Substituting Biofuel for Coal: Determining the Optimal Perennial Grass Species and Nitrogen Fertilization Level for Bioenergy Production in Eastern Ontario

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Abstract

Renewable energy production has become increasingly important in recent years due to climate change and energy security issues, and bioenergy crops may be able to supply a large amount of the world's energy needs. Bioenergy is a carbon-neutral energy source, and perennial grasses are an ideal bioenergy crop due to their high growth rates and ability to grow under a range of conditions. To enhance grass productivity, nitrogen fertilization is often applied to these crops, but this can lead to increased emissions of nitrous oxide (N₂O), a potent greenhouse gas (GHG), from the soil. In collaboration with Lafarge Cement in Bath, Ontario, three perennial grass species (*Panicum virgatum*, *Schizachyrium scoparium* and *Andropogon gerardii*) were grown in conjunction with three fertilization treatments (0, 50 and 150 lbs/acre of nitrogen as urea) to explore the tradeoffs associated with using nitrogen fertilizer in these bioenergy systems. Soil N₂O emissions were measured weekly during the growing season, following fertilizer application in May 2013. At the same time, various soil properties known to influence N₂O emissions (pH, soil temperature and soil moisture) were measured. Crop productivity was measured at the end of the growing season to determine the species and fertilization level at which GHG emission benefits are maximized. N₂O fluxes were significantly higher in the 150 lbs./acre treatment, with no significant difference between the 0 and 50 lbs./acre treatments. Grass species had no effect on N₂O fluxes from the different fertilization treatments. Total GHG fluxes, which incorporate CO₂ and CH₄ in addition to N₂O, exhibited similar results among the fertilization treatments, and were also shown to be higher in the control treatment than the other three species treatments due largely to differences in soil CO₂ production.

Nitrogen fertilizer, however, did not significantly increase grass aboveground biomass. My results indicate that these perennial grass species are viable bioenergy crops in Eastern Ontario, and that the addition of nitrogen fertilizer at this site would reduce the benefits of the bioenergy crop to the climate system.

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List of Common Abbreviations

CH₄ - Methane

CO₂ – Carbon Dioxide

CO₂eq – Carbon Dioxide Equivalent

GHG – Greenhouse Gas

GWP – Global Warming Potential

ha – Hectare

KCl – Potassium Chloride

lbs. – Pounds

m² – square metre

Mg – Megagrams

N - Nitrogen

N₂ – Dinitrogen

N₂O – Nitrous Oxide

NH₄⁺ - Ammonium

nmol - nanomole

NO – Nitric Oxide

NO₂⁻ - Nitrite

NO₃⁻ - Nitrate

O₂ – Oxygen Gas

ppb – Parts Per Billion

ppm – Parts Per Million

s - second

μmol – micromole

1.0 Introduction and Literature Review

1.1 Introduction

Renewable energy production has become increasingly important in recent years, mainly due to climate change and the increasing monetary costs associated with non-renewable energy sources (Gallagher, 2013; Karp and Shield, 2008). Energy consumption is currently three times higher than it was in 1960 (Hein, 2005), and this increase is likely to continue into the future. Increasing consumption has created interest in finding alternative fuels that result in fewer greenhouse gas (GHG) emissions, and are sustainable over the long term. Biomass energy, or bioenergy, may be part of a potential solution to this problem. Bioenergy refers to energy derived from plant materials. This source of energy is close to being carbon-neutral, as it releases only carbon that has been captured during the growth of the plant, rather than carbon that has been locked up for millions of years in the form of fossil fuels (Sims et al., 2006). Previous work has suggested that bioenergy crops may provide a significant amount of the world's energy within the next half-century (Sims et al., 2006).

While woody plants have higher lignin content and are more energetically dense (Clinch et al., 2009; Volk et al., 2006), herbaceous species, such as grasses, are often considered to be more suitable biofuels. Perennial grasses can be harvested annually and have relatively high growth rates (Karp and Shield, 2008). Switchgrass (*Panicum virgatum*) has been examined as a potential bioenergy crop in North America due to its tolerance of a wide variety of environmental conditions, high productivity, and ability to be integrated into conventional farming (Lewandowski et al., 2003). Other species, including

big bluestem (*Andropogon gerardii*) and little bluestem (*Schizachyrium scoparium*) have also been shown to be productive bioenergy crops, particularly on dry and marginal soils (Gonzalez-Hernandez et al., 2009).

Adding nitrogen fertilizers to bioenergy crops is a common agricultural practice, as this increases the productivity of these crops. However, it may also increase GHG emissions from the soils of these crops through the processes of nitrification and denitrification (Kaiser et al., 1998). Nitrification involves the oxidation of ammonium to nitrate and generally occurs in aerobic conditions, while denitrification involves the reduction of nitrate to nitrogen gases (N_2 , N_2O and NO) and occurs in anaerobic conditions. N_2O emissions from agricultural soils are one of the major sources of anthropogenic N_2O , a potent GHG. When using nitrogen fertilizers to increase the productivity of bioenergy crops, it is important to consider how this fertilization affects GHG benefits. GHG benefits are defined as the difference between the GHG decreases associated with reduced fossil fuel use and the GHG increases associated with the fertilized soils, in terms of global warming potential (GWP). Soil moisture is also believed to play a role in N_2O release, as higher water content within a soil often results in increased denitrification, due to anaerobic conditions. However, nitrogen fertilizers increase these N_2O emissions primarily through increased nitrification (Bremner and Blackmer, 1978).

One industry that may be able to benefit from the use of bioenergy crops as an energy source is the cement industry. Cement making accounts for approximately 5% of global CO_2 emissions (CDIAC, 2013). Approximately 50% of these emissions occur from the decarbonation of calcium carbonate to calcium oxide, which makes up 60% of typical cement (Benhelal et al., 2013). The other half of the emissions occur from the energy

needed to produce the heat that is required for this reaction to occur. While there is not currently a viable way to reduce emissions associated with the chemical reaction during cement production, replacing coal with alternative energy sources can reduce GHG emissions associated with the energy production process.

This study examines the potential productivity of perennial grass species as bioenergy crops for use in the cement making process at Lafarge Inc. in Bath, Ontario, as well as the tradeoffs associated with using nitrogen fertilizer in these bioenergy systems. Three perennial grass species (switchgrass, little bluestem and big bluestem) and three nitrogen fertilizer treatments (0 lbs/acre, 50 lbs/acre and 150 lbs/acre urea) are studied. The objectives of this study are:

1. Determine the fertilization level at which GHG benefits are maximized. This will be done by determining the amount of GHG's released from the soil, and the amount of biomass produced, in each fertilization treatment.
2. Determine the grass species that maximizes GHG benefits, and whether this differs for the fertilization levels. This will be done by determining the amount of GHG's released from the soil, and the amount of biomass produced, for each grass species treatment.
3. Determine the interaction between environmental variables (pH, soil moisture and soil temperature) and GHG fluxes. This will be done by determining GHG fluxes, pH values, soil moisture, and soil temperatures for each plot over the course of the growing season.

1.2 Bioenergy Production

Climate change has resulted in increased interest in renewable energy production. (Gallagher, 2013; Karp and Shield, 2008). While anthropogenic CO₂ emissions are known to be the primary cause of climate change (Cook et al., 2013), these emissions continue to rise, and atmospheric CO₂ levels are projected to be approximately twice as high as pre-industrial levels by 2050 (IPCC, 2013). Fossil fuels currently supply approximately 85% of the world's energy demand (Hein, 2005), but it is projected that reserves of oil, coal and gas could be depleted as soon as 2040, 2042, and 2112, respectively (Shafiee and Topal, 2009). Renewable energy is likely to play a large role in meeting the world's energy needs in the future, and bioenergy has the potential to be an important part of this.

Bioenergy refers to energy that is derived from plant materials. This source of energy is essentially carbon-neutral, as it produces energy while only releasing carbon that has been captured during the growth of the plant, rather than carbon that has been locked up for millions of years in the form of fossil fuels (Sims et al., 2006). Bioenergy currently makes up 13.4% of the world's energy usage; most of this comes from traditional biomass sources such as non-commercial firewood, charcoal and dung, which are often used for cooking and heating in developing countries (Sims et al., 2006). Dedicated bioenergy crops currently represent a small percentage of the overall energy supply from bioenergy. However, Sims et al. (2006) suggest that these dedicated energy crops could meet a significant portion of the world's energy needs within the next half-century. In their analysis, which was based on potential energy crop areas for each IPCC SRES scenario in 2025, they found that these crops could realistically provide between 2 and 22 EJ of energy per year, which could account for almost 5% of global energy needs and offset up to 2070

Mt of CO₂ equivalent per year. Other studies support the idea that biomass could produce a large amount of the world's energy in the future and agree that increasing the overall contribution of bioenergy will require the active growth of energy crops, rather than just relying on traditional biomass sources (Hall and Rosillo-Calle, 1998; Hoogwijk, 2004). While Sims et al. (2006) acknowledge that bioenergy crops alone will not be sufficient to stabilize atmospheric CO₂ concentrations, the potential exists for this energy source to contribute to future climate change mitigation.

1.3 Perennial Grasses as Bioenergy Crops

A wide variety of plants have been studied as potential bioenergy crops. These include annual grains such as wheat (*Triticum*), maize (*Zea mays*) and sugarcane (*Saccharum officinarum*); perennial rhizomatous grasses such as switchgrass (*Panicum virgatum*), miscanthus (*Miscanthus sacchariflorus*), little bluestem (*Andropogon gerardii*) and big bluestem (*Schizachyrium scoparium*); and fast growing woody plants such as willows (*Salix*) and poplars (*Populus*) (Karp and Shield, 2008; Kole et al., 2012).

Karp and Shield (2008) investigated a wide range of bioenergy crops and their abilities to produce sustainable yields. A sustainable yield refers to the ability of a crop to maintain high levels of productivity over long periods of time. Yield rates and efficient growth strategies are key aspects of successful bioenergy crops. While woody plants have higher lignin content and are more energetically dense (Clinch et al., 2009; Volk et al., 2006), herbaceous species, such as grasses, are often considered to be more suitable bioenergy crops. Karp and Shield (2008) noted that herbaceous plants generally have higher yield rates than woody plants, especially when nitrogen (N) fertilizer is applied, and

they can be easily managed with common farming equipment. Perennial herbaceous species were found to be particularly advantageous. These species have the advantage of being able to be harvested annually, and they are also able to produce higher yields in low-N conditions than annual species, due to their ability to efficiently remobilize reserves. These perennial species have greater nutrient use efficiency, as they are able to achieve similar yields to annual species with 20-50% less fertilizer applied. They can be grown more effectively on marginal lands than other bioenergy crops due to their efficient recycling of nutrients, reducing competition with food crops over land. Perennial grasses have also been found to be relatively unaffected by pests and pathogens in temperate climates.

1.3.1 Switchgrass

Switchgrass (*Panicum virgatum*) is one of the most commonly studied bioenergy crops, particularly within North America. It is an herbaceous warm-season plant native to North America, and like other warm-season grasses, switchgrass uses the C₄ photosynthetic pathway (Heaton et al., 2004). This pathway allows plants to enhance the efficiency of converting solar radiation to stored biomass energy (Long, 1994). C₄ plants are able to use more of the available light energy, and they are able to convert this light energy to stored energy much more efficiently than plants that utilize the C₃ pathway (Caemmerer and Furbank, 2003; Ehleringer et al., 1992). Radiation use efficiency experienced by C₄ plants can be up to 40% greater than C₃ plants (Long, 1999).

