GREENHOUSE GAS EMISSIONS FROM PRODUCTION OF *MISCANTHUS X GIGANTEUS* ON A MOLLISOL

BY

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THESIS

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**ABSTRACT**

Biofuels have a great potential to alleviate our dependence on non-renewable fossil fuel products; however the beneficial effects of substituting biomass for fossil fuel is reduced if the biofuel crops also emit large amounts of greenhouse gases (GHGs), such as carbon dioxide ($CO_2$) and nitrous oxide ($N_2O$) during their production. Most crops require nitrogen (N) fertilization to maximize productivity, but the amount of N fertilizer *Miscanthus* x *giganteus* might need is currently not known. Because *M.* x *giganteus* is highly efficient in its N use, it is a great potential energy crop because it has had large yields even at low N inputs from fertilizers (including no additions of N fertilizer). Therefore, it is critical to determine the response of *M.* x *giganteus* to N fertilizer rates and determine the effect of fertilization on GHG emissions. The main objective of this study was to examine the effect of N fertilization rates (0, 60 and 120 kg N ha$^{-1}$ of urea) on GHG emissions from production of *M.* x *giganteus* on a central Illinois Mollisol. The study had twelve, 10x10 m plots organized in four replicate rows with each of the three N fertilizer treatments placed randomly in each row. Gas samples to determine $N_2O$ and $CO_2$ fluxes were taken near noon throughout the year (March-November) when soil temperatures were warm enough to support microbial activity. In addition, soil moisture and soil temperature were continuously measured, and soils were regularly sampled for inorganic N to make specific inferences about abiotic factors affecting the GHG emissions. Furthermore, inorganic N leaching was assessed using resin lysimeters buried at 50 cm in each plot; the lysimeters placed in the soil in April of each year and excavated the following April. *M.* x *giganteus* biomass was measured on each plot, along with N and C concentrations in the harvested material. At the end of 2009 cumulative $N_2O$ and $CO_2$ emissions did not have a significant response due to fertilization. However, at the end of 2010, cumulative $N_2O$ emissions significantly increased with fertilizer additions (0.35, 0.77, and 2.91 kg N ha$^{-1}$ for the 0, 60, and 120 kg N ha$^{-1}$ fertilizer treatments, respectively). Carbon dioxide emissions did not respond to fertilization in 2010. Larger $NO_3^-$ concentrations were significantly related with larger $N_2O$ emissions. Greater temperature

and greater soil moisture at 10 cm were significantly related to larger $N_2O$ emissions. Higher

temperature at 10 cm was significantly related to larger $CO_2$ emissions; conversely, soil moisture was

not related to $CO_2$. In 2010, several large precipitation events occurred following fertilization, leading to

greater $N_2O$ emissions due to greater soil moisture. This study shows the potential for large $N_2O$ releases

in fertilized *M.* x *giganteus* when rates were greater than 60 kg N ha$^{-1}$, but this response is dependent on

precipitation and resulting soil moisture status following fertilizer application. Soil $CO_2$ emissions were

unaffected by N fertilization. During the first year of the study, $NO_3^-$ leaching was not significantly

affected by fertilization, but by the second year, the treatment plots had significantly different $NO_3^-$

leaching at 50 cm soil depth (8.9, 15.3, and 28.9 kg N ha$^{-1}$ yr$^{-1}$, for the 0, 60, and 120 kg N ha$^{-1}$ fertilizer

treatments, respectively). Yield data the first year of the study showed no significant difference among

treatments and were quite small, likely due small to the previous crop failure during the establishment

year and replanting (1.1, 4.1, and 4.0 Mg ha$^{-1}$, for the 0, 60, and 120 kg N ha$^{-1}$ treatments, respectively).

After the second year, biomass was much larger for all the treatments, but was still not significantly

different due to fertilization with N (14.9, 15.8, and 17.0 Mg ha$^{-1}$, for the 0, 60, and 120 kg N ha$^{-1}$

treatments, respectively). The amount of N removed from harvesting the biomass was significantly

larger with additional fertilizer in year 2; therefore, fertilization removed more N while yielding relatively

the same biomass. Overall, fertilization of *M. x giganteus* can lead to important $N_2O$ releases, which

reduces the overall favorable GHG balance; increased fluxes of inorganic N (primarily $NO_3^-$) through the

soil profile; and increases in harvested N without a significant increase in biomass.

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**INTRODUCTION**

Global greenhouse gas (GHG) emissions are inherently large and, prior to human intervention, carbon (C) absorbed from the atmosphere each year from photosynthesis was roughly balanced by soil and plant respiration (Dondini et al., 2009a). This balance has been disturbed by changing land use and fossil fuel combustion and this has led to global climate change and warming trends (Hughes et al., 2010). Therefore, governmental and political trends towards renewable energy resources have led to many recent studies on biofuel crops. The Intergovernmental Panel on Climate Change reported that even though agriculture is one of the largest sources of atmospheric GHG emissions, it also provides several innovative possibilities for GHG reduction (Solomon et al., 2007). The main policy driver is the goal of an overall net reduction in atmospheric emissions of carbon dioxide ($CO_2$), the major GHG emitted from the combustion of fossil fuels.

Fast growing plant species have the potential to be serious contenders for reducing GHG emissions compared to burning fossil fuels. Two categories of plants being examined in current research programs in the United States are fast growing woody species, such and willow and poplar (also known as coppice) and perennial rhizomatous grasses (PRGs), such as switchgrass (*Panicum virgatum* L.), reed canary grass (*Phalaris arundinacea* L.), and Miscanthus (*Miscanthus x giganteus*) (Clifton-Brown et al., 2007). The advantage of PRGs compared with trees is PRGs can be established more quickly and also produce an annual biomass with low moisture content (Clifton-Brown et al., 2007). Substituting plant biomass for fossil fuels is beneficial because plant-derived biomass is termed a $CO_2$ neutral fuel because the only $CO_2$ released from utilizing the biomass is what was photosynthetically fixed from the atmosphere during growth (Christian et al., 2008). However, the benefits of using energy crops to replace fossil fuels would be dramatically reduced if they also emit GHGs during production (Jørgensen et al., 1997). In addition, new agricultural practices aimed at alleviating the global food demand has dramatically disrupted the nitrogen (N) cycle and has led to many environmental problems including the

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potent GHG, $N_2O$ (Canfield et al., 2010). For this reason, it is critical to develop crop management systems that have the least potential for $N_2O$ and $CO_2$ emissions from soil per unit of energy produced (Jørgensen et al., 1997).

Assessments of global sources of $N_2O$ using modeling indicate that agricultural activities have been responsible for more than 70% of the increase in atmospheric concentrations of $N_2O$ occurring since 1850 (Kroeze et al., 1999). Fixen and West (2002) also found that from 1960 to 2000, the use of fertilizers containing N has increased by around 800%; wheat, rice and corn accounted for near 50% of the current fertilizer use. With N use efficiencies below 40%, these crops can cause fertilizer runoff, nitrification and denitrification depending on soil conditions. From 1990 to 2005, global agricultural $N_2O$ emissions increased 17% (USEPA, 2006) and more importantly, the Food and Agriculture Organization projects a 35-60% increase in global agricultural $N_2O$ emissions by 2030 (Bruinsma, 2003). With the development and research on cellulosic biofuel, Melillo et al. (2009) project even greater $N_2O$ emissions from such systems due to the reliance on N fertilizer to facilitate crop yields. As with any production, the costs of synthetic N fertilizer have been rising and this overuse is not only costly, but has severe environmental consequences (Galloway et al., 2008). For this reason, $N_2O$ is a major contributor to atmospheric pollution.

The global warming potential of $N_2O$ per molecule has been estimated at about 300 times greater than that of $CO_2$ (Solomon et al., 2007); it is the single most important ozone-depleting compound and expected to remain so throughout the 21st century (Ravishankara et al., 2009). The demand for more fuel has caused a need for either increased imported fossil fuel or substantial growth in biofuel production (Crutzen et al., 2008). Biofuel crops are used as substitutes for combusting fossil fuels to reduce the net $CO_2$ emission, but agricultural practices, such as fertilization that manipulate the plant-soil system, implies that $N_2O$ emissions can be a potential risk.

