.7a	1.03a	8.11a	0.426b	6.11a	10.7a	0.590a	8.47a	-1.09a	-0.301a	-1.51a	-0.417b
BV	7.67b	0.468b	10.8a	0.662b	6.33b	0.374b	5.41a	8.95a	0.523a	7.65ab	-1.86a	-0.110a	-1.97a	-0.290ab
Means	9.31	0.654	11.2	0.791	7.97	0.474	6.08	9.40	0.562	7.41	-1.58	-0.178	-1.75	0.273

Significant differences in a column divided by soil layer indicated by lowercase letters at $P < 0.05$.

VVV

V

No early changes according to crop effect were observed at any of the sites, suggesting that the PAO-C fraction may not be a quality indicator to differentiate early changes in labile SOC fractions between SS and maize. While PAO-C can reveal differences between some more drastic land-use differences, such as no-till farming to agroforestry systems, given by Veum et al. (2011), no differences were elucidated between maize and SS two years after converting the marginal lands from perennial grasses to the annual row crops. Continued PAO-C measurements beyond the first two years may reveal diverging carbon pools in the cropping systems. With limited research involving this method, it is difficult to predict long-term changes.

Correlations

Total SOC concentrations and stocks did not correlate to other soil fractions or yields across location and depth. Labile-C concentrations were highly correlated to PAO-C concentrations (R^2=0.932), but Labile-C concentrations and Labile-C amounts were negatively correlated to SOC concentrations and total SOC stocks, indicating differences between the SOC and more labile pools over time. Stocks of PAO-C and Labile-C followed similar trends (R^2=0.562), but PAO-C did not follow total SOC stocks (R^2=0.037). Crop DM yields weakly correlated to soil characteristics. Labile-C and PAO-C fractions may relate to yields. It is hard to determine whether high R values for correlations indicated relationship or they are circumstantial. DM yields had the highest relationship to Labile-C concentrations (R^2=0.5402) and PAO-C concentrations (R^2 =0.5727), suggesting that a crop’s DM may affect soil concentrations of the labile or active carbon pools. Although, Culman et al. (2013), are among the first researchers to correlate maize growth and yields to Labile-C pools; they reported significant positive relationships between DM to SOC and Labile-C at later plant growth stages.

Conclusions

No SOC differences were observed between maize and SS-based rotations, suggesting that these two crops' impacts on soil C are similar in the top 0-200mm soil depth and across latitudinal gradient and soil type. Measuring labile-C and PAO-C fractions of SOC did reveal similar trends in soil carbon changes within these smaller active C pools, but there was a decrease in total SOC and labile-C concentrations and pools from 2010-2011, showing that transitioning marginal lands, that are mostly perennial grasslands and pastures in Central U.S., to annual biofuel feedstock crops may result in increased soil carbon loss, especially under drought conditions with limited crop biomass growth. However, choosing a SS-based crop rotation may not negatively affect these marginal lands more so than maize-based systems. Environment and soil properties are strong determining factors of yield and SOC changes. Across the three study sites, New Franklin had the highest yields for SS and maize, over Mount Vernon and Booneville.

Marginal lands may need more careful management if switching to annual biofuel feedstock crops is considered. It may be possible to stem SOC loss by leaving larger dry matter portions as ground cover for the field, and using reduced-tillage options. As well, this study does not clearly predict whether the early loss in SOC and more labile fractions will continue beyond the first two years. This is the first study comparing SS and maize-based rotations' effects on SOC and labile pools, and more long-term studies on SOC changes under SS-soy rotations are needed to elucidate any possible benefits.

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CHAPTER 5: CONCLUSION AND REVIEW

Missouri production agriculture has an array of crops that are predominated by maize and soybean. This is true for a large part of the Midwest U.S., as much of our nation's arable land is devoted to producing this steady rotation to serve the food, livestock, and fuel industries. Ethanol production is increasing in the U.S. with the investment by private industry into technology and research to better convert plant carbohydrates and cellulose to a renewable fuel source to stay the increasing petroleum costs. Maize production's divided interest in the livestock, human food, and ethanol industries is stressing the maize supply chains and more crops are needed to add to the supply for the ethanol industry. Therefore, many crops, including sorghum, switchgrass, and miscanthus, are under investigation for their potential yields and suitability to supplement the ethanol industry.

Sweet sorghum is a dynamic plant. It can adapt well to changing climatic stresses, as observed in this study. Drought and temperature are influential in sweet sorghum's growth and yield, but because grain production is not its differential sink for resources it is able to make stem sugar juice and sugar yields even when maize yields no grain. Despite this grand difference in above ground biomass and potential ethanol yields, maize and sweet sorghum under stress appear to have similar affects on soil organic carbon stocks. Yet, sweet sorghum is a strong candidate for continued biofuel feedstock research because it almost always produces a yield, except in the most extreme drought conditions (i.e. Booneville, AR 2011). This makes it suited for broad-scale production across latitudes in the U.S., especially in the Midwest, where transitioning moisture regimes and variable climates are not perhaps as limiting to sorghum growth and yields compared to other grain and biomass crops discussed in literature.

Nitrogen fertilization definitely has been demonstrated here and in previous literature to affect dry matter yields. It may not affect stem juice and sugar yields above 168 kg N ha^{-1} and the efficient accumulation of applied N and subsequent use of that in some cases may result in minor yield decreases, although not significant in this study. Realistically, the optimum N range of 56-112kg N ha^{-1} determined in this study will differ depending on location, soil type, climate, and a plethora of crop management choices. Sweet sorghum does prove to be a suitable alternative feedstock for ethanol production in Missouri.

Continued research on the sweet sorghum plant is needed. The crops physiology at the plant level is fascinating, but when plant by plant, row by row is amalgamated, the benefits of producing sweet sorghum on an areal basis is hard to ignore, as its dry matter yields approach upwards of 30 Mg ha^{-1} (higher in other published studies) and total theoretical ethanol yields top 10,000 L ha^{-1} (again, higher in other studies), as demonstrated here.

Of further interest to the author is specifically how sweet sorghum achieves such growth. Root growth allowing for more water uptake deeper into the soil profile may be a decipherable explanation for sorghum’s competitive growth and yields, is little understood, and merits in-depth investigation.

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