Switchgrass has a number of features that make it a good bioenergy crop. The species is able to tolerate a variety of environmental conditions, and it has a very wide

geographical distribution in North America, from approximately 20°N latitude to 55°N latitude (McLaughlin and Walsh, 1998). It generally maintains high productivity throughout this wide range, although its effectiveness as a bioenergy crop in the most northern parts of its range may be limited by colder temperatures and shorter growing seasons (Lewandowski et al., 2003; Madakadze et al., 1998). The crop is also easily integrated into conventional farming practices, as it can be managed with common farming equipment (Lewandowski et al., 2003).

Lignocellulosic biomass is generally considered to be better for bioenergy production, and switchgrass is comprised of more than 60% lignocelluloses (Ghosh and Prelas, 2011; Samson et al., 2005). Additionally, switchgrass can be used as a viable bioenergy source through the densification of the grass into a pellet form (Samson et al., 2000). In this pellet form, Samson et al. (2000) argue that switchgrass offers the highest net energy yield per hectare, the highest energy output to input ratio, the greatest economic advantage over fossil fuels and the most significant potential to reduce greenhouse gas emissions.

Many studies have indicated that switchgrass as a bioenergy crop may have several positive environmental consequences. McLaughlin and Walsh (1998) found that these crops can improve soil quality and stability, provide cover for wildlife, and grow with limited energy, water and fertilizer inputs. McLaughlin et al. (1994) found that switchgrass adds significant amounts of organic matter to soils through their rooting systems. This improves soil structure, increases water-holding capacity and infiltration through changes to soil structure and porosity, improves nutrient availability, and decreases soil erosion (McLaughlin and Walsh, 1998; Hall, 1991).

Sanderson et al. (1996), in a study in the United States, examined the possibility that phenotypic selection could lead to the development of new cultivars with desirable traits that could result in improved biomass yield. Their projects focused on switchgrass breeding and physiology, and included field trials. The identification of key beneficial traits allowed them to develop high yielding cultivars, improved management practices, and improved knowledge of key physiological attributes that may limit yields. While these traits varied depending on the location and environmental conditions, this study helped show that it is possible to create high yielding bioenergy crop cultivars using various crop improvement strategies.

Optimal establishment practices vary between regions, but there are many known ways to maximize the success of switchgrass crops in the initial planting year. N fertilization at planting is inadvisable, due to the ability of switchgrass to effectively scavenge for nutrients (Wolf and Fiske, 1995). Switchgrass also requires a soil pH level of 5.0 or more. Other establishment strategies include using seed of known viability, and limiting weeds (Wolf and Fiske, 1995). Switchgrass typically attains 33-66% of maximum production within the first two years of growth. Full production capacity is often not reached until the third year after it has been planted (McLaughlin and Kszos, 2005).

In a review of a 10-year study on switchgrass by the United States Department of Energy, McLaughlin and Kszos (2005) found that switchgrass crops yielded approximately 13-23 Mg per hectare in the southern United States, and approximately 9-13 Mg per hectare in the central and northern United States. The decline in yield as the crops move north is mainly due to the shorter growing season. Additionally, they found that breeding research has resulted in switchgrass yield gains of 1-7% per year, which is significantly higher than

the 0.7-1.2% yield gains associated with corn breeding. Optimizing cutting frequency and timing can also play an important role in maximizing yield. A single-cut system is generally advisable, particularly in arid conditions (Sanderson et al., 1999). Where two cut systems are used, it is important to time the first cut in July, or later (McLaughlin et al. 1999). In their review, McLaughlin and Kszos (2005) also found that reducing N fertilization levels by approximately 40% also helped maximize crop yields; while fertilization increases biomass productivity to a certain point, excess fertilizer can limit the GHG benefits of bioenergy systems. This study concludes that switchgrass energy crops compare favourably as an agricultural crop and a fuel source to most other potential bioenergy sources.

1.3.2 Little Bluestem and Big Bluestem

While not as commonly studied as switchgrass, little bluestem (*Andropogon gerardii*) and big bluestem (*Schizachyrium scoparium*) may have higher yield potentials than switchgrass (Gonzalez-Hernandez et al., 2009). These species are particularly productive in marginal areas with dry soils. Little bluestem is one of the most abundant grass species throughout the prairies. Weaver (1960) studied 63 typical prairies in the central and northern United States and found that little bluestem made up 58% of the grassland cover, with big bluestem making up 38%. In the driest areas of this region (61-66 cm of annual precipitation), little bluestem and big bluestem composed 69% and 28% of the grassland, respectively. These species are dominant in the southern plains of the US, with geographic ranges extending north to the Dakotas and southern Manitoba. Many of the attributes that have made switchgrass such an attractive and widely studied bioenergy

crop are also exhibited by little bluestem and big bluestem, and thus, Gonzalez-Hernandez et al. (2009) argue that knowledge of local environmental conditions and possible limitations due to biotic and abiotic stresses should play an important role in the choice of a bioenergy crop for each region.

Little bluestem and big bluestem are also warm-season C4 plants that are able to survive over a wide geographic range (Gonzalez-Hernandez et al., 2009). They can attain their maximum root growth in shallow soils, allowing them to thrive in areas with decreased soil depth (Van Auken et al., 1994). They are also very successful in dry soils, and can survive drought conditions (Zhang et al., 2014). This may make them a suitable bioenergy crop in dry areas, as well as areas with fluctuating climatic conditions. Additionally, like switchgrass, little bluestem and big bluestem require minimal N inputs. Propheter and Staggenborg (2010), found that big bluestem, along with other perennial grasses, required only one tenth of the N fertilization levels that are required by annual crops. These perennial crops were also found to have soil N removal rates that are approximately 5 times lower than annual crops.

Little bluestem and big bluestem are well adapted to lands that are considered to be class V land under the Land Capability Class system (Gonzalez-Hernandez et al., 2009). This class refers to land that is unsuitable for cultivation for reasons such as salinity or frequent flooding (Gonzalez-Hernandez et al., 2009). In the United States alone, there are 13.2 million hectares of class V land that could potentially be used for these bioenergy crops. Using this marginal land, which is unable to be cultivated, for bioenergy crop production may help reduce the need for these crops to have to compete with food

production and other forms of land use (Gamborg et al., 2012; Gonzalez-Hernandez et al., 2009).

A number of ethical concerns have been raised regarding bioenergy production. Gamborg et al. (2012) suggested that these ethical issues are often unclear, and they attempted to analyze them from a land use perspective. Bioenergy production gives rise to ethical problems primarily because it competes with other forms of land use, which can be problematic for two reasons. First, bioenergy production may compete with food production, either directly or indirectly. Second, the production of bioenergy may lead to environmental issues such as deforestation and other land use changes, which can negatively affect GHG's. These issues are important to consider when working with bioenergy systems. Ethical problems associated with bioenergy production can be minimized by using plant species that have limited negative environmental impacts, and by using soils that are not suitable for food production.

1.4 Soil Greenhouse Gas Emissions

Carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O) are the three most important anthropogenic GHG's in the atmosphere, and the concentration of all three gases is increasing rapidly. As of 2011, the concentration of atmospheric CO₂ was 390.5 ppm (an increase of 40% since 1750), the concentration of atmospheric CH₄ was 1803.2 ppb (an increase of 150% since 1750), and the concentration of atmospheric N₂O was 324.2 ppb (an increase of 20% since 1750) (IPCC, 2013). Of these three, N₂O is the most potent. Nitrous oxide has a global warming potential (GWP) of 310 over 100 years, compared to 21 for CH₄ and 1 for CO₂ (IPCC, 2007).

N_2O release from soils, through the processes of nitrification and denitrification, is a natural source of atmospheric N_2O (IPCC, 2007). Both nitrification and denitrification are driven by microbial activity. Nitrification is a two-step process that generally occurs under aerobic conditions, involving the oxidation of ammonium (NH_4^+) first to nitrite (NO_2^-), and then to nitrate (NO_3^-) (Norton, 2008). The first reaction is generally catalyzed by *Nitrosomonas* bacteria, and the second by *Nitrobacter* bacteria (Norton, 2008). The second reaction occurs quickly after the first, and nitrite rarely accumulates to high concentrations in the soil, except in heavily fertilized and high pH soils (Schmidt, 1982). Denitrification involves the opposite reaction, in which NO_3^- is reduced to N gases, including N_2O and dinitrogen gas (N_2). This process is an incomplete reduction, and while N_2 is the end product of the complete reaction, denitrification generally results in both gases (N_2 and N_2O) being released in varying amounts. Denitrification usually requires anaerobic conditions, so that microbes reduce nitrogen oxides rather than oxygen gas (O_2) in the soil (Coyne, 2008).

Many studies have investigated the controls over the relative proportions N_2O and N_2 produced during denitrification. The availability of organic carbon as an electron donor has an effect on the distribution of products, with more available carbon resulting in more N_2O relative to N_2 (Firestone and Davidson, 1989). A study by Dendooven and Anderson (1995) found that the relative proportions of the two gases is also influenced by the length of the aerobic period prior to the onset of anaerobic conditions that favour denitrification. This period of aerobic conditions affects the concentration of reduction enzymes involved in the denitrification process. Without any preceding period of aerobic conditions, the proportion of N_2 relative to N_2O was found to be 49%, but this was found to decrease to

28% after 3 days of aerobic conditions, 21% after 49 days and 0% after 70 days. These results indicate that soils with fluctuating moisture levels are likely to emit more N_2O than soils that are constantly anaerobic.

Approximately 40% of current atmospheric N_2O originates from anthropogenic sources, and emissions from agricultural soils are the major source of this anthropogenic N_2O (IPCC, 2013). Nitrification and denitrification are also controlled by soil mineral N availability. N fertilizer often results in increased N_2O emissions, as it increases the amount of available mineral N, which increases the rates of nitrification and denitrification (Dobbie et al., 1999; Kaiser et al., 1998). N_2O emissions are generally highest in the period immediately following fertilizer application, and after large precipitation events (Dobbie et al., 1999). Agricultural N_2O emissions are highly variable and depend on soil properties such as temperature, soil moisture and mineral N availability; it is estimated that 0.7 to 6.8% of N applied as fertilizer is lost as N_2O (Bouwman, 1996). However, this number may be lower in extremely dry climates. A study done by Barton et al. (2010) in a semi-arid region of southwestern Australia resulted in only 0.06% of the applied N fertilizer being emitted as N_2O . This indicates that soil GHG fluxes from bioenergy crops in more arid regions are likely to have less of an influence on the net climate system impact of bioenergy crop production than those in more temperate regions (Barton et al., 2010).