During the past 25 years agricultural impacts on $N_2O$ emissions have been studied. Results have shown that management practices such as fertilizer (type, timing, and application), crop tillage, residue management, and water (precipitation, irrigation) influence $N_2O$ emissions from agricultural soils (Parkin and Kaspar, 2006). The flux of $N_2O$ between the soil and the atmosphere depends on the $N_2O$ production and consumption by denitrification (conversion of nitrate to nitrous oxide and N gas) and nitrification (conversion of ammonia to nitrate and nitrous oxide) in the soil. The $N_2O$ flux is directly influenced by abiotic factors such as the concentration of inorganic N and temperature; the $N_2O$ flux also requires a low oxygen environment for the microbial production of $N_2O$, which is largely determined by soil water content (Aulakh et al., 1992). Since nitrification can also emit $N_2O$ as an intermediate step in the N cycle, agricultural systems represent large sources of $N_2O$ pools to the atmosphere (Mosier et al., 1998). Crutzen et al. (2008) estimated that agricultural systems are responsible for 80% (4.3-5.8 Tg $N_2O$ yr.$^{-1}$) of current annual global anthropogenic emissions.

Atmospheric $CO_2$, like $N_2O$, is a GHG; the dominant GHG to be precise. The atmospheric concentration of $CO_2$ is currently increasing at a rate of 1.4 ppmv yr$^{-1}$ (Solomon et al., 2007). Agriculture presents itself as a major source of $CO_2$ and GHG emissions alike because fossil-derived $CO_2$ is emitted as a result of cultivating, planting and harvesting crops, and of manufacturing agrochemicals (Christian et al., 2008). The manufacturing of N fertilizer often represents the largest emission of $CO_2$ due to the intensive energy requirement in the Haber-Bosch process; emissions of 0.86 kg $CO_2$-C per kg N have been calculated (Polwson et al., 2005). In addition, agriculture inherently manipulates the soil-plant ecosystem which has dramatic implications to the environment because the soil reserve is the largest pool of C on land (Schlesinger, 1997). The idea of using soil organic carbon (SOC) to sequester atmospheric $CO_2$ is not a viable solution to the global $CO_2$ and climate change issue because SOC increases at only an annual rate of 0.9±0.3 Pg C yr$^{-1}$, while the atmospheric concentration of $CO_2$ will

continue to increase at the rate of 2.0-2.6 Pg C yr$^{-1}$, even if C sequestration with SOC is implemented (Lal, 2004). Therefore it is critical to develop other methods to sequester C.

Just as agriculture is one of the major sources in emitting GHG, it could be an important solution to the problem. With the advent of bioenergy crops such as *M.* x *giganteus*, switchgrass and other potential biofuel crops, the global climate change problem could be mitigated by the cultivation of these novel crops. *M.* x *giganteus* utilizes N efficiently which makes it a great potential energy crop because it sustains large yields even at low N fertilizer inputs. Several studies have confirmed that *M.* x *giganteus* has no significant yield response to fertilizer treatments (Christian and Riche, 1998, Christian et al., 2008, Heaton et al., 2008a, Lewandowski et al., 2000, Miguez et al., 2008). Because agriculture is a major contributor of $N_2O$ and $CO_2$, several studies have focused on GHG emissions from production of *M.* x *giganteus* (Jørgensen et al., 1997; Christian et al., 2006, 2008), but none in the U.S. Midwest where much of the best soils in the world are located.

**OBJECTIVES**

The overall, general objective of this study was to examine the effect of N fertilization on GHG emissions from production of *Miscanthus x giganteus* on a central Illinois Mollisol.

Questions specifically addressed include:

1. Will N fertilization rate affect the emissions of soil $CO_2$ and $N_2O$ from production of *M. x giganteus*?

2. Does precipitation and N fertilizer application timing affect soil $N_2O$ flux from *M. x giganteus*?

3. How do soil $N_2O$ and $CO_2$ gas fluxes relate to other abiotic parameters, such as soil moisture and temperature as well as soil inorganic N concentrations?

4. Does N fertilization rate affect inorganic N leaching and yield production of *M. x giganteus*?

**LITERATURE REVIEW**

*Miscanthus x giganteus*

The genus *Miscanthus* comprises perennial grasses originating from the tropical, subtropical, and temperate regions of Southeast Asia ranging from the Pacific islands to the mountain regions of Japan and the Himalayas (Greef and Deuter, 1993; Heaton et al., 2010). The hybrid, *Miscanthus* x *giganteus*, used in most biofuel experiments is a naturally occurring sterile hybrid with parents *M. sacchariflorus* and *M. sinensis* (Greef et al., 1997). *Miscanthus* x *giganteus* is a triploid hybrid that can grow vigorously and tolerate some stress, but cannot produce a viable seed, so it poses no risk for weed invasion (Jorgensen and Muhs, 2001); it is environmentally adaptable and can photosynthesize over a wide range of temperature regimes due to its $C_4$ physiology (Beale et al., 1996). The $C_4$ photosynthetic pathway, which helps reduce respiration during the hot and dry summer months, is also shared by corn. The $C_4$ pathway results in high water use efficiency, and *M. x giganteus* needs only about half as much water input as cereal crops and a third of coppice, such as willows or poplar, per unit of biomass (Lewandowski et al., 1995). Moreover, the $C_4$ pathway inherently has high N-use efficiency and also $C_4$ plants store N in the rhizomes (Brown, 1978). *Miscanthus* x *giganteus*, like other perennial grasses, can retranslocate nutrients from aboveground to belowground storage organs for overwintering and thus may provide cleaner biomass feedstock than annual crops that do not directly cycle nutrients (Heaton et al., 2009, 2010).

In addition, *M. x giganteus* can obtain N from sources other than fertilizer (Christian and Riche, 1998); some N, between 20-46%, is provided by mobilization of N stored in roots and rhizomes, (Himken et al., 1997). Christian and Riche (1998) indicate that other sources of N are likely to be from mineralization of soil organic matter and decomposition of leaf litter. Nitrogen deposition at Rothamsted, England, where Christian and Riche (1998) conducted their study, was estimated to

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contribute 35-40 kg N ha$^{-1}$ yr$^{-1}$ (Goulding, 1990). In addition, significant decreases in aboveground N content between August and December indicate further evidence of N used by *M.* x *giganteus* (Davis et al., 2010).

The C$_4$ physiology also helps maintain high photosynthetic rates over a wider temperature range (Beale et al., 1996), helping it exploit a longer-than-average growing season (Heaton et al., 2008a; Dohleman et al., 2009). Therefore, the C$_4$ physiology in *M.* x *giganteus* increases nutrient, water, and solar radiation use efficiencies (Himken et al., 1997). This leads to twice the biomass compared to switchgrass (22 Mg ha$^{-1}$ vs. 10 Mg ha$^{-1}$) (Heaton et al., 2004b). Similarly, Clifton-Brown et al. (2000) estimated an average of 33 t dry mass ha$^{-1}$ over the state of Illinois predicted by an agro-climactic computer model using actual climatic data, with yields ranging from 20-40 Mg dry mass ha$^{-1}$.

In addition to the C$_4$ pathway aiding in the high N use efficiency of *M.* x *giganteus*, a recent study conducted by Davis et al. (2010) hypothesized that *M.* x *giganteus* has the ability to biologically fix N. Modeling exercises conducted by Davis et al. (2010) indicated that N fixation was an important component of the overall N budget, providing up to 250 kg N ha$^{-1}$ y$^{-1}$. *Miscanthus* x *giganteus* produced more standing aboveground C and soil C than switchgrass, prairie, and corn, which could be attributed to the ability to host N fixing bacteria. This hypothesis was supported by their results that confirm nitrogenase (N fixing) activity in *M.* x *giganteus* rhizomes and in bacteria isolated from *M.* x *giganteus* (Davis et al., 2010). N fixation may also explain the at least two times greater C yield and plant N uptake compared to switchgrass when grown on the same soils without fertilizer (Heaton et al., 2008a). It is likely that rates of N fixation will vary geographically and could account for the variations in yields outside Illinois. However, another study indicated that *M.* x *giganteus* yields were maintained after repeated harvests that inherently removed N (Christian et al., 2008) and the grass responded to little or no N fertilization (Miguez et al., 2008).