Many studies have indicated that that switchgrass requires N fertilization in order to achieve desirable yields (Madakadze et al., 1999; McLaughlin and Kszos; 2005 Samson, 2007), and it is believed that other bioenergy crops with similar traits require fertilization as well. A field study conducted by Madakadze et al. (1999) in eastern Canada, examined the productivity of switchgrass under three different fertilization treatments: 0, 75 and 150 kg

ha⁻¹. For the one-cut system studied, the 75 and 150 kg ha⁻¹ treatments produced 11% and 41% more biomass, respectively, than the 0 kg ha⁻¹ treatment. While fertilization rates of 100-300 kg ha⁻¹ are often used in warmer climates, such as those in the southern United States, many bioenergy crops are overfertilized. McLaughlin and Kszos (2005) argued that reducing the level of nitrogen fertilization by approximately 40% could result in higher switchgrass yields in some areas of the United States. A fertilization rate of approximately 50 kg ha⁻¹ is generally considered to be acceptable for bioenergy crops in Canada (Samson, 2007). However, optimal fertilization rates are largely dependent on a number of soil properties, such as temperature, moisture and available mineral N. It is thus impossible to establish a standard fertilization rate for bioenergy crops, and each fertilization application must be tailored to the conditions of the specific field site being examined.

Some evidence suggests that agricultural N₂O emissions do not increase linearly relative to the fertilizer application rate. A study by Kaiser et al. (1998) investigated this by measuring N₂O emissions for crops that were fertilized at three rates of N application: the usual amount of fertilization (200 kg ha⁻¹), half the usual amount of fertilization (100 kg ha⁻¹), and no fertilization. This study was done at a field site in Germany, with the usual amount of fertilization referring to the typical application level used at this site to achieve economically desirable yields. While N₂O emissions were highest on the plot fertilized at the usual level, there was not a significant difference in N₂O emissions between the other two plots. The results of this study also showed that significant N₂O emissions could occur in agricultural fields that are not fertilized.

Kaiser et al. (1998) also investigated the temporal variability of N₂O release at this agricultural field site. The study, which measure N₂O release rates over a continuous 33-

month period, found that 50% of the annual emissions occurred during the winter months (October to February). This was attributed to the physical release of subsurface N₂O when the soil froze as well as microbial N₂O release during the daily freezing and thawing cycles. Winter N₂O release also increased with increased N content in the soil. The temporal variability of N₂O release is dependent on a number of factors, including available soil N and soil temperature (Kaiser et al., 1998).

N fertilization does not appear to have a significant impact on CH₄ and CO₂ emissions from bioenergy crop soils. A study by Barton et al. (2010), which investigated both fertilized and unfertilized bioenergy crop plots at a field site in Australia, found no significant differences in CH₄ emissions between the treatments. Similar results have been found by studies done in more temperate climates (Drewer et al., 2012). A study conducted by Masse et al. (2010) investigated CH₄ emissions from a switchgrass crop grown in Eastern Canada. This study utilized both a one-cut and two-cut harvesting strategy, and found that 25% more CH₄ per hectare was produced by the two-cut strategy. These results indicate that a single-cut strategy is advisable from a GHG emission perspective, providing further evidence that this strategy is optimal in temperate climates (Masse et al., 2010; Sanderson et al., 1999). CH₄ production in perennial grass bioenergy crop fields is fairly small compared to other crops (Masse et al., 2010).

1.5 Summary

While several other studies have explored the greenhouse gas benefits of perennial grass bioenergy systems, there is still some uncertainty about how these crops should be managed to maximize benefits to the climate system. Discrepancies in suggested

fertilization rates and biomass productivity highlight the importance of local environmental conditions on biomass productivity and fertilization effectiveness. This makes it very important to conduct research on bioenergy crops in new locations, before large-scale bioenergy production is implemented. The results of this study will help us understand the impacts of nitrogen fertilizer on biomass production and soil GHG emissions under different grass species, as well as the relationship between GHG fluxes and several environmental variables in Bath, Ontario. This will allow us to optimize bioenergy crop production and the GHG emission benefits of this bioenergy system.

2.0 Methods

2.1 Experimental Area

The sampling area for this study consisted of four 120 x 120 metre fields, or blocks, near the Lafarge Cement Factory in Bath, Ontario. Each of these blocks consisted of four 120 x 30 metre strips; three of these strips contained a different grass species (switchgrass (*Panicum virgatum*), little bluestem (*Andropogon gerardii*) or big bluestem (*Schizachyrium scoparium*)), and the fourth was a control with no grass species planted. Three 10 x 10 metre plots were located within each strip. Each small plot randomly received one of three fertilization levels: 0 lbs/acre, 50 lbs/acre or 150 lbs/acre of nitrogen as urea. Within each plot a collar (20 cm diameter by 15 cm deep) was inserted into the soil for connecting to the chambers. For each collar, roughly 5cm of the collar was left aboveground for attachment of the chamber. The same collars were used in both years of sampling.

2.2 Experimental Design

At the beginning of June in 2012 and 2013, the fields were mowed, and the plots were then fertilized with the appropriate amount of fertilizer in the form of urea ($\text{CH}_4\text{N}_2\text{O}$). The same plots were used in 2012 and 2013. After the initial mowing and fertilization of the plots in early June, gas flux and soil samples were collected from each plot every 1 to 2 weeks from June to September. Soil samples were taken starting from the day of fertilization in 2013 (June 5; Julian Day 156), while gas samples were taken starting one week after fertilization (June 12; Julian Day 163). Gas samples were taken to determine fluxes of CO_2 , CH_4 and N_2O from the soil. Soil samples were taken to determine soil

moisture content and soil pH. All samples were taken at least 24 hours after any major precipitation occurred.

2.3 Gas Flux Measurements

Gas flux measurements were made by placing a cylindrical chamber (20 cm diameter, 4.5 litre volume) on the collars that were inserted 10cm into the ground. Each chamber was outfitted with a vent designed to maintain atmospheric pressure in the chamber during sampling and a syringe connected to a 3-way valve that had a needle fitted on one port. Vent length and diameter were based on data from Hutchinson and Mosier (1981). At the start of the sample collection, chambers were sealed onto the top of the collars using rubber gaskets. Gas samples were collected from the chambers after 0, 10, 20 and 30 minutes to measure changes in concentration over time. Samples were collected by drawing 25 mL of gas from the chamber using a syringe attached to the 3-way valve, and then injecting into gas vials using the needle attached to the valve port. Air temperature, atmospheric pressure, wind speed, and wind direction were also recorded at each plot at the time of sampling, using a weather meter (Kestrel 3500). Gas samples were stored at room temperature, and were analyzed using a gas chromatograph (Shimadzu GC-2014), which determined the CO₂, CH₄ and N₂O concentrations of each sample. CO₂ and CH₄ concentrations were measured using a flame ionization detector, and N₂O concentrations were measured using an electron capture detector (Shimadzu Manual, 2012).

To calculate gas fluxes, concentration measurements in parts per million (ppm) of N₂O, CH₄ and CO₂ had to be converted to moles. This was done by first calculating the

number of moles of each gas in the chamber at each sample time using the ideal gas law (equation 1):

$$n \text{ air mol} = (P \text{ (hPa)} \times V \text{ (L)}) / (R \text{ 83.14472} \times T \text{ (K)}) \quad (1)$$

where n is the number of moles of air in the headspace, P is the atmospheric pressure in hectopascal (hPa), V is the volume of air in the headspace in litres (L), T is the air temperature in kelvins (K), and R is the Universal Gas Constant. A gas flux value was then calculated for each chamber using equation 2:

$$\text{Flux (nmol/m}^2\text{s)} = n \text{ air mol} \times (\text{umol/mol}) \text{ ppmv} \times 10^9 \times 1 / (A \text{ m}^2 \times 60) \quad (2)$$

where ppmv is the slope of the linear trend line for the four gas measurements taken at different times at each chamber (Figure 2.1), and A is the area of the collar in square metres (m^2).

Figure 2.1. An example of a plot used to generate N₂O flux values.

To calculate a total GHG flux value that included all the gases, individual GHG fluxes for each plot were multiplied by their GWP and then added together. The GWP values used were 310, 21 and 1 over 100 years for N₂O, CH₄ and CO₂, respectively (IPCC, 2007). The resulting value gave a total GHG flux value for each plot, in CO₂eq.

2.4 Soil Temperature

Soil temperature was determined using an electronic temperature probe (Thermor (Model #PS100)). Soil temperatures were measured at 10 cm depth within 0.5 m of the collar in each plot.

2.5 Soil Moisture

Soil samples (2cm diameter) were collected to a depth of 10 cm. Three cores were taken randomly from within the 10 x 10 metre plot. These soil samples were then sieved using a 2 mm sieve. Approximately 10 g of sieved soil was weighed out into a small container and oven dried for 24 hours at 105 degrees Celsius. These 10 g samples were then weighed again after drying, to determine the soil moisture content.

2.6 Soil pH

Ten grams of sieved soil were measured into small plastic containers. Ten ml of deionized water was added to each container and then shaken for two minutes at 150 rotations per minute on a mechanical shaker. A pH meter (Oakton pH/CON 5-10 Series) was then used to measure the pH of each sample. The pH meter was calibrated after every ten samples using buffered solutions of pH 4, 7 and 10.

2.7 Soil Available Nitrogen Concentration

Using the sieved soil, 10g of soil was measured from each sample into small (120 ml) plastic containers. Seventy-five ml of 2M KCl was then added to each sample. The plastic containers were then placed on a mechanical shaker for 45 minutes at 250 rotations per minute. Samples were then filtered through 45 μ m filter paper (Whatman 40 equivalent) placed inside small plastic funnels. The filtrate was collected in plastic scintillation vials, with the first few drops of each sample discarded, as this material is likely to contain any contaminants in the filter paper. Samples that were not transparent, due to soil contamination of the filtrate, were re-filtered. Two blank samples consisting of 2M KCl only were filtered for each sampling date. Samples were then frozen for future analysis.

2.8 Biomass Sampling

Biomass samples were collected from each plot in the fall of 2012 by locating a 1 x 1m quadrat within each plot, and collecting all the biomass within each quadrat. Samples were weighed in the field in large paper bags, and a subsample was removed for moisture determination. In the lab, biomass subsamples were dried at 60° C until the sample weight stabilized (approximately 48 hours). Subsample moisture values were used to calculate the dry weight of the larger samples. Biomass sampling was not conducted after the 2013 growing season due to weather conditions.

2.9 Statistical Analysis

All data analysis was done using R 2.15.2 statistical software. All individual variables (N_2O flux, CH_4 flux, CO_2 flux, total GHG flux, moisture, temperature and pH) were first tested for normality for each sampling date using the Shapiro-Wilk Test of Normality. Normality tests for flux values were further separated by fertilization level. Variables were then tested for homogeneity of variance for each individual sampling date using Levene's Test. Homogeneity tests for gas flux values were also separated by fertilization level, within each sampling date. Data were divided into these different groups as it would not be expected for the data to satisfy these assumptions given the large effects of some of the treatments, and the large differences in values over time (for some of the variables). Between subject treatment effects (grass species, fertilization level) were compared across sampling dates using repeated measures ANOVA. A post hoc test for fertilization rate and grass species was done using the Tukey HSD test, but only applied when there was an overall significance. For biomass data, a two-factor ANOVA (grass species, fertilization level) was used. Correlation tests were conducted to determine the relationships between the gas flux rates and the various environmental variables. These tests were conducted using data from all ten sampling dates.

3.0 Results

3.1 Normality and Homogenous Variance Tests

A majority of the normality tests for soil gas fluxes passed ($p > 0.05$) (Appendix I). Fourteen of 27 tests run on the N_2O flux data passed (51.85%), 20 of 27 tests passed for CH_4 fluxes (74.07%), 26 of 27 tests passed for CO_2 flux data (96.30%), and 13 of 27 tests passed for the total GHG flux results (48.15%). Flux data were then $\log_{10}$ transformed to see if results for the normality tests improved. This transformation did not result in a noticeable increase in the number of normality tests being passed, so the original untransformed data were used. A majority (68.97%) of the normality tests run on the environmental variables (soil temperature, moisture, and pH) passed as well.

A majority of the tests for homogeneity of variance passed as well ($p > 0.05$) (Appendix I). Twenty-two of 27 tests passed for N_2O fluxes (81.48%), 26 of 27 tests passed for CH_4 fluxes (96.30%), 25 of 27 tests passed for CO_2 flux results (92.59%), and 21 of 27 tests passed for the total GHG flux data (77.78%). Like the normality tests, the $\log_{10}$ transformation of gas flux data did not make a noticeable improvement in the results of the homogeneity of variance tests, so the untransformed data were used. Almost all (96.55%) of the homogeneity of variance tests passed for the environmental variables. The biomass data also passed both the normality ($p = 0.855$) and homogeneity of variance tests ($p = 0.076$). The relative success of these tests allowed me to use parametric statistical tests to explore treatment effects on soil gas fluxes (Appendix II).

3.2 Gas Fluxes

N₂O fluxes changed significantly over the sampling period ($p < 0.001$). N₂O fluxes, particularly for the fertilized treatments, were higher early in the growing season compared to the latter portion. They were also significantly different between fertilization treatments (Figure 3.1a; $p < 0.001$). N₂O fluxes were significantly higher for the 150 lbs/acre treatment than the 0 and 50 lbs/acre treatments ($p < 0.001$), but did not differ between the 0 and 50 lbs/acre treatments ($p = 0.847$). N₂O fluxes also did not differ for the different grass species (Figure 3.1b; $p = 0.804$).

Figure 3.1. Average N₂O fluxes over the sampling period for a) fertilization treatments and b) grass species.

CH₄ fluxes did not exhibit any significant change over the sampling period ($p=0.299$).

They were also not significantly different between the different fertilization treatments

(Figure 3.2a; $p=0.552$) or the different species treatments (Figure 3.2b; $p=0.436$).

Figure 3.2. Average CH₄ fluxes over the sampling period for a) fertilization treatments and b) grass species.

CO₂ fluxes changed significantly over the sampling period ($p < 0.001$), increasing over the first few weeks of June before declining over the remainder of the growing season. There was no significant difference in CO₂ fluxes between the different fertilization treatments (Figure 3.3a; $p = 0.307$). However, CO₂ fluxes were significantly different between species treatments (Figure 3.3b; $p < 0.001$). CO₂ fluxes were significantly higher in the control plots than the plots with switchgrass ($p < 0.001$), big bluestem ($p < 0.001$) and little bluestem ($p < 0.001$).

Figure 3.3. Average CO₂ fluxes over the sampling period for a) fertilization treatments and b) grass species.

Total GHG fluxes exhibited significant change over the sampling period ($p < 0.001$), being highest early in the growing season primarily because of differences in N_2O fluxes (Figure 3.4a). These GHG fluxes were significantly different between the fertilization treatments as they were significantly higher for the 150 lbs/acre treatment than the 0 and 50 lbs/acre treatments ($p < 0.001$ and $p = 0.009$, respectively). Again, this was largely a result of the high N_2O fluxes early in the growing season. Total GHG fluxes did not differ between the 0 and 50 lbs/acre treatments ($p = 0.529$). Total GHG fluxes were also significantly different between species treatments (Figure 3.4b; $p < 0.001$), being highest in the control treatment compared to any other treatment ($p < 0.001$ for all species comparisons).

Figure 3.4. Average total GHG fluxes over the sampling period for a) fertilization treatments and b) grass species. Total GHG fluxes were calculated by multiplying each individual gas flux value by their GWP, and then adding these values together for each plot.

3.3 Environmental Variables

Soil temperature changed significantly over the sampling period ($p < 0.001$). Soil temperature increased over the first five sampling dates, before decreasing over the latter half of the growing season (Figure 3.5). Soil temperature was significantly different between the fertilization treatments (Figure 3.5a; $p < 0.001$). It was also significantly different between the different species treatments (Figure 3.5b; $p < 0.001$). The temperatures of the control plots were significantly higher than in any of the perennial grass plots ($p < 0.001$), but there was no significant difference among the perennial grass species.

Figure 3.5. Average soil temperature over the sampling period for a) fertilization treatments and b) grass species.

While soil moisture changed significantly over the sampling period ($p < 0.001$), it did not differ by fertilization treatments (Figure 3.6a; $p = 0.992$). Moisture percentages were significantly different between the different species treatments ($p < 0.001$), as moisture was significantly lower in plots with little bluestem than those with switchgrass ($p < 0.001$), big bluestem ($p = 0.007$) and the control plots ($p = 0.004$) (Figure 3.6b).

Figure 3.6. Average soil moisture over the sampling period for a) fertilization treatments and b) grass species.

Soil pH changed significantly over the sampling period ($p < 0.001$). Soil pH was significantly lower in the 150 lbs/acre treatment (Figure 3.7a; $p < 0.001$), and was significantly higher in the 0 and 50 lbs/acre treatments ($p < 0.001$). Soil pH was also higher in the 0 lbs/acre treatment than the 50 lbs/acre treatment ($p < 0.001$). Soil pH also differed significantly among the different grass species ($p = 0.001$), with pH values of the control plots being significantly higher than those of switchgrass ($p = 0.007$), big bluestem ($p = 0.010$) and little bluestem ($p = 0.004$) (Figure 3.7b).

Figure 3.7. Average soil pH over the sampling period for a) fertilization treatments and b) grass species.

3.4 Biomass Production

The average biomass yield for all treatments was 4.20 Mg ha^{-1} (Figure 3.8). Two strips that exhibited minimal biomass growth were considered to be outliers, and they were removed for the purposes of this analysis. Biomass yield did not differ among the fertilization treatments ($p=0.382$) or the different species treatments ($p=0.106$) (Figure 3.8).

Figure 3.8. Biomass production (in Mg/ha/year) for a) fertilization treatment and b) grass species. Error bars represent one standard deviation. There was no significant difference in biomass production between the different fertilization and species treatments.

3.5 Relationships Between Environmental Variables and Gas Fluxes

Correlation tests were run to examine how variation in various gas fluxes (N_2O , CH_4 , CO_2 and total GHG) are explained by various environmental factors (Table 3.1). For the environmental variables and gas fluxes, data from all sampling dates was used.

Table 3.1. The relationships and correlation coefficients (R-values) between the gas fluxes and environmental variables.

<i>N₂O Fluxes</i>		
Variable	Relationship	R-value
Temperature	Negative	0.226
Moisture	Positive	0.117
pH	Negative	0.087
<i>CH₄ Fluxes</i>		
Variable	Relationship	R-value
Temperature	Negative	0.048
Moisture	Positive	0.054
pH	Positive	0.037
<i>CO₂ Fluxes</i>		
Variable	Relationship	R-value
Temperature	Positive	0.500
Moisture	Negative	0.200
pH	Negative	0.140
<i>GHG Fluxes</i>		
Variable	Relationship	R-value
Temperature	Positive	0.136
Moisture	Negative	0.033
pH	Negative	0.157

Correlation analyses show a strong correlation between CO_2 fluxes and temperature (Figure 3.9; $r=0.500$). Methane fluxes correlated poorly with the environmental variables (Table 3.1).

Figure 3.9. Correlation plot between CO₂ flux and soil temperature ($r=0.500$).

4.0 Discussion

4.1 Differences Between Fertilization Treatments

Both soil gas fluxes and soil environmental characteristics varied in response to fertilization. While N_2O fluxes did not differ between the 0 and 50 lbs/acre treatments ($p=0.847$), fluxes were significantly higher for the 150 lbs/acre treatment than either the 0 and 50 lbs/acre treatments ($p<0.001$) (Figure 3.1a). Urea first forms NH_4 in soil, which then gets nitrified to NO_3 . N_2O fluxes are driven by nitrification rates, and higher fertilization levels likely resulted in more available nitrogen, and thus, higher rates of nitrification. It is therefore not surprising to see higher N_2O fluxes in the more heavily fertilized plots.

Total GHG fluxes exhibited the same trends as N_2O fluxes. These GHG fluxes were significantly higher for the 150 lbs/acre treatment than the 0 and 50 lbs/acre treatments (Figure 3.4; $p<0.001$ and $p=0.009$, respectively). As CH_4 and CO_2 fluxes did not differ between the different fertilization treatments, N_2O fluxes appear to be the primary driving force behind this difference. This is apparent by looking at Figure 3.1a and Figure 3.4a; while GHG trends over the sampling period follow CO_2 trends in general, the stark differences in flux rates that can be seen at the beginning of the sampling period diverge sharply from the trends seen on the CO_2 graph (Figure 3.3a), closely resembling trends seen in Figure 3.1a.

Soil pH also varied significantly among the fertilization treatments (Figure 3.8a). These differences between the fertilization treatments can likely be attributed to differences in nitrification which, as previously mentioned, is an acidifying process (Van Miegroet and

Cole, 1984). After fertilization, pH dropped sharply in the fertilized plots, and pH continued to decline over several sampling dates. After this, pH values started to increase for these treatments, as nitrification rates likely decreased as the amount of ammonium available for nitrification likely declined. By the end of the sampling period, all soil pH values were once again fairly similar. Furthermore, the pH of the 0 lbs./acre fertilization treatment remained fairly steady over the course of the sampling period (Figure 3.7a), with the exception of a small drop after the first sampling date. Nitrification levels (and therefore N₂O fluxes) were much lower in this treatment than the others, and as such, the acidification of these plots was much less noticeable.

4.2 Differences Between Grass Species

Four of the variables measured (CO₂ flux, total GHG flux, soil temperature and soil pH) varied significantly between the control treatments and the other 3 grass species treatments. CO₂ fluxes were significantly higher in the control treatment than the other three treatments (Figure 3.3b; $p < 0.001$). This may have been due to differences in root biomass, with higher root biomass in the control plots leading to higher respiration rates. Previous studies have shown that little bluestem and big bluestem are capable of attaining their maximum root depth in very shallow soils, and that they are able to thrive even when root length is limited (Van Auken et al., 1994). Switchgrass has been shown to have a very flexible root distribution strategy compared to other species (Xu et al., 2010). Lower CO₂ fluxes in the control treatment could also be due to litter being more decomposable. Alternatively, these differences could be due to the higher temperatures in these plots; temperatures were significantly higher for the control treatment (Figure 3.5b), and higher

soil temperatures are known to increase soil respiration rates (Davidson et al., 1998; Liu et al., 2014). Total GHG fluxes were also significantly higher in the control treatment than the other three treatments (Figure 3.4b; $p < 0.001$), following the patterns shown by the CO₂ flux results.