As previously written, *M.* x *giganteus* is a highly efficient N-use crop making it a potential energy crop because of high energy output even at low N fertilizer inputs (Heaton et al., 2010). Studies by Christian and Riche (1998) and Christian et al. (2008) found that there was no significant yield response to N fertilizer. Likewise, Miguez et al. (2008) found that considerably high yields can be obtained with little N fertilizer because the difference in the response to N fertilizer between 0 and 100 kg N ha$^{-1}$ was small compared with more typical row crop responses. Heaton et al. (2008a) found similar results. In a review of several studies compiled by Lewandowski et al. (2000), numerous field trials at different locations in Austria, Germany, and Greece confirmed that *M.* x *giganteus* showed no significant response to N fertilizer from the second or third year onwards. This phenomenon was due to several different characteristics: soil type, previous cropping system, the plant's $C_4$ photosynthetic pathway and the natural recycling of N and other minerals from the stems and leaves into rhizomes at senescence (Christian et al., 2008; Lewandowski and Kicherer, 1997).

*Miscanthus* x *giganteus* is harvested annually when the stems have senesced and mineral nutrient content is at its lowest due to re-mobilization to rhizomes and natural weathering. Even though *M.* x *giganteus* has yield reductions of 30-50% dry matter when harvested after winter (Jørgensen, 1997), field drying reduces the mineral and water content of the crop, which allows for cleaner fuel and allows nutrients to remain in the field (Lewandowski et al., 2003). When compared to other lignocellulose plants, the low mineral concentrations (Cl, K, N, S, and ash) and water content at *M.* x *giganteus* harvest is advantageous for biomass thermal conversion because it minimizes the impact on combustion efficiency and lowers stack emissions (Christian et al., 2008; Lewandowski and Kicherer, 1997). *Miscanthus* x *giganteus* specifically has low N and sulfur (S) content in its biomass which results in lower $N_2O$ and sulfur dioxide emissions during combustion (Lewandowski et al., 1995). Also, it reduces mineral removal in the harvested biomass which could lower future input costs and improve production sustainability (Christian et al., 2008).

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The physiology of *M.* x *giganteus* makes it an ideal biofuel crop and when compared to maize, switchgrass, and prairie grasses (Heaton et al., 2010). However, the current US ethanol fuel source is corn-based (*Zea mays*, or maize) and the US is also the major producer of maize ethanol (Heaton et al., 2008a). The Advanced Energy Initiative (AEI) recommends the conversion of 30% of the 2005 petroleum use in the transportation sector to domestically grown renewable bioethanol in the coming future (Milliken et al., 2007). Unfortunately, maize production in the US cannot meet the renewable fuel target because it supplied less than 2% of the 2004 transportation energy demand (Davis and Diegel, 2004). And more importantly, maize is an annual crop which requires large annual energy and financial inputs: tillage and planting, energy intensive N fertilizer, herbicides, and pesticides (Heaton et al., 2008a). Due to this, ethanol from maize grain has only a small net positive C balance at best because GHG emissions from ethanol made from conventionally grown corn can be slightly more or slightly less than from gasoline per unit of energy, but ethanol requires much less petroleum inputs (Farrell et al., 2006). Therefore, ethanol produced from cellulosic material, switchgrass or *M.* x *giganteus,* reduces both GHGs and petroleum inputs substantially (Farrell et al., 2006). This interest in GHG emissions, specifically $N_2O$ and $CO_2$, is of paramount interest in the understanding and growing of biofuel crops, such as *M.* x *giganteus*. Nonhebel (2002) determined that for a plant to be considered a potential energy crop, the bioenergy yield must be produced with minimal inputs of energy, so as to have a positive energy balance.

Biomass energy is near 'carbon neutral' because all the combusted C released to the atmosphere was fixed by the plant during its growing cycle, rather than emitting C that has been stored in the earth for millions of years (Dondini et al., 2009b; Hughes et al., 2010). The biomass produced from biofuel crops can be used as a substitute for fossil fuels and may help to reduce the dependence on imports of such fossil fuels and also can help reduce $CO_2$ emissions and even sequester C (Smeets et al., 2009; Dondini et al., 2009b). *Miscanthus* x *giganteus* is an ideal bioenergy crop which can be used to

generate heat, power and fuel, and alleviate $CO_2$ emissions (Heaten et al., 2004a). *Miscanthus* x

*giganteus* has a reported energy output in comparison to input to be approximately 15-20:1

(Lewandowski and Kircherer, 1997). The energy content of *M.* x *giganteus* harvested from just one

hectare was approximately 20 tons of dry matter, which corresponds to the energy content in 12 tons of

hard coal; for each gigajoule of hard coal, 96.6 kg of $CO_2$ are emitted during combustion, but by

combusting *M.* x *giganteus* instead of hard coal, 90% of the $CO_2$ emissions are alleviated (Lewandowski

et al., 1995). If *M.* x *giganteus* were combined with coal in a 50% blend, it would avoid the net emission

of 39 million tons of $CO_2$ to the atmosphere each year, based on replacement of coal with the C neutral

*M.* x *giganteus* (Heaton et al., 2004a). *Miscanthus* x *giganteus* also produced an average maximum

annual biomass of 20 Mg $ha^{-1}$ compared with 10 t $ha^{-1}$ of switchgrass (Heaton et al., 2004b). Heaton et

al. (2008a) showed that *M.* x *giganteus* could provide 260% more ethanol per hectare than maize grain

and, if grown on land currently devoted to maize ethanol, *M.* x *giganteus* would meet the 35 billion

gallon US biofuel goal for 2016; it would take 24.4% of total agricultural land for corn and 26.5% for

switchgrass to meet that same goal, while only 9.3% for *M.* x *giganteus* to meet the 2016 goal. In

addition, *M.* x *giganteus* appears to be profitable even without government subsidies, unlike the

traditional corn/soybean rotation; much of the expense is in planting the *M.* x *giganteus.* Since it is a

perennial species, subsequent years require limited maintenance, and therefore, the stand will be highly

profitable if maintained for 10 years (Heaton et al., 2004a).

*Carbon Dioxide*

Biofuel crops possess a variety of environmental benefits, one of the most attractive being the

reduction of $CO_2$ emissions produced when substituting biomass for fossil fuel combustion

(Lewandowski et al., 1995). Simulations conducted by Hughes et al. (2010) reveal that by replacing fossil

fuel consumption with *M.* x *giganteus* biofuel, $CO_2$ concentrations could be lowered by up to 323 ppmv

by the end of the current century. The $CO_2$ balance shows that $CO_2$ emissions can be reduced by as much as 90% compared to hard coal combustion; combustion of *M.* x *giganteus* emits 2 Mg C-$CO_2$ (Lewandowski et al., 1995). The $CO_2$ balance is highly dependent on efficient N fertilizer application; N fertilizer contributes about 43% of the $CO_2$ emissions (Lewandowski et al., 1995). Likewise, Powlson et al. (2005) found that for winter wheat, N fertilizer accounts for about 60% of the total $CO_2$ emissions. Fertilizer rate application affects soil emissions of $CO_2$ and $N_2O$ and tends to increase during the first few weeks following fertilization (Phillips et al., 2009). This emphasizes the importance of using N fertilizer efficiently and minimizing losses (Powlson et al., 2005). Clifton-Brown et al. (2007) found that when *M.* x *giganteus* is harvested and combusted instead of coal, the grass mitigates 3.6-4.8 Mg C ha$^{-1}$ yr$^{-1}$ in emissions and C sequestration in the soil, potentially removing 0.5 Mg C ha$^{-1}$ yr$^{-1}$ from the atmosphere. In addition, *M.* x *giganteus* had the greatest aboveground C content compared with switchgrass, maize and prairie and up to four times as much biomass due to its N use efficiency (Davis et al., 2010).