Soil temperature was higher in the control treatment than the other species treatments (Figure 3.5b; $p < 0.001$). This is largely due to the greater amounts of solar radiation reaching the soil surface in these plots. While biomass production in the different treatments did not differ, it is possible that plant growth in these plots created less of a canopy, and thus, allowed more radiation to reach the soil surface. Visual observations at the field site support this, as the grass species plots (switchgrass, little bluestem and big bluestem) had more dense canopies, and they were much more difficult to walk through than the control plots.

Soil pH values were also significantly higher in control plots than the plots with switchgrass ($p = 0.007$), big bluestem ($p = 0.010$) and little bluestem ($p = 0.004$) (Figure 3.7b). This may have been due to differences in organic inputs into the soil between the different treatments. Increased rates of nitrification in the grass species treatments could theoretically explain the differences in pH, but the fact that N₂O flux rates did not vary between the species treatments make it unlikely that this was a significant factor.

Interestingly, soil moisture was significantly lower in the little bluestem treatment than the switchgrass treatment ($p < 0.001$), the big bluestem treatment ($p = 0.007$) and the control treatment ($p = 0.004$) (Figure 3.6b). This is somewhat surprising. This result may indicate that little bluestem has lower water use efficiency than the other grass species studied, which contradicts previous studies that have shown little bluestem to be well

adapted to dry conditions (Gonzalez-Hernandez et al., 2009; Zhang et al., 2014). Averaged over the entire growing season, soil moisture for the little bluestem plots was approximately 24.6%, while the other species ranged from 26.8% to 27.5%; while the differences are significant, they are not particularly large. Additionally, with the exception of soil moisture, there were no significant differences between the different perennial grass species in terms of the environmental variables (soil temperature and pH), gas fluxes and biomass productivity.

4.3 Seasonal Changes

All variables, with the exception of CH₄ fluxes, exhibited significant change over the sampling period. N₂O fluxes decreased throughout the sampling period, as the time since fertilization increased (Figure 3.1; $p < 0.001$). These fluxes were highest at the beginning of the sampling period, in the period immediately following fertilization. It is likely that fertilization resulted in high levels of available nitrogen in the soil, which lead to high levels of nitrification. N₂O fluxes dropped sharply within 2 weeks of fertilization, and remained at close to baseline levels for the duration of the sampling period.

CH₄ fluxes did not significantly change over the sampling period (Figure 3.2; $p = 0.299$). This is consistent with previous literature that indicates that nitrogen fertilization does not influence CH₄ soil emissions (Barton et al., 2010; Drewer et al., 2012; Masse et al., 2010).

CO₂ fluxes exhibited significant change over the sampling period (Figure 3.3; $p < 0.001$). These fluxes increased until the third sampling date (Julian Date 177), and then declined over the rest of the sampling period. CO₂ fluxes are often related to temperature

and soil moisture, with higher temperatures and higher moisture levels resulting in increased soil respiration rates (Davidson et al., 1998; Liu et al., 2014). Temperature often has the largest influence on these rates. Soil temperature trends observed during the study period paralleled CO₂ flux results, as temperatures rose over the first three sampling dates, peaked in late June and early July, and then declined over the rest of the sampling period. CO₂ flux and temperature correlated positively ($r=0.250$) over the study period, suggesting an important role for soil temperature as a control on soil respiration rates.

Total GHG fluxes varied over the course of the sampling period (Figure 3.4; $p<0.001$). Total GHG fluxes generally mirrored the CO₂ trends, as CO₂ soil emissions contributed a much larger proportion of the overall GHG soil emissions than CH₄ and N₂O. The only exception to this general trend was the first sampling date (Julian Date 163), which occurred one week after fertilization. High N₂O fluxes for this sampling date resulted in higher GHG fluxes, particularly for the 150 lbs/acre fertilization treatment.

Soil temperature also changed significantly over the sampling period (Figure 3.5; $p<0.001$). Temperatures increased over the first several sampling dates, reaching a peak on July 9th (Julian Date 190). From that point on, the temperatures exhibited a steady decline. The increase over the early part of the sampling period can be attributed primarily to rising air temperatures, which caused the soil to warm. Decreased soil temperatures over the last five sampling dates likely occurred due to increased grass biomass. Air temperatures did not exhibit a noticeable decline over this period (Environment Canada, 2013), so decreased temperatures were likely due to an increase in grass biomass that inhibited soil radiation from warming the soil.

Soil moisture changed significantly throughout the sampling period (Figure 3.6; $p < 0.001$). Soil moisture did not, however, show any particular trend over the growing season. Soil moisture is largely dependent on precipitation events; and these events are unpredictable and varied over the course of the sampling period.

Soil pH also changed significantly throughout the sampling period (Figure 3.7; $p < 0.001$). pH declined over the first several sampling dates, and then increased steadily over the rest of the season. The relatively sharp pH decline at the beginning of the period is likely due to high levels of nitrification. Nitrification is an acidifying process (Van Miegroet and Cole, 1984), and nitrification rates were likely highest in the beginning of the sampling season following fertilizer application (Bremner and Blackmer, 1978). The particularly sharp drop in pH that occurred between the first and second sampling date is likely due to these extremely high rates of nitrification in the immediate aftermath of fertilization. The increase in pH over the rest of the sampling season was likely due to organic inputs, which likely increased as crop biomass increased, as well as slowing rates of nitrification.

4.4 Relationships Between Environmental Variables and Gas Fluxes

Most of the correlations between environmental variables, gas fluxes and biomass production were relatively low (Table 3.1). The strongest correlation was between CO₂ flux and temperature (Figure 3.9; $r = 0.500$). The strong positive correlation between CO₂ flux and temperature is not surprising, as previous studies have shown strong correlations between soil temperature and soil respiration rates (Davidson et al., 1998; Liu et al., 2014).

Soil moisture correlated weakly and positively with N_2O flux ($r=0.117$). Previous studies have shown that these two variables are more closely correlated when denitrification is the dominant N_2O -emitting process in soil (Dendooven and Anderson, 1995; Dobbie et al., 1999). This is because higher moisture levels generally result in more anaerobic soil conditions, which favours denitrification. The fact that there was only a weak correlation between these two variables in this study confirms the idea that nitrification is the dominant N_2O -emitting process in this fertilized bioenergy crop system.

4.5 Lack of Increased Productivity at Higher Fertilization Levels

A surprising result of this study was the fact that grass biomass production did not increase with fertilization. These results contradict results obtained in a number of other studies (e.g. Karp and Shield, 2008; Madakadze et al., 1999; McLaughlin and Kszos, 2005; Samson, 2007). This site may have a large amount of available nitrogen in the soil due to prior management, making increased fertilization rates mainly irrelevant. It is also possible that some other resource may be limiting productivity at this site, negating any effect of N addition. An important next step in this study will be analyzing the soil samples for nitrate and ammonium concentration; this may give a better indication of the amount of available nitrogen in the soil, and it may help explain these results. Additional fertilization experiments, perhaps including elements like phosphorus and potassium, may also be warranted.

Higher available nitrogen levels in the soil could result from nitrogen inputs from previous crops. In a typical bioenergy system, the entire crop is harvested at the end of the growing system. However, in this study, a year-end harvest did not occur. Only a 1 m^2

quadrat in each plot was sampled for biomass, and the rest of the crop was left on the field. This may have avoided any soil nutrient depletion, as nutrients may have simply been recycled back into the soil. Overall, these results highlight the importance of local environmental factors on bioenergy crop production, and the importance of investigating each site to determine how bioenergy crop production can be optimized at each specific location.

4.6 The Potential of Perennial Grasses to Offset GHG Emissions

Big bluestem contains about 17,680,000 Btu Mg⁻¹, which is slightly greater than the heating value of switchgrass (17,480,000 Btu Mg⁻¹) and little bluestem (17,270,000 Btu Mg⁻¹) (Clark and Preto, 2011; Kidnie, 2009). Burning bituminous coal, one of the most commonly used types of coal, has a heating value of approximately 27,500,000 Btu Mg⁻¹ (Longwell et al., 1995). From these values, it can be concluded that it takes 1.55, 1.57 and 1.59 Mg of big bluestem, switchgrass and little bluestem, respectively, to produce the same amount of energy as 1 Mg of coal. Furthermore, since every Mg of coal that is burned produces approximately 2.86 Mg of CO₂ (McParland, 2011), every 1.55, 1.57 and 1.59 Mg of big bluestem, switchgrass and little bluestem, respectively, can offset 2.86 Mg of CO₂ emissions by replacing coal as an energy source.

The average biomass yield for the big bluestem, switchgrass and little bluestem plots were 3.26, 4.99, and 3.61 Mg ha⁻¹, respectively. By multiplying these values by the amount of each crop that can replace 1 Mg of coal, it was found that that each hectare of big bluestem, switchgrass and little bluestem grown at this site has the potential to offset 6.02, 9.21, and 6.49 Mg of CO₂ emissions, respectively. While this may seem to indicate

that switchgrass is the best perennial grass species for bioenergy production at this site, it is important to note that there were no significant differences in biomass production among the different species. There was a great deal of variability in biomass production (Figure 3.9b), and the results of this study do not conclusively indicate that a particular bioenergy species is best suited for bioenergy production at this location.

Since nitrogen fertilizer did not significantly increase grass production, the results of this study indicate that using no fertilizer will result in the largest GHG gains at this site. As previously mentioned, however, these results contradict a number of different studies at different field sites (Karp and Shield, 2008; Madakadze et al., 1999; McLaughlin and Kszos, 2005; Samson, 2007). This does not necessarily invalidate the results of this study; bioenergy systems are notoriously sensitive to local environmental factors, and it is certainly possible that nitrogen fertilization is not beneficial at this particular site. However, quantifying the amount of GHG's released at higher fertilization levels can still be useful, as it can help determine how much more productive these plots would have to be in order to offset the increased GHG's emitted from the soil as a result of increased nitrogen fertilization. To calculate this, the 0 and 150 lbs./acre treatments were examined, as there was no significant difference in total GHG fluxes between the 0 and 50 lbs./acre treatments. Average GHG fluxes from each fertilization treatment were first calculated for each of the nine sampling dates. A representative time period was then established for each sampling date; this time period generally consisted of approximately 3.5 days on either side of sampling dates in June and July (when sampling dates were approximately one week apart), and approximately 7 days on either side of sampling dates in August (when sampling dates were approximately two weeks apart) (Appendix III). The number of

seconds was then calculated for these established time periods to convert flux rates to total ecosystem losses and gains. Flux values from the sampling periods were then summed for both fertilization treatments.

These calculations found that 3.23 Mg more of CO₂eq were emitted by the 150 lbs/acre fertilization treatment than the 0 lbs/acre fertilization treatment (Appendix III). This figure is significantly higher than previous studies (McParland, 2011). This may be due to the fact that this study sampled closer to the time of fertilization than other studies, which is when N₂O fluxes are highest. For this higher fertilizer level to be beneficial, it would have to increase biomass productivity enough to offset these additional emissions. Assuming that this site is capable of producing 3.26, 4.99, and 3.61 Mg ha⁻¹ of big bluestem, switchgrass and little bluestem, respectively, at a fertilization level of 0 lbs/acre (these are the overall averages achieved at this site, where there was no significant difference between fertilization levels). Biomass production for these species would need to be raised to 5.01, 6.83 and 5.40 Mg ha⁻¹ when fertilized at 150 lbs./acre in order to offset the additional GHG emission due to the nitrogen fertilizer. These calculations support the idea that nitrogen fertilizer is unlikely to be beneficial at this site.

Additionally, the proportion of nitrogen fertilizer that was emitted as N₂O was quantified. This was done by quantifying N₂O emissions over the entire growing season, and then dividing this figure by the amount of N fertilizer added at the beginning of the season (Appendix IV). It was determined that for the most fertilized plots, approximately 9.52% of the added fertilizer was released as N₂O. This value is higher than the 0.7% to 6.8% range observed in other studies (Bouwman, 1996), which may indicate higher levels of nitrification at this site than most others.

4.7 Improvements and Future Research

Several improvements to this study would enhance its value. Analyzing the soil samples for this sampling period is an important next step. Knowing the levels of available nitrogen in the soil will allow us to better understand how this affected various gas fluxes and biomass productivity. It will be interesting to see the differences in available nitrogen between the various fertilization and species treatments, as well as how its availability in the soil changed over the course of the growing season. Additionally, sampling biomass from the same year that the gas flux and soil samples were taken may have increased the accuracy of the results. While biomass sampling could not be done in late 2013 due to inclement weather conditions, sampling is planned for the spring of 2014. Having this 2013 biomass data will allow us to compare it to both the 2012 biomass data, and the 2013 gas flux data and environmental variables.

Another improvement would be to include more frequent sampling during the early growing season to get more detail on the controls of soil GHG production, particularly during the early summer when GHG fluxes are highest. Almost all the GHG soil emissions in this study occurred within the first few weeks of fertilization; however, since there was only one sampling date during this time, the total GHG emission calculations from the flux values are very coarse (Appendix III). Having more frequent sampling in the period immediately following fertilization would allow us to gain a better understanding of N₂O fluxes at this time, and how they change after fertilization. Additionally, it would be good to conduct biomass harvests at the end of the growing season. As previously mentioned, nutrient inputs from the previous years crops may result in inflated nitrogen levels within the soil. Harvesting would minimize the amount of nutrients being recycled back into the

soil, and it would more accurately mimic a fully functional bioenergy crop system. It would also be a good idea to conduct additional fertilization experiments to include elements such as phosphorus and potassium. It is possible that N may not be limiting productivity at this site, which would negate any effect of N addition. Overall, the results of this study may warrant further research in this area.

Summary

In this study, the effects of nitrogen fertilization on different potential bioenergy crops were examined at a field site in Bath, Ontario. Three perennial grass species (switchgrass, little bluestem and big bluestem) were grown in conjunction with three fertilization treatments (0, 50 and 150 lbs/acre of nitrogen as urea) to explore the tradeoffs associated with using nitrogen fertilizer in these bioenergy systems. Soil properties known to influence N₂O emissions (pH, soil temperature and soil moisture) were also measured. Crop productivity was measured at the end of the growing season to determine the species and fertilization level at which GHG emission benefits are maximized.

It was determined that increasing N fertilizer application resulted in increased soil N₂O emissions. Total GHG fluxes were also significantly higher because of this. Fluxes were highest in the beginning of the growing season, immediately after fertilizer application, likely due to high levels of available nitrogen and nitrification in the soil. Grass species had no effect on N₂O fluxes from the different fertilization treatments, although the perennial grasses emitted significantly less CO₂ from the soil than the control plots. Despite resulting in higher GHG fluxes, N fertilizer did not significantly increase grass aboveground biomass. There was also no significant difference in aboveground biomass production between the different grass species.

The biomass production of these species indicates that bioenergy crops at this site have the potential to offset 6.02 to 9.21 Mg of CO₂ emissions per year. The highest fertilization level (150 lbs/acre) emitted 3.23 Mg more of CO₂eq than the 0 lbs/acre

treatment. Biomass production for these perennial grass species would need to be raised by approximately 40 to 50% at this higher fertilization level in order to offset the additional soil GHG emissions produced.

Overall, the results of this study indicate that using no fertilizer will result in the largest GHG gains at this site. These results contradict some past studies in other locations, highlighting the importance of local environmental conditions on biomass productivity and fertilization effectiveness. The results of this study will help optimize bioenergy crop production and management, through the increased understanding of the impacts of fertilizer on perennial grass productivity and soil GHG emissions at this site.

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Appendix I: Normality and Homogenous Variance Test Data

[illegible]

Appendix II: Repeated Measures ANOVA Test Results (p values)

ANOVA (Repeated Measures) - Bold values indicate significance									
		Temperature	Moisture	pH	N2O Flux	CH4 Flux	CO2 Flux	GHG Flux	Biomass*
Within Subject	Date	0.000	0.000	0.000	0.000	0.299	0.000	0.000	N/A
	Date * Fertilization	0.931	0.675	0.162	0.000	0.971	0.977	0.000	N/A
	Date * Species	0.039	0.813	0.788	1.000	0.217	0.002	0.418	N/A
	Date * Fertilization * Species	1.000	0.945	1.000	1.000	0.826	0.996	1.000	N/A
Between Subject	Fertilization	0.000	0.992	0.000	0.000	0.552	0.307	0.000	0.382
	Species	0.000	0.000	0.001	0.804	0.436	0.000	0.000	0.106
	Fertilization * Species	0.015	0.079	0.002	0.898	0.258	0.776	0.804	0.757
Tukey HSD	Fertilization								
	0 - 50	1.000	0.989	0.000	0.847	0.569	0.522	0.529	0.716
	0 - 150	0.053	0.900	0.000	0.000	0.574	0.928	0.000	0.808
	50 - 150	0.057	0.952	0.000	0.000	1.000	0.313	0.009	0.350
	Species								
	Control - BB	0.000	0.999	0.010	0.769	0.725	0.000	0.000	0.351
	Control - LB	0.000	0.004	0.004	0.998	0.991	0.000	0.000	0.612
	Control - SG	0.000	0.811	0.007	1.000	0.879	0.000	0.000	0.924
	LB - BB	0.983	0.007	0.995	0.855	0.542	0.409	0.415	0.975
	SG - BB	0.456	0.725	0.999	0.778	0.991	0.875	0.635	0.131
	SG - LB	0.254	0.000	0.999	0.999	0.728	0.856	0.985	0.289
*Biomass data analyzed with two-factor ANOVA (not repeated measures)									

Growing Season N ₂ O Emissions	11.75227548		Just N componenet of N ₂ O	7.482362131					
(kg/ha)									
Growing Season N ₂ O Emissions	10.45952518		Just N componenet of N ₂ O	6.659302296					
(lbs/acre)									
Just N Component of 150lbs/acre urea	69.98								
% of N fertilizer emitted as N ₂ O:	9.52%								

Appendix V: Gas Flux Data

N2O Flux Values												
Plot	Block	Fertilization	Species	12-Jun	19-Jun	26-Jun	09-Jul	16-Jul	24-Jul	02-Aug	14-Aug	28-Aug
1	D	0	LB	0.145733	0.063107	0.006511	0.098180	0.014648	0.038378	0.042034	0.013425	-0.033272
2	D	50	LB	0.201740	0.147284	0.160684	0.290961	0.049350	-0.045634	0.148907	-0.039503	0.00942
3	D	150	LB	89.055686	2.835666	0.730465	0.513723	0.358079	0.051210	0.070673	0.059446	0.071795
4	D	0	SG	-0.202363	0.03725	0.080069	0.018509	-0.217781	-0.158864	0.025900	0.026404	0.507893
5	D	50	SG	0.596277	0.082734	0.193827	0.386825	0.024949	0.038610	0.098600	0.105168	0.014069
6	D	150	SG	14.173854	0.323844	0.127222	0.099101	0.030159	0.002718	0.075355	0.04009	0.092688
7	D	0	Control	0.164485	0.091413	0.111751	0.048675	0.063322	0.004889	0.085189	-0.001248	0.039586
8	D	50	Control	3.642170	0.256519	0.205766	0.265981	0.023235	0.047394	0.047774	-0.056924	0.055306
9	D	150	Control	69.552089	3.640052	2.714889	3.030963	0.273155	0.085391	0.123331	0.088885	0.098011
10	D	0	BB	-0.122766	0.083514	0.011765	0.029174	0.067032	0.146368	0.041004	0.04406	-0.010643
11	D	50	BB	0.104705	0.096974	0.027671	0.300447	-0.008168	0.005808	0.096836	0.082282	-0.053055
12	D	150	BB	68.977660	1.652014	0.560606	0.435632	0.076199	0.089732	0.142197	0.037804	0.051153
13	C	0	LB	0.087583	-0.030164	-0.089315	-0.058395	0.006726	0.028951	-0.024345	-0.080924	0.017218
14	C	50	LB	2.100953	0.236835	0.054813	0.017877	0.045782	0.089413	0.090549	0.008752	0.035234
15	C	150	LB	65.814774	1.378203	0.338251	0.270176	0.075947	0.037981	0.123051	0.025917	0.015592
16	C	0	Control	0.017363	0.105332	0.015622	0.186778	1.420717	0.488617	0.085804	0.076585	1.13103
17	C	50	Control	3.226298	0.459471	0.263830	0.176481	0.098666	0.047957	0.125574	0.029915	0.106057
18	C	150	Control	13.535587	5.520946	1.134332	3.763558	0.041705	0.200683	0.300161	0.069517	0.010148
19	C	0	BB	0.196778	0.153173	0.060902	0.069727	0.098925	-0.006862	0.042444	0.087671	0.011655
20	C	50	BB	1.971900	0.247759	0.095133	0.080295	0.030192	0.041352	0.072420	0.068948	0.068782
21	C	150	BB	0.126056	1.782893	3.214108	0.824928	0.069357	0.089643	0.236116	0.176072	0.080241
22	C	0	SG	0.041370	-0.010537	0.044388	0.099292	0.063420	0.113974	0.175179	0.04188	0.08607
23	C	50	SG	27.464284	1.384393	0.940107	0.475609	0.114752	0.021784	0.080443	0.024727	0.032245
24	C	150	SG	111.184313	5.336813	1.400433	0.626509	0.199776	0.106208	0.302979	0.134456	0.223032
25	B	0	SG	0.060071	0.069897	0.034847	0.027501	-0.005665	0.058850	-0.028483	0.034752	0.102463