The physiology of *M.* x *giganteus,* and agricultural practices associated with its cultivation, supports a large potential for soil C sequestration (Dondini et al., 2009b). *Miscanthus* x *giganteus*, like any energy crop, takes in $CO_2$ from the atmosphere for photosynthesis; when the crop is burned for energy, the $CO_2$ released was previously taken out of the atmosphere in the recent past, unlike fossil fuels that have been sequestered for millions of years (Hughes et al., 2010). Hillier et al. (2009) found that on average *M.* x *giganteus* sequesters more C in the soil in comparison with annuals (winter wheat and oilseed rape). In addition, Schneckenberger and Kuzyakov (2007) indicated that the amount of SOC derived from *M.* x *giganteus* was higher than that of maize; a higher contribution of *M.* x *giganteus* derived C to SOC in the deeper soil layers compared to maize indicates the possibility of belowground C sequestration by planting the perennial grass. Therefore, it is believed that *M.* x *giganteus* accumulates stored C in the soil due to its extensive below ground biomass and rootstock, as well as enhanced organic matter content (Dondini et al., 2009a). Using changes in stable C isotope ratios, Hansen et al.

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(2004) observed that a large fraction of the SOC pool was C derived from *M.* x *giganteus*; after 9 and 16 years, 13% and 31%, respectively, of the SOC present at 0 – 20 cm soil depth originated from *M.* x *giganteus*. Hansen et al. (2004) also indicated that *M.* x *giganteus* increased soil C by 14 t ha$^{-1}$, representing 29% of the C input after 16 years. Simulated levels of soil C for several biofuel crops indicated that *M.* x *giganteus* accumulates soil C at a faster rate than other species due to the greater litterfall and belowground plant growth (1041-1194 g C m$^{-2}$ y$^{-1}$) (Davis et al., 2010). This leads to enhanced soil organic matter as result of *M.* x *giganteus* losing its leaves and top shoot in autumn and winter; the biomass retains residues on the soil surface to keep the humidity in the top soil and supply C to the soil for growth in the spring (Lewandowski et al., 2000; Kuzyakov and Domanski, 2000; Clifton-Brown et al., 2007).

In addition to C and nutrient cycling, Clifton-Brown et al. (2007) found that *M.* x *giganteus* grown on previously arable land in the absence of conventional farming practices resulted in lower decomposition rates and increased soil C stabilization. *Miscanthus* x *giganteus* systems that typically receive little or no N fertilizer inherently have a higher C:N ratio resulting in a slower decomposition rate of plant residue (Schneckenberger and Kuzyakov, 2007). Dondini et al. (2009b) found that throughout the whole soil profile, the total soil organic C content under *M.* x *giganteus* is significantly higher than under an arable crop (unidentified C$_3$ crops; 131.3 vs. 105.8 Mg C ha$^{-1}$). The results from Dondini et al. (2009b) suggest that the potential of soil C storage under *M.* x *giganteus* largely depends on the land that it is replacing; arable lands provide the largest potential as a C sink. Smith et al. (2000) confirm that arable soils are often depleted in soil C and therefore have a great potential to store large quantities of C; furthermore, Hillier et al. (2009) found that replacement of arable and grassland by perennial crops leads to GHG savings.

*Nitrous Oxide*

In a study by Jørgensen et al. (1997), fertilizer was applied before the plants began growing and also when the soil water content was highest, so this provided ample condition for $N_2O$ emissions to occur. In addition, as soil water content and concentration of inorganic N in the fertilized *M. x giganteus* decreased, so did the $N_2O$ emissions. Therefore, rainfall is a major regulator on $N_2O$ fluxes; events of heavy rainfall during the early growing season soon after fertilization result in high fluxes in fertilized *M. x giganteus.* This was seen by in the study by Jørgensen et al. (1997), where peak $N_2O$ fluxes were recorded in mid-July after a temporary rainfall event. The reasoning behind the $N_2O$ fluxes after rain events is most likely because the water filled the soil pore space and supplied the best anaerobic conditions for microbial production of $N_2O$ by nitrification and denitrification. In addition, other authors have recorded similar events; Groffman and Tiedje (1998) observed a pulse of $N_2O$ when the soil is re-wetted after a dry period due to an increase in nutrient availability for microbes. This was probably the reason for the $N_2O$ peaks in the autumn after the dry summer period recorded by Jørgensen et al. (1997). Phillips et al. (2009) also found that the magnitude of GHG emissions is likely to vary with temperature and timing of N fertilizer application since microbial respiration is strongly related to temperature. Therefore applying N at the start of the growing season may be unnecessary and could result in N losses and consequently $N_2O$ emissions (Christian et al., 2008). Only 38% of ^{15}N-labeled fertilizer applied in the spring to a one-year-old crop was taken up by the plant; however, recovery of ^{15}N-labeled fertilizer on a more established crop was greater (Christian et al., 2006).

Crutzen et al. (2008) found that when biofuel crops are grown in replacement for fossil fuel consumption, harmful emissions of $N_2O$ can offset the intended benefits of growing the biofuel crop in the first place. Depending on the N use efficiency of the crop being grown, $N_2O$ emissions can be large enough to cause climate warming instead of the intended climate cooling due to "saved fossil $CO_2$"

(Crutzen et al., 2008). The underlying factor for growing biofuel crops is the agricultural practices associated with the specific crop. Stehfest and Bouwman (2006) found that soil $N_2O$ emissions are best influenced by N application rate, N source, crop type, soil pH, soil texture, climate, and soil organic matter (SOM). The easiest factor to control without disrupting crop rotations or other agricultural practices or without crop yield reduction is the N-application rate (Hoben et al., 2011). The N-application rate was tested in a study by McSwiney and Robertson (2005). They analyzed variable N fertilizer rates applied to maize; maize grain yields in their three year study increased in response to N additions from 0 to 101 kg-N $ha^{-1}$ and then leveled off at larger additions of N fertilizer. They found that at greater additions of N fertilizer, no significant yield gain was observed, but $N_2O$ did increase with excess N fertilizer rates. A similar study by Hoben et al. (2011) confirmed their findings that $N_2O$ emissions increased exponentially with increasing N fertilizer amount and yield reaches a plateau just as the $N_2O$ response sharply increases. Providing farmers an incentive to apply N at rates where maximum yield is achieved with no excess N added, could offer a unique opportunity for mitigating $N_2O$ emissions from agricultural soils and also lowered leaching of N from the soil.

*Inorganic Nitrogen*

In addition to $N_2O$ and $CO_2$ emissions, N can leave the system via leaching of nitrate ($NO_3^-$) through drainage tiles. Since plant yields are influenced greatly by the N content in the soil and removal of that biomass through modern agricultural techniques removes much of the organic N, natural supplies of N are supplemented by fertilizer to maximize plant productivity. The form of N that is most susceptible to losses is $NO_3^-$, which is a combination of N from fertilizer and soil mineralized N. Over-fertilization and inappropriate timing of fertilizer application can lead to $NO_3^-$ enriched soil water solution and the result is leaching of that $NO_3^-$ to ground and surface water (Christian and Riche, 1998). If fertilization occurs before the crop can incorporate the N, large amounts of $NO_3^-$ can be leached in short

periods of time around precipitation events (David et al., 1997). Likewise, when *M.* x *giganteus* replaces a managed agriculture field with previously high inorganic N mineralization (long-term grass with incorporation of bean residues), as seen in Christian and Riche (1998), significantly higher $NO_3^-$ leaching is possible; Christian and Riche (1998) go on to indicate that this would not be the case if *M.* x *giganteus* replaces arable land, low in inorganic N, and they also report that *M.* x *giganteus* has $NO_3^-$ losses comparable to forests and natural areas even when fertilized.

The inappropriate timing of fertilizer application was indicated by Gentry et al. (2009), when spring tile $NO_3^-$ concentrations were large due to 50% of N fertilizer applied the prior fall. This leaching of $NO_3^-$ began when tile drainage was introduced to the United States; most importantly, tile drainage changed the hydrology of the Midwestern United States resulting in large quantities of $NO_3^-$ leaching from agricultural fields to streams and rivers (David et al., 1997; McIsaac and Hu, 2004; Gentry et al., 2009). Results from an intensive study of the N balance from a highly agricultural watershed in East-Central Illinois indicated the risk of $NO_3^-$ leaching occurs mostly during the winter and spring with very large $NO_3^-$ leaching concentrations bound to high flow events. To further emphasize this, Gentry et al. (1998) found that in a study on maize over three years, record rainfall in only one month can contribute to 75% of the $NO_3^-$ loss for an entire year, as much as 148 kg N in one day and concentration of 49 mg N $L^{-1}$; this is an excellent example of high fertilization rates and large precipitation events leading to very large $NO_3^-$ losses. In contrast, David et al. (1997) found that $NO_3^-$ concentrations in late summer and early fall showed almost no $NO_3^-$ concentrations due to denitrification in the stagnant water caused by no tile inputs; this emphasizes the fact that $NO_3^-$ leaching is a direct result of agricultural practices and precipitation events. These results show that agricultural land is the source of nearly all the $NO_3^-$ in this particular watershed, rather than urban inputs, with leached $NO_3^-$ through tile drainage accounting for 25-85% of the field N balance (David et al., 1997).