26	B	50	SG	11.175092	0.409845	0.079435	0.021040	0.007198	0.004629	0.086222	0.061419	0.011578
27	B	150	SG	23.866607	3.919493	1.885040	0.834784	0.140692	0.069709	0.132941	0.10323	0.045173
28	B	0	LB	0.037489	0.030985	0.039318	0.063291	0.062310	0.025694	0.070415	0.005553	0.019338
29	B	50	LB	2.647883	1.133364	0.285091	0.082885	0.034188	-0.017572	-0.009197	-0.001875	-0.001001
30	B	150	LB	26.083890	1.900401	1.040847	0.583479	0.090519	0.121639	0.097157	0.090839	-0.002953
31	B	0	Control	0.061527	0.112076	0.243930	0.077894	0.090779	0.005522	0.015420	0.016702	0.031056
32	B	50	Control	1.040634	0.982779	0.283002	0.522168	0.055928	-0.026298	0.015795	0.018491	-0.00482
33	B	150	Control	49.561475	10.7319	4.491461	1.132296	0.254853	0.062951	0.027756	0.009625	0.007996
34	B	0	BB	0.083577	-0.081519	0.077117	0.020876	-0.005582	0.071019	0.041660	0.0562	0.022204
35	B	50	BB	0.200826	0.111976	0.074372	0.109192	0.137492	0.051452	0.186694	0.000529	0.060926
36	B	150	BB	4.580297	0.649018	0.302500	0.198516	0.104156	0.111111	0.208961	0.02824	0.105583
37	A	0	Control	0.036142	0.134808	0.190227	0.098047	0.030660	0.127657	0.035726	0.002874	0.031942
38	A	50	Control	0.599574	0.348138	0.502044	0.322624	0.172195	0.086242	0.034092	0.044741	0.083689
39	A	150	Control	15.381160	14.161103	5.277956	1.443669	0.209837	0.076307	0.038057	-0.017283	0.552266
40	A	0	SG	0.045902	0.044544	0.033067	0.057394	0.037703	0.000243	0.002012	-0.012762	0.021718
41	A	50	SG	0.122618	0.059198	0.119432	0.098823	0.313608	-0.030849	0.109463	-0.018704	-0.015577
42	A	150	SG	8.688732	2.617965	1.350372	0.849208	0.168345	-0.006428	0.149772	0.032717	0.150102
43	A	0	BB	0.139804	0.037355	0.064203	0.073091	0.157955	-0.082711	-0.095171	0.000418	0.013919
44	A	50	BB	1.930712	0.75908	0.417414	1.607774	0.421041	0.022172	-0.030733	0.054817	0.103742
45	A	150	BB	23.810616	1.673225	0.444709	0.519793	0.182291	0.148510	0.141032	0.055127	0.292846
46	A	0	LB	-0.013951	0.040739	0.147231	0.061605	0.091720	0.105164	0.072070	0.073522	0.054706
47	A	50	LB	0.908878	0.139398	0.083043	0.076915	0.310026	0.202915	0.022673	0.063163	0.031892
48	A	150	LB	2.405060	1.288114	0.410090	1.059963	0.444321	0.167649	0.124684	0.055719	0.67687

CH4 Flux Values												
Plot	Block	Fertilization	Species	12-Jun	19-Jun	26-Jun	09-Jul	16-Jul	24-Jul	02-Aug	14-Aug	28-Aug
1	D	0	LB	-0.110064	-0.013911	-0.277398	3.469438	-0.277770	-1.221514	0.315559	0.679762	0.237045
2	D	50	LB	-0.517876	-0.500478	-0.184365	1.601478	0.628060	-9.201835	0.698525	-2.250171	0.677277
3	D	150	LB	-1.677664	-0.124558	-0.097936	-1.462013	-0.636665	2.277395	-0.249480	1.834368	-0.073234
4	D	0	SG	1.111779	0.765895	-0.135874	0.677271	-1.635769	-2.627477	0.958286	0.316346	1.243934
5	D	50	SG	-0.080744	-2.019789	-0.063964	2.102251	0.677222	0.249235	1.596830	1.953170	-0.291229
6	D	150	SG	0.112028	0.130725	-1.004903	0.246126	1.768705	-3.099819	-1.379732	-3.461840	-0.504586
7	D	0	Control	0.471655	-0.183392	-0.228752	2.416369	-0.649342	0.748712	-0.215906	-0.379390	0.853193
8	D	50	Control	-0.669300	0.693885	-0.133348	2.126718	0.412458	0.883977	2.047796	-3.149855	-0.310040
9	D	150	Control	0.553117	-0.27084	-0.162896	-1.695303	-0.186988	0.558789	-1.744863	-0.195683	0.604706
10	D	0	BB	0.259320	-0.072339	-0.164798	2.144798	1.742499	1.724093	2.376243	1.944406	0.265353
11	D	50	BB	-1.169909	0.670809	-0.128566	-1.504592	1.340549	-0.515948	0.177773	0.884787	-0.216318
12	D	150	BB	1.112573	0.173166	-0.232628	1.058817	-0.238532	-0.094415	-0.380700	-1.089945	-0.531399
13	C	0	LB	0.636773	-0.004976	-0.155904	-0.342759	-1.649698	0.578668	-1.284050	0.000000	-1.666164
14	C	50	LB	-1.091009	-0.263743	-0.336052	-0.326838	0.360908	1.369658	0.048978	0.340113	-0.620205
15	C	150	LB	0.202178	-0.334326	-0.141013	-0.068257	-1.959761	-0.997284	-0.212239	-0.875703	-1.117568
16	C	0	Control	-0.113201	0.753524	0.128898	0.963190	-0.502930	0.576306	1.945818	2.295363	-0.390000
17	C	50	Control	0.048657	-0.11801	-0.195450	-2.441180	0.326848	0.644367	-2.025793	-0.426914	0.367258
18	C	150	Control	0.461247	-2.72965	-0.479784	2.516446	-2.611821	-2.397489	0.585898	-1.465091	1.211139
19	C	0	BB	0.086291	1.7147	-0.050755	-0.437879	-0.607781	-0.883076	0.412386	1.537800	0.123933
20	C	50	BB	0.453473	-0.09815	-0.245962	-3.412930	-1.437942	0.674992	0.069976	0.159473	-1.207708
21	C	150	BB	-0.549250	-0.766616	-0.018265	0.650754	-0.027456	2.280994	-0.637701	-0.401345	-0.599053
22	C	0	SG	0.521674	0.567445	-0.150574	-0.453582	-1.211665	-1.893121	1.556757	-0.363486	-0.212171
23	C	50	SG	1.095739	1.567567	-0.077764	0.293833	0.307803	-2.141536	-1.417578	-0.232694	-0.226169
24	C	150	SG	-0.824619	-2.241091	-0.136184	-0.447481	-0.516830	-1.699406	0.555801	-0.274442	0.644425
25	B	0	SG	-0.815473	-0.445994	-0.099005	-1.430725	-0.341947	-1.104622	-0.454800	-0.154695	-0.597372
26	B	50	SG	-0.807999	-0.038247	1.048486	1.285321	0.716532	-3.018309	3.313568	0.095373	-0.315615
27	B	150	SG	0.318974	-0.104525	0.099094	-2.093746	0.021297	0.581844	2.708220	0.685800	3.221178

28	B	0	LB	-0.608094	-0.267575	-0.521101	1.116533	-1.580322	-1.144958	-1.845680	-1.437894	1.461954
29	B	50	LB	0.338407	-0.170907	-0.123632	0.447352	-0.801403	-2.912218	-3.073678	-0.492975	-0.395457
30	B	150	LB	-1.509529	-0.487225	0.061661	-1.282830	-1.100041	1.437256	-1.053012	-0.655241	0.401495
31	B	0	Control	-0.466261	0.01805	-0.322639	-0.897200	-0.435680	-0.719482	-3.242108	-0.638841	0.481011
32	B	50	Control	0.128462	-0.320307	-0.273426	-0.739698	-0.405940	-1.133475	-1.568892	-0.222774	-1.244262
33	B	150	Control	0.373152	0.035688	0.060026	-0.594987	-0.104910	1.324932	1.000924	-0.435553	0.778550
34	B	0	BB	-0.028345	-0.050484	-0.032042	0.030181	0.500337	-1.593825	-3.170732	-0.242140	-0.919151
35	B	50	BB	0.123931	-0.036839	0.724039	0.731095	0.517170	-2.008800	-1.499056	-0.641189	-1.178091
36	B	150	BB	-0.253536	0.100036	-0.794306	-1.184145	0.029659	0.938200	-3.826516	-0.476699	-0.594504
37	A	0	Control	-0.257563	0.25662	-0.117324	1.166667	2.053891	-1.067303	-2.363419	0.154965	-1.079897
38	A	50	Control	-1.698998	-0.633537	-4.289755	1.142209	-1.167983	0.868629	-3.265291	-1.802353	-1.481868
39	A	150	Control	-0.210783	0.353799	-0.172315	-0.197924	-1.327720	-1.439758	-1.167978	0.051789	0.675265
40	A	0	SG	0.066472	-0.256595	-0.322285	-0.280845	0.092255	-1.985067	-0.309942	-0.151193	-0.829544
41	A	50	SG	-0.351885	0.950075	-0.052069	-0.197084	0.125157	-0.959057	-0.045468	0.516198	2.147373
42	A	150	SG	-0.094357	0.656887	-0.170894	-0.097396	-1.932076	-2.573298	2.893598	-0.327341	-2.191618
43	A	0	BB	-0.393491	0.321122	-0.079001	-0.098402	0.711209	0.289398	-0.622964	-0.802543	-0.279474
44	A	50	BB	0.033123	-0.327223	-0.184777	-0.443059	-1.999859	2.551861	-0.935144	-0.605495	2.411200
45	A	150	BB	0.195384	-1.354676	-0.144420	-0.146666	0.681496	-0.866688	2.302161	0.375130	0.416521
46	A	0	LB	-0.489566	-0.353767	-0.215318	-0.224177	-1.126374	3.252253	2.547313	-4.227730	-1.741111
47	A	50	LB	0.149991	0.983217	-0.168224	-0.174153	0.136752	0.201632	1.518434	-0.512840	0.606345
48	A	150	LB	-0.068188	0.644224	-0.194441	-0.278977	0.819105	-3.668754	-1.249530	2.469825	0.870327