The reason $NO_3^-$ is a major concern is that it causes drinking water problems and eventually leads to hypoxia in the Gulf of Mexico as it is transported down the Mississippi River; the hypoxic zone in the northern Gulf of Mexico occurs annually and, since 1950, has increased significantly. Anthropogenic activities such as deforestation, channelization, wetland drainage, loss of riparian zones, and large increases in N fertilizer application have caused the increase in the Gulf of Mexico hypoxic zone (Rabalais et al., 2002). The area intended to grow ethanol biofuel is projected to increase due to increased demand for "clean energy", which if used to grow maize – the current main biofuel crop, harmful water quality consequences could ensue due to increased $NO_3^-$ leaching from N fertilizer losses (Simpson et al., 2008). However, if perennial grasses, such as *M.* x *giganteus*, were substituted for maize grown on the same land devoted to solely for ethanol production, it could provide 260% more ethanol per hectare. Even more promising is the fact that *M.* x *giganteus* is a highly efficient N use crop even at low to no inputs of N fertilizer (Lewandowski et al., 2000; Christian et al., 2008; Heaton et al., 2008a; Miguez et al., 2008; Dohleman and Long, 2009) and could have reduced $NO_3^-$ losses as well (Powlson et al., 2005).

A study by McIsaac et al. (2010), found that in each year of the study, the corn-soybean system was dominated by $NO_3^-$ losses and were statistically greater than then losses of switchgrass or *M.* x *giganteus*; therefore, *t*otal inorganic N losses from corn-soybean were on average 7.5 times greater than switchgrass and 9 times greater than *M.* x *giganteus*. McIsaac et al. (2010), did indicate that first-year *M.* x *giganteus* had a high amount of $NO_3^-$ losses (154 kg N ha$^{-1}$ yr$^{-1}$), but then quickly decreased to 8 and 3 kg N ha$^{-1}$ yr$^{-1}$ in years 2 and 3, respectively. In another Illinois field study, Mitchell et al. (2000) indicated that $NO_3^-$ losses from an unfertilized, unharvested, perennial grass field was 3.8 kg N ha$^{-1}$ yr$^{-1}$, which is similar to the study by McIsaac et al. (2010). *Miscanthus* x *giganteus* is typically harvested during the dormant season, which may increase soil evaporation in the spring, and in turn, would reduce drainage and leaching of $NO_3^-$ (McIsaac et al., 2010). Also, harvesting an unfertilized *M.* x *giganteus* crop removes

between 5-40 kg N ha$^{-1}$ yr$^{-1}$, which may also reduce $NO_3^-$ leaching by N removal (Heaton et al., 2009; McIsaac et al., 2010). Their findings indicate that well-managed perennial grasses have considerable potential for lower N leaching compared with corn-soybean systems because perennial grasses have more extensive root systems, a longer growing season, and can supply additional C to the soil to promote immobilization. However, perennial grasses do influence the hydrological cycle by either flood reduction, which would benefit the ecosystem, or prolonged low stream flow, which could be a possible detriment to the ecosystem (McIsaac et al., 2010).

**MATERIALS AND METHODS**

**Site Description**

This study was conducted on experimental plots located at the Department of Energy funded *M. x giganteus* plots on the University of Illinois Bioenergy Farm located approximately 3 km south of the University of Illinois at Urbana-Champaign (-88.19W, 40.06 N), between April 2009 and November 2010. The mean annual temperature is 10.8°C and the mean annual precipitation is 104 cm (data from long-term weather station located at the Energy Biosciences Institute farm where plots reside). Mean maximum and minimum temperatures from April to November are 22.4°C and 10.8°C, respectively (ISWS Official Data 1971-2000). The precipitation total for 2009 was 106 cm and for 2010 was 81 cm (Data from long-term weather station located at the Energy Biosciences Institute farm where plots reside).

The experimental plots were initially thought to be located on deep loess Mollisols with 4-7% organic matter in the top 30 cm like much of east-central Illinois, and the soil was originally classified as being 76% Dana silt loam (Fine-silty, mixed, superactive, mesic Oxyaquic Argiudolls) for the northern portion of the plots and 24% Drummer silty clay loam (Fine-silty, mixed, superactive, mesic Typic Endoaquolls) for the southern portion of the plots using the Web Soil Survey. But after further investigation, the soil included a fine cap of sandy loam sediments throughout the top 30 cm and was classified into the Wyanet series (Fine-loamy, mixed, active, mesic Typic Argiudolls). See Table 1 for further soil profile descriptions. The site was in row crop production prior (1993-2005) to *M. x giganteus* planting (Figures 1-3).

Figure 1. Aerial map view of the experimental DOE site in 1993, with plots marked by the pin.

Figure 2. Aerial map view of the experimental DOE site in 1998, with plots marked by the pin.
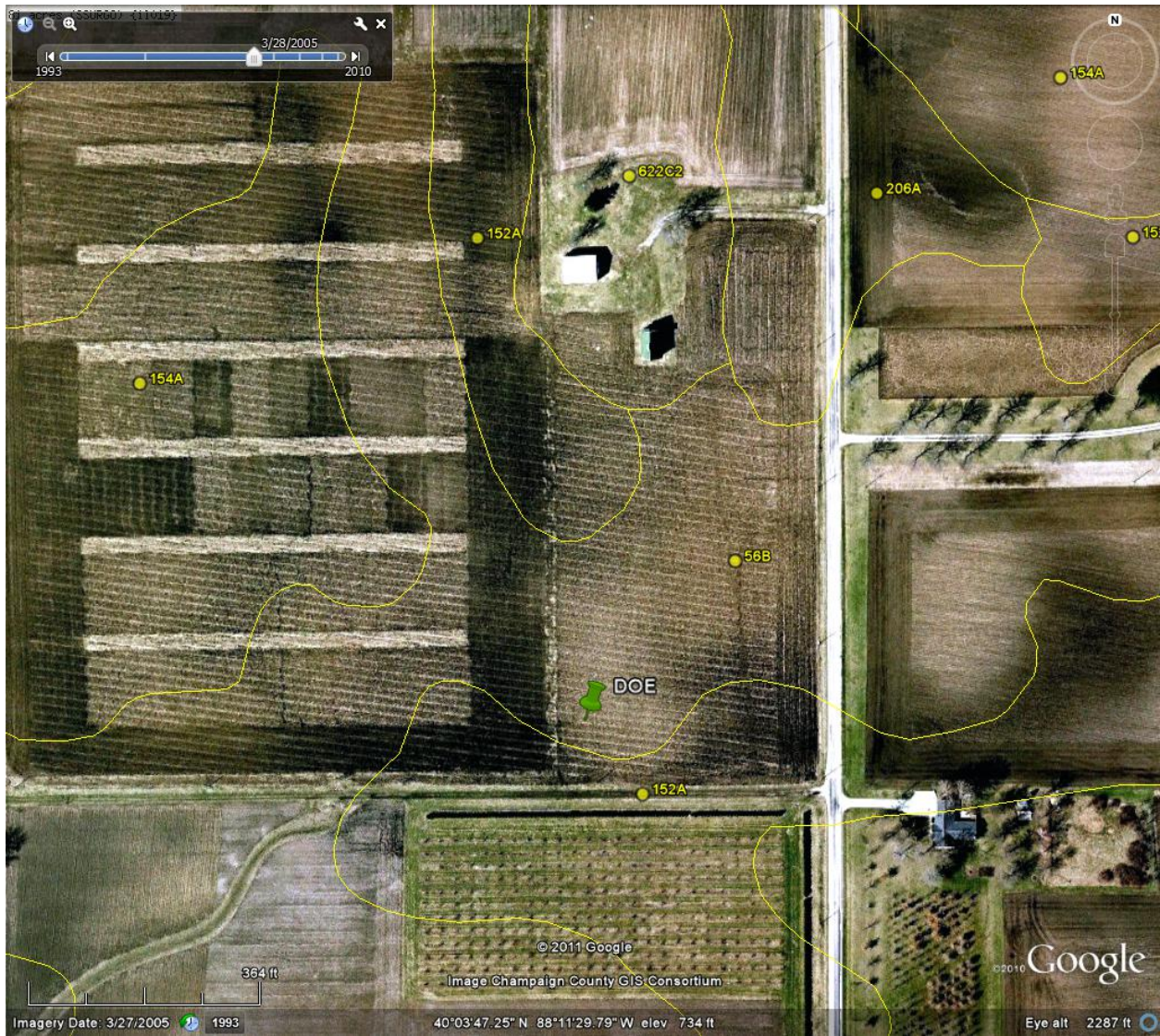
Figure 3. Aerial map view of the experimental DOE site in 2005, with plots marked by the pin.
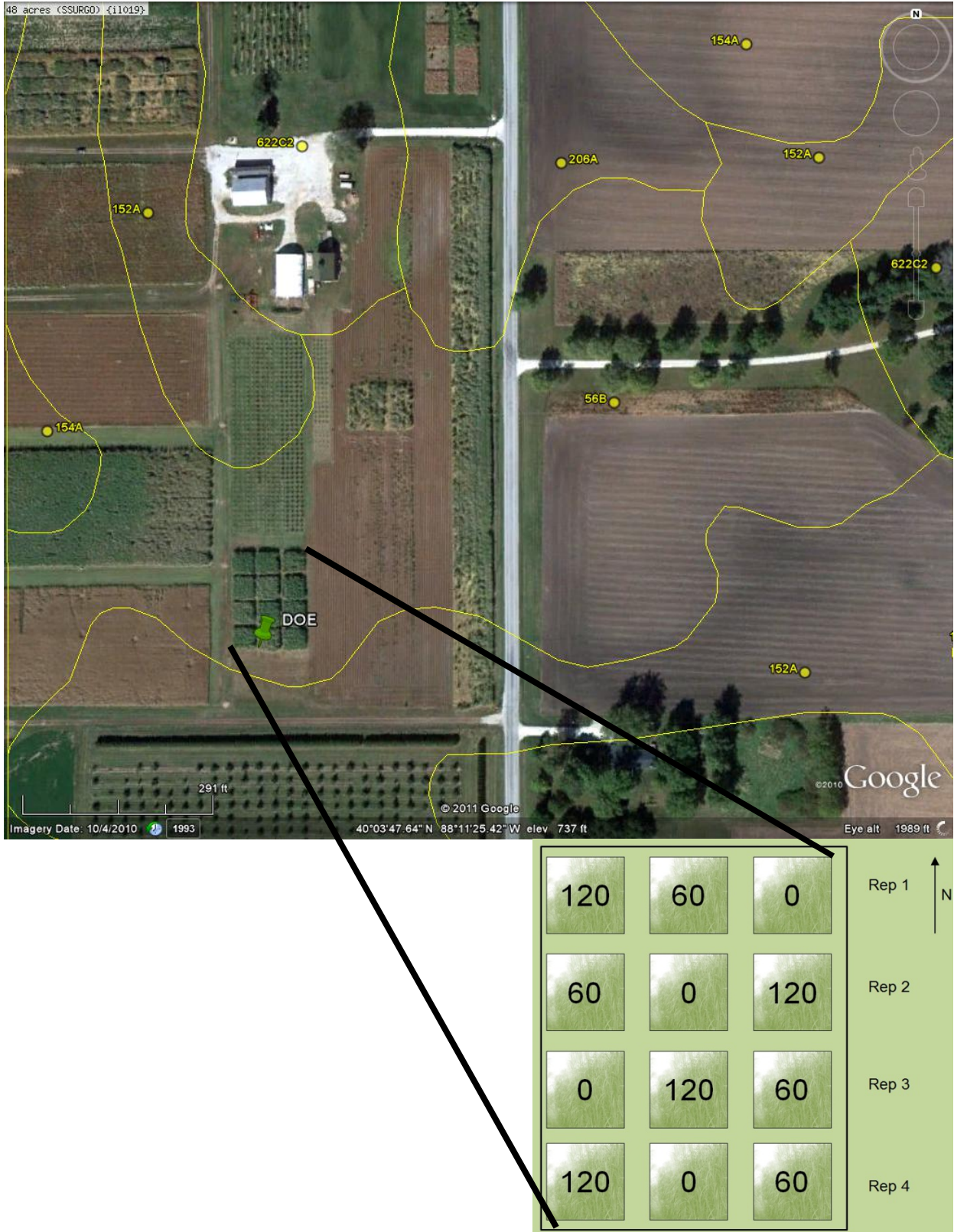
Figure 4. Aerial map view of the DOE *M. x giganteus* plots in 2010 with an expanded plot design layout. Replicate plots are fertilized in kg N ha$^{-1}$, were randomly assigned, and 10x10m.

**Experimental Design and Crop Management**

Twelve, 10 m x 10 m plots of *M. x giganteus* were planted in July 2008 using greenhouse produced plants grown in 2 cm square pots using commercially available soilless potting mix. The study had four replicates each with three N fertilizer treatments arranged in a randomized complete block design. Each plot was planted with 100 *M. x giganteus* plants spaced 1 m apart. The plots received 0, 60, or 120 kg N ha$^{-1}$ using urea fertilizer once each growing season, applied on June 23, 2009 and May 6, 2010. Approximately 80% of the grasses failed to survive the 2008-2009 winter because of mid-summer planting in July 2008 and severe winter conditions. The dead plants were removed and replanted in spring, 2009, thus 805 of the plants were first-year plants in 2009 and 20% were second year plants. The plots were hand weeded as necessary during the summer of 2009 and sporadically during 2010 to reduce weed competition. The plots were also irrigated during the summer of 2009 to ensure establishment.

**Sampling Procedures**

*Nitrous Oxide Measurement*

Nitrous oxide measurements were sampled following the GRACEnet chamber-based trace gas flux measurement protocol (Baker et al., 2003). Beginning in late April 2009, two 0.031m$^2$ Polyvinyl chloride (PVC) chamber bases were installed on the west half and east half of each of the twelve plots prior to sampling to allow for settling. A total of 24 chamber bases were used in the experiment following the vented chamber technique. The chamber tops were made of reflective, white PVC, had a vent tube, sampling ports, and insulation foam to make an air-tight seal with the chamber bases. The chamber bases were left in place for the entire trial except for the final biomass harvest.

Nitrous oxide fluxes were measured near noon, when the temperature was near the average for the day. Chambers were located between rows and did not include any plants. Before gas sampling, 4 chamber offsets were taken with a ruler, recorded, and averaged in order to calculate head space and then flux. Nitrous oxide measurements were taken by placing the closed chamber tops on the chamber bases and taking 15 mL samples of air using a PrecisionGlide® Needle syringe at 0, 10, 20, and 30 minute intervals through the 20 mm Pharma-Fix® Butyl septa sealed with 20mm Alum Tops. The samples were then injected into 10 mL vials and placed in a plastic bag and to be processed on a gas chromatograph machine with an electron capture detector (ECD), (Shimadzu® GC 2014 with AOC-5000). Standards were prepared using Scott Specialty Gas® in the laboratory directly before being analyzed on the gas chromatograph. Standards include: 0.1, 0.32, 0.65, 0.98, and 1.98 ppm $N_2O$. Around fertilization periods, additional standards of 3.65 ppm and 7.46 ppm were used to ensure that the standard concentrations encompassed the samples taken in the field for a more accurate regression line. Nitrous oxide emission was calculated using the regression coefficients obtained from $N_2O$-N concentrations against sampling time. In addition to collecting $N_2O$ emissions, soil temperature at 10 cm and air temperature was recorded during the 30-minute interval for each of the 24 rings.