CO2 Flux Values												
Plot	Block	Fertilization	Species	12-Jun	19-Jun	26-Jun	09-Jul	16-Jul	24-Jul	02-Aug	14-Aug	28-Aug
1	D	0	LB	6.571543	5.760793	7.333555	7.866664	8.893848	4.647883	4.570072	3.816397	6.011270
2	D	50	LB	4.329204	5.380027	7.412822	7.332220	9.254373	4.825789	5.291169	4.510034	6.655896
3	D	150	LB	6.176880	5.23037	6.291809	4.305435	8.265165	5.134143	5.394521	4.700428	6.340093
4	D	0	SG	4.776622	4.65238	6.024663	8.329216	9.309448	6.362038	6.060246	4.365587	7.191254
5	D	50	SG	3.839973	3.770187	6.248464	6.718235	9.865599	5.354267	5.323028	4.028663	5.467599
6	D	150	SG	4.203111	3.868005	7.223509	6.207780	6.520843	3.961583	5.053517	3.955399	5.396982
7	D	0	Control	6.945356	7.345905	11.076735	9.446133	11.625712	6.712014	8.631722	6.369475	10.686686
8	D	50	Control	5.999025	7.222549	11.582824	8.801767	12.238742	5.162882	6.328764	4.006327	6.132947
9	D	150	Control	4.901398	7.548309	12.345057	10.439253	13.033208	5.011247	6.676707	4.608270	6.227881
10	D	0	BB	4.368486	5.597941	6.643204	6.465047	6.877006	4.210894	4.924499	4.338303	4.023367
11	D	50	BB	4.645303	5.893437	7.796002	7.918901	7.553216	4.562900	6.623616	5.042075	-0.879825
12	D	150	BB	5.546496	8.064464	9.302378	9.592892	7.306901	4.225451	5.992002	4.216919	4.586275
13	C	0	LB	5.410513	6.858193	7.886952	4.500877	8.087451	4.468549	4.485791	5.679855	5.911520
14	C	50	LB	7.926003	10.712963	9.923349	6.214355	9.060600	7.035217	6.064903	5.859212	7.047306
15	C	150	LB	8.068520	8.630337	9.498023	7.539998	9.225229	7.867203	6.817220	3.518719	6.539884
16	C	0	Control	4.635228	4.470882	8.724742	7.398895	9.710594	10.863534	6.136033	6.521355	4.605420
17	C	50	Control	8.866906	11.448434	16.554535	11.373563	12.559655	9.480062	7.420290	5.734404	6.443260
18	C	150	Control	7.287115	9.461583	10.887874	11.349969	14.190934	10.177316	7.722402	8.475482	6.276008
19	C	0	BB	6.303288	5.647192	8.996603	8.111419	10.201848	7.261884	7.078201	5.917598	6.340267
20	C	50	BB	7.832010	10.355642	11.986981	9.290229	10.406705	8.111392	8.312373	9.409808	10.147637
21	C	150	BB	1.669673	4.558587	5.934876	7.159424	7.221839	5.967324	7.558653	6.634064	5.435397
22	C	0	SG	6.554292	7.472543	9.030387	7.643118	8.353256	6.782727	6.583510	5.199033	5.367009
23	C	50	SG	5.322689	2.956736	10.654162	9.344134	9.060926	7.520249	7.490074	5.821810	5.767956
24	C	150	SG	6.863037	7.19124	9.062574	7.584060	7.741758	5.415317	5.600870	3.300836	4.226681
25	B	0	SG	3.578474	2.762512	5.180249	6.035730	4.562460	5.894352	4.178183	4.876310	3.944684
26	B	50	SG	4.499538	6.18903	10.534716	8.504942	8.714566	6.786671	9.412207	8.270384	7.141034
27	B	150	SG	3.364738	5.183975	8.593893	8.967193	8.643183	7.349297	10.681109	7.977560	9.047066
28	B	0	LB	9.505292	6.408426	9.214641	7.883138	6.399729	7.073916	7.454237	5.381708	6.431860

29	B	50	LB	4.970790	7.601164	10.419023	8.543432	7.060378	6.686313	7.738363	5.431727	5.476675
30	B	150	LB	6.994741	6.508478	7.499125	5.923406	6.014350	5.873347	5.796424	4.213336	6.090226
31	B	0	Control	5.328534	6.214571	12.915715	12.718232	10.952476	9.070260	9.333955	6.695762	3.205035
32	B	50	Control	7.797631	9.610198	13.355410	12.314959	7.381835	6.874435	9.675594	4.930376	9.009773
33	B	150	Control	6.733421	4.273973	13.888229	13.190073	11.095354	7.599613	8.085908	4.334505	6.443347
34	B	0	BB	6.335517	6.735904	8.998184	6.807382	5.857082	4.711009	5.171134	3.171719	3.444794
35	B	50	BB	6.282635	5.022768	7.127753	5.687781	4.907326	4.809657	4.077743	2.966718	3.172758
36	B	150	BB	5.588625	5.621158	7.476865	5.349909	3.947663	3.351793	3.304397	1.959457	2.514271
37	A	0	Control	7.351154	3.594154	13.215322	11.348233	7.133333	7.561800	9.567640	11.404498	7.404255
38	A	50	Control	5.945743	4.978992	9.754890	12.203998	8.285134	6.118912	6.935659	4.759089	6.877313
39	A	150	Control	5.009840	6.864662	12.051697	11.950761	6.356459	7.173189	5.823090	4.464772	5.610661
40	A	0	SG	6.501544	7.900321	7.888764	7.552049	8.149426	5.313042	5.784731	4.036395	5.737518
41	A	50	SG	5.383093	4.613273	5.155821	6.592896	5.586274	3.149159	3.660421	2.198510	3.732425
42	A	150	SG	7.551062	6.198202	8.179381	10.070695	5.987597	5.532418	6.049535	4.166567	5.023575
43	A	0	BB	4.194611	5.140154	7.728267	6.843365	5.614992	6.402964	5.053650	6.962398	6.697801
44	A	50	BB	4.105827	4.857459	6.525908	7.720539	6.753702	6.778203	5.908784	4.651605	5.193298
45	A	150	BB	3.766467	4.747511	7.694691	6.246651	7.118670	7.375423	6.245173	6.271483	6.453689
46	A	0	LB	5.285728	5.355313	8.796874	9.279942	6.370392	5.775418	6.296789	5.818815	5.677217
47	A	50	LB	7.189070	5.766381	8.338063	8.636742	5.198866	5.070699	4.046598	4.613987	4.235343
48	A	150	LB	6.634568	5.691089	7.905279	7.647718	6.492649	3.030288	3.586878	2.251051	4.797736

GHG Flux Values												
Plot	Block	Fertilization	Species	12-Jun	19-Jun	26-Jun	09-Jul	16-Jul	24-Jul	02-Aug	14-Aug	28-Aug
1	D	0	LB	6.614409	5.780064	7.329748	7.969958	8.892555	4.634128	4.589730	3.834834	6.005934
2	D	50	LB	4.380869	5.415175	7.458762	7.456049	9.282861	4.618404	5.351999	4.450534	6.673039
3	D	150	LB	33.748912	6.106811	6.516197	4.433987	8.362800	5.197843	5.411191	4.757378	6.360812
4	D	0	SG	4.737237	4.680011	6.046631	8.349176	9.207584	6.257613	6.088399	4.380416	7.374823
5	D	50	SG	4.023123	3.753419	6.307207	6.882298	9.887555	5.371470	5.387128	4.102281	5.465845
6	D	150	SG	8.599359	3.971142	7.241845	6.243670	6.567335	3.897329	5.047902	3.895129	5.415119
7	D	0	Control	7.006251	7.370392	11.106574	9.511965	11.631706	6.729253	8.653597	6.361121	10.716875
8	D	50	Control	7.114042	7.316641	11.643811	8.928882	12.254606	5.196137	6.386578	3.922534	6.143581
9	D	150	Control	26.474161	8.671037	13.183252	11.343250	13.113960	5.049453	6.678298	4.631715	6.270963
10	D	0	BB	4.335874	5.622311	6.643391	6.519132	6.934379	4.292474	4.987112	4.392794	4.025640
11	D	50	BB	4.653193	5.937586	7.801880	7.980443	7.578835	4.553866	6.657369	5.086163	-0.900815
12	D	150	BB	26.952935	8.580225	9.471280	9.750173	7.325514	4.251285	6.028089	4.205750	4.590973
13	C	0	LB	5.451036	6.848738	7.855991	4.475577	8.054892	4.489676	4.451279	5.654768	5.881868
14	C	50	LB	8.554387	10.780843	9.933284	6.213034	9.082371	7.091698	6.094002	5.869067	7.045204
15	C	150	LB	28.475346	9.050559	9.599919	7.622319	9.207617	7.858034	6.850909	3.508363	6.521249
16	C	0	Control	4.638234	4.519359	8.732292	7.477023	10.140455	11.027107	6.203495	6.593299	4.947849
17	C	50	Control	9.868081	11.588392	16.632217	11.377007	12.597105	9.508461	7.416677	5.734713	6.483851
18	C	150	Control	11.492834	11.115754	11.229442	12.569517	14.149014	10.189181	7.827756	8.466266	6.304588
19	C	0	BB	6.366101	5.730684	9.014416	8.123839	10.219751	7.241212	7.100018	5.977070	6.346483
20	C	50	BB	8.452822	10.430386	12.011307	9.243449	10.385868	8.138386	8.336293	9.434531	10.143598
21	C	150	BB	1.697216	5.095185	6.930866	7.428817	7.242763	6.043014	7.618457	6.680218	5.447691
22	C	0	SG	6.578072	7.481193	9.040986	7.664373	8.347472	6.778303	6.670508	5.204383	5.389235
23	C	50	SG	13.859627	3.418817	10.943963	9.497743	9.102963	7.482030	7.485243	5.824589	5.773203
24	C	150	SG	41.312857	8.798589	9.493848	7.768881	7.792836	5.412554	5.706465	3.336754	4.309354
25	B	0	SG	3.579971	2.774814	5.188972	6.014210	4.553523	5.889399	4.159803	4.883834	3.963903
26	B	50	SG	7.946849	6.315279	10.581359	8.538456	8.731845	6.724721	9.508520	8.291427	7.137995
27	B	150	SG	10.770085	6.396823	9.180336	9.182007	8.687245	7.383126	10.779194	8.023963	9.128714
28	B	0	LB	9.504143	6.412412	9.215886	7.926205	6.385859	7.057838	7.437307	5.353234	6.468556

29	B	50	LB	5.798740	7.948918	10.504805	8.578521	7.054146	6.619709	7.670965	5.420794	5.468060
30	B	150	LB	15.049047	7.087371	7.823082	6.077345	6.019310	5.941238	5.804429	4.227736	6.097742
31	B	0	Control	5.337816	6.249694	12.984557	12.723538	10.971468	9.056863	9.270650	6.687524	3.224764
32	B	50	Control	8.122925	9.908133	13.437399	12.461297	7.390648	6.842479	9.647544	4.931430	8.982149
33	B	150	Control	22.105314	7.601611	15.281842	13.528590	11.172155	7.646952	8.115532	4.328342	6.462175
34	B	0	BB	6.360830	6.709573	9.021417	6.814487	5.865858	4.699555	5.117464	3.184056	3.432375
35	B	50	BB	6.347494	5.056707	7.166014	5.736984	4.960809	4.783423	4.104138	2.953417	3.166905
36	B	150	BB	7.003193	5.824454	7.553960	5.386582	3.980574	3.405940	3.288818	1.958200	2.534517
37	A	0	Control	7.356949	3.641334	13.271829	11.403127	7.185969	7.578961	9.529083	11.408643	7.391480
38	A	50	Control	6.095932	5.073611	9.820439	12.327998	8.313986	6.163888	6.877657	4.735109	6.872138
39	A	150	Control	9.773573	11.262034	13.684245	12.394142	6.393626	7.166609	5.810360	4.460502	5.796044
40	A	0	SG	6.517170	7.908741	7.892246	7.563944	8.163052	5.271431	5.778846	4.029264	5.726830
41	A	50	SG	5.413715	4.651576	5.191751	6.619392	5.686120	3.119455	3.693400	2.203552	3.772691
42	A	150	SG	10.242588	7.023566	8.594408	10.331904	5.999211	5.476386	6.156730	4.169835	5.024082
43	A	0	BB	4.229687	5.158478	7.746511	6.863957	5.678894	6.383401	5.011064	6.945674	6.696247
44	A	50	BB	4.705044	5.085902	6.651426	8.209645	6.842228	6.838665	5.879619	4.655883	5.276093
45	A	150	BB	11.151861	5.237763	7.829518	6.404707	7.189491	7.403261	6.337238	6.296450	6.553218
46	A	0	LB	5.271122	5.360513	8.837994	9.294332	6.375171	5.876316	6.372624	5.752824	5.657612
47	A	50	LB	7.473972	5.830242	8.360274	8.656928	5.297846	5.137837	4.085514	4.622798	4.257962
48	A	150	LB	7.378705	6.103933	8.028323	7.970448	6.647590	3.005215	3.599290	2.320190	5.025842