*Carbon Dioxide Measurement*

Using the same chamber bases and offsets described in the previous section, a LI-COR LI-8100 device (Lincoln, NE, USA) was used to measure the $CO_2$ fluxes. Carbon dioxide measurements were taken at the same time as the $N_2O$ measurements and during the first year, twice a week for much of the growing season. The LI-COR® was placed on each chamber base for a time of 90 seconds and $CO_2$ flux was recorded; in addition, the LI-COR® also measured soil temperature at 5 cm using an attached temperature probe. The LI-8100 was equipped with a Wi-Fi card to enable communication with a Palm[TM] LifeDrive[TM] handheld PDA.

*Soil Moisture and Soil Temperature*

Twelve Decagon® Em5b data loggers were installed to measure soil temperature using ECH2O® ECT temperature sensors, at 10 cm, and also soil moisture using ECH2O® EC-5 moisture sensors, at 10 (two sensors placed in opposite directions), 30, and 50 cm continuously (once every hour) for the entire study period. The ECH2O® EC-5 determines volumetric water content (VWC) by measuring the dielectric constant of the media using capacitance/frequency domain technology with a 70 MHz frequency (Decagon®, 2010). A measurement from the five sensors was taken every hour for the duration of the project.

*Soil Analysis*

Before the *M.* x *giganteus* in the plots was established, two separate soil cores were taken from each of the twelve plots using a Giddings probe hydraulic soil sampling and coring machine mounted to the back of a truck. Soil cores (2 cm in diameter) were 100 cm long, and divided into 0-10, 10-20, 20-30, 30-50, and 50-100 cm sections. Each plot's soil characteristics were averaged into the respective corresponding fertilizer treatment and then summarized (Table 1). Soil samples were sent to Brookside Laboratories, Inc., New Knoxville, OH, where soil pH, extractable P, and C to N ratio (C:N) were determined. Soil pH was determined using a 1:1 $H_2O$ solution. Extractable P was determined following the Mehlich III soil test extractant. Total C and N were determined by combustion (Brookside Laboratories, Inc., 2000). Soil texture for determining sand, silt, and clay percentages was conducted at the Pedology Laboratory at the University of Illinois Urbana-Champaign. Soil samples were first air dried and ground to pass through 2 mm and 50 μm sieves to retrieve all the sand sized particles, leaving only the silt and clay particles. The silt and clay particles were then determined using the hydrometer method (American Society for Testing and Materials, 2000).

In addition to initial soil sampling, two soil cores (0-10 cm depth) were collected from each plot during every other gas sampling, composited, and $NH_4$-N and $NO_3$-N concentrations determined. Soil bulk density was also determined for each sample to allow the content of soil $NH_4$-N and $NO_3$-N per kg of soil area to be determined.

*Inorganic Nitrogen Leaching*

Fields fertilized with N will inherently lead to some inorganic N leaching, especially in soils with a low C:N ratio as is common with east-central Illinois Mollisols; therefore it is critical to quantify the inorganic N leaching from the agricultural system. Four ion exchange resin lysimeters were installed in each of the twelve plots following the design and methods described in Susfalk and Johnson (2002) and Langlois et al. (2003). The lysimeters were constructed with a 5.1 cm diameter PVC pipe and a coupling section that were a total of 7 cm long. Nitrate and $NH_4^+$ were absorbed by a layer of ion exchange resin placed between two layers of washed sand and held in the lysimeter with a permeable nylon membrane. The lysimeters were installed at a depth of 50 cm under undisturbed soil. Water draining though the soil profile above the lysimeter carries $NO_3^-$ and $NH_4^+$ dissolved in the soil water solution, which is then captured and adsorbed onto the resin in the lysimeter. After a year in the soil, the lysimeters are removed and replaced with new lysimeters containing fresh resin and allowed to remain in the soil for another year; the same process is conducted each next year. The mass of $NO_3^-$ and $NH_4^+$ absorbed on the year-old resin was extracted using 1M KCl. Nitrate-N and $NH_4^+$-N concentrations in the extract were measured colormetrically by flow injection analysis with a Lachat QuickChem 8000 (Lachat, Loveland, CO). The mass of the $NO_3^-$ and $NH_4^+$ absorbed on the resin was determined by multiplying the concentrations by the extract volume [procedure was the same as in McIsaac et al. (2010)]. Due to the small surface area of the resin lysimeters, individual lysimeters could produce highly variable data. Therefore, to lessen the influence of individual lysimeters, outliers were trimmed and means calculated.

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To calculate the trimmed means, the maximum and minimum observations were excluded from the calculation.

*Yield and biomass*

At the end of each season, final yield data was calculated following the DOE Sun Grant procedure. Harvest date was noted, fresh weight was measured, percent moisture was determined, and dry biomass yield was calculated in Mg ha$^{-1}$. To determine which *M.* x *giganteus* plants would be harvested, 1 m$^2$ quadrats were constructed out of PVC pipe and placed randomly around a *M.* x *giganteus* plant, preferably not during wet conditions and not on the border row of the plot. Then the *M.* x *giganteus* plant and all tillers within the 1 m$^2$ quadrat were clipped at 10 cm above the ground. Only the standing residue within the quadrat was taken, none of the fallen litter on the ground was used. After the first quadrat was sampled, the quadrat was flipped to the north and harvested. The same procedure was used again in each plot, so as to take a total of 4 plants from each of the 12 plots (Figure 5.). Next, the entire fresh sample was weighed on a field scale to determine wet biomass weight. After determining the fresh weight of each subsample, 5 randomly selected stems from each subsample were used to determine % moisture by drying them at 60°C for at least 48 hours. Percent moisture from these 5 dried tillers was used to determine dry biomass yield in Mg ha$^{-1}$ for each large subsample. Total C and N were determined using an Elemental Analyzer (EAS 4010, Costech).

**Data Analysis**

To calculate annual N$_2$O and CO$_2$ fluxes, field sampled fluxes were extrapolated to predict Flux (*F*) at one-hour intervals for the entire year based on measured soil temperature (*T*) at 10 cm. We assumed that *F* varied with temperature in the same manner as CO$_2$ efflux, as described using a standardized Boltzmann- Arrhenius equation (e.g., (McCoy and Gillooly, 2008):

$$F = F_{ref} * e^{\left[\frac{-E}{k}*\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right]}$$

Here, $F_{ref}$ and $T_{ref}$ are the reference (measured) flux and temperature (in Kelvin), $E$ is a the average

activation energy of heterotrophic soil respiration (0.65 eV) (Allen et al., 2005), and *k* is Boltzmann's

constant ($8.62 \times 10^{-5}$ eV K$^{-1}$). Between 0 and 30 °C, this temperature relationship roughly equates to

$Q_{10}$=2, which is a widely used standard for describing the temperature dependence of biological

processes (Davidson and Janssens, 2006). We choose to use the Arrhenius equation, as opposed to $Q_{10}$,

because of its grounding in physical chemistry and the fact that it correctly predicts the observed decline

in $Q_{10}$ with increasing temperature (Davidson and Janssens, 2006).

Statistical analyses were performed using SAS v9.2 (SAS Institute Inc., 2011). Dependent

variables were analyzed for a significant response due to fertilizer treatment using regression analysis.

Each year of results were analyzed separately. Significance was set at either α = 0.05 or α = 0.01

depending on statistical model; correlation coefficients are expressed as r.

| 10 Meters | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| X | X | X | X | X | X | X | X | X | X |
| X | X | X | X | X | X | X | X | X | X |
| X | X | X | X | X | X | X | X(1b) | X | X |
| X | X | X | X | X | X | X | X(1a) | X | X |
| X | X | X | X | X | X | X | X | X | X |
| X | X | X | X(2b) | X | X | X | X | X | X |
| X | X | X | X(2a) | X | X | X | X | X | X |
| X | X | X | X | X | X | X | X | X | X |
| X | X | X | X | X | X | X | X | X | X |
| X | X | X | X | X | X | X | X | X | X |

10 Meters

Figure 5. Schematic for harvesting the *M.* x *giganteus* biomass in each plot. The schematic below represents 1 plot with 100 *M.* x *giganteus* plants spaced one meter apart with each "X" representing a *M. x giganteus* plant. 1a represents the first plant sampled and 1b represents the plant harvested to the north of the first selected *M.* x *giganteus* plant. 2a and 2b represent the replication sampled in each plot to give the total of 4 plants harvested in each 1 $m^2$.

**RESULTS**

**Soil Characteristics**

The Drummer soil series, which is typical of what is found in central Illinois and found close to the experiment site, normally has a depth to horizons with greater than 15 percent sand ranging from 102 to 152 cm. The other soil series mapped near the experiment site is in the Dana soil series. It is also typical of soils in central Illinois; the depth to horizons with more than 20 percent fine sand or coarser ranges from 60 to 102 cm. The DOE plots contain greater than 50% sand in the surface 10 cm. This phenomenon can be explained by Fig. 1 that shows an aerial map of the DOE site in 1993. The site is under row crops, but a faint stream can be made out near where the DOE site is currently located; this stream break is most likely where the sandiness of the DOE plots originated. The soil series of the DOE plots therefore has to be the Wyanet soil series (Fine-loamy, mixed, active, mesic Typic Argiudolls) because it contains a cap of fine sandy loam sediments at the surface.

The soil from each of the fertilizer treatments did not differ much in the percentages of sand, silt and clay (Table 1). For the most part, each plot had more than 50% sand in the upper 30 cm. The silt for the control plots was slightly higher in percentage compared to the fertilizer treatment plots. In each of the fertilizer treatment plots, the percentage of sand decreased when moving down the soil profile. The silt content in each of the fertilizer treatments increased with depth to approximately 50% in the 50-100 cm depth. The clay content also increased with depth to approximately 35% in the 50-100 cm depth. In each of the fertilizer treatments, the soil pH increased with depth to 6.5 for all plots in the 50-100 cm depth. The soil pH at the top 0-10 cm was 5.7 in each of the fertilizer treatments. The extractable P was high in the surface soils with little in the deeper layers, no doubt from past fertilization. The C/N ratios were generally all around 10 and typically decreased with depth.

Table 1. Soil pH, extractable P, total C, total N and C/N ratio and soil texture by treatment before the study (n = 8)

| Treatment | Soil Depth (cm) | pH | Extractable P (mg/kg) | Total C (g kg$^{-1}$) | Total N (mg kg$^{-1}$) | C/N | Texture (%) Sand | Silt | Clay |
|---|---|---|---|---|---|---|---|---|---|
| Control | 0-10 | 5.7 | 34 | 1.1 | 0.11 | 10.4 | 53 | 32 | 16 |
| (0 kg ha$^{-1}$) | 10-20 | 5.9 | 40 | 1.1 | 0.11 | 10.2 | 51 | 32 | 17 |
| | 20-30 | 6.0 | 30 | 1.0 | 0.10 | 10.1 | 47 | 35 | 18 |
| | 30-50 | 6.0 | 8 | 0.7 | 0.08 | 9.8 | 29 | 45 | 26 |
| | 50-100 | 6.5 | 1 | 0.4 | 0.04 | 10.3 | 8 | 55 | 37 |
| 60 kg ha$^{-1}$ | 0-10 | 5.7 | 45 | 1.1 | 0.11 | 10.1 | 58 | 27 | 15 |
| | 10-20 | 5.8 | 50 | 1.1 | 0.11 | 10.0 | 57 | 28 | 15 |
| | 20-30 | 5.9 | 44 | 1.1 | 0.11 | 10.5 | 57 | 29 | 15 |
| | 30-50 | 6.2 | 12 | 0.7 | 0.08 | 9.5 | 46 | 37 | 18 |
| | 50-100 | 6.5 | 1 | 0.4 | 0.05 | 8.1 | 8 | 55 | 37 |
| 120 kg ha$^{-1}$ | 0-10 | 5.7 | 38 | 1.2 | 0.11 | 10.2 | 55 | 30 | 16 |
| | 10-20 | 6.0 | 52 | 1.1 | 0.12 | 9.9 | 55 | 31 | 15 |
| | 20-30 | 6.1 | 40 | 1.1 | 0.11 | 9.5 | 51 | 32 | 17 |
| | 30-50 | 6.0 | 11 | 0.7 | 0.08 | 8.9 | 35 | 49 | 22 |
| | 50-100 | 6.5 | 1 | 0.4 | 0.04 | 9.1 | 15 | 50 | 35 |

Table 2. Average soil moisture and soil temperature taken hourly for the entire growing season (May 1 – October 31) for 2009 and 2010.

| Year | Treatment (kg ha$^{-1}$) | Soil Moisture (m³/m³ VWC) @10 cm | @30 cm | @50 cm | Soil Temperature (°C) @10 cm |
|---|---|---|---|---|---|
| 2009 | 0 | 0.23 (0.02)‡ | 0.28 (0.07) | 0.34 (0.06) | 20.1 (0.3) |
| | 60 | 0.22 (0.01) | 0.27 (0.02) | 0.30 (0.06) | 20.2 (0.4) |
| | 120 | 0.22 (0.03) | 0.26 (0.06) | 0.29 (0.07) | 19.6 (0.4) |
| 2010 | 0 | 0.21 (0.02) | 0.22 (0.05) | 0.29 (0.03) | 18.9 (1.7) |
| | 60 | 0.16 (0.02) | 0.21 (0.03) | 0.27 (0.08) | 19.9 (0.5) |
| | 120 | 0.19 (0.02) | 0.21 (0.04) | 0.25 (0.07) | 19.1 (0.8) |

‡ Values in parentheses are standard deviations.

Soil moisture at any of the three depths, 10, 30 or 50 cm, during the growing season (May 1 –
October 31) in 2009 were not affected by fertilizer treatment (p = 0.5772, p = 0.5150, and p = 0.2470,
respectively); likewise, soil temperature at 10 cm did not significantly respond to fertilizer treatment(p =
0.0834) (Table 2). Soil moisture at 10 cm was not significantly affected by fertilizer treatment during the
growing season in 2010 (p = 0.4157). Soil moisture at 30 and 50 cm also did not respond to fertilizer
treatment (p = 0.6555, and p = 0.2969, respectively). Likewise, soil temperatures at 10 cm did respond
to fertilizer treatment during the growing season in 2010 (p = 0.7674).

**Nitrous Oxide**

In 2009, mean cumulative $N_2O$ fluxes (Table 3) were not affected by fertilizer treatments. In
2010, however, the cumulative nitrous oxide fluxes were significantly different (p = 0.0152, $\alpha$ = 0.05 for
all statistical comparisons hereafter). In 2009, mean cumulative $N_2O$ fluxes ranged from 0.75 kg-N ha$^{-1}$ in
the control plots, to 1.30 kg-N ha$^{-1}$ in the 60 kg N ha$^{-1}$ treatment plots, to 1.35 kg-N ha$^{-1}$ in the 120 kg N
ha$^{-1}$ treatment plots (p = 0.0859). In 2010 mean cumulative $N_2O$ fluxes ranged from 0.35 kg-N ha$^{-1}$ in the
control plots, to 0.77 kg-N ha$^{-1}$ in the 60 kg N ha$^{-1}$ treatment plots, to 2.91 kg-N ha$^{-1}$ in the 120 kg N ha$^{-1}$
treatment plots. Mean daily $N_2O$ fluxes (Table 3) were calculated by taking the mean cumulative $N_2O$
flux and dividing the value by 365 to obtain a daily flux mean. The mean daily fluxes followed the same
pattern as mean cumulative $N_2O$ fluxes in both years. In 2009, mean daily $N_2O$ fluxes ranged from 2.06 g-
N ha$^{-1}$ day$^{-1}$ kg-N ha$^{-1}$ in the control plots, to 3.65 g-N ha$^{-1}$ day$^{-1}$ in the 60 kg N ha$^{-1}$ treatment plots, to
3.68 g-N ha$^{-1}$ day$^{-1}$ in the 120 kg N ha$^{-1}$ treatment plots and observed no difference among treatments (p
= 0.0859). In 2010, mean daily $N_2O$ fluxes ranged from 0.95 g-N ha$^{-1}$ day$^{-1}$ kg-N ha$^{-1}$ in the control plots,
to 2.12 g-N ha$^{-1}$ day$^{-1}$ in the 60 kg N ha$^{-1}$ treatment plots, to 7.98 g-N ha$^{-1}$ day$^{-1}$ in the 120 kg N ha$^{-1}$
treatment plots. In 2010, mean daily $N_2O$ fluxes were different among treatment plots (p = 0.0152).

In 2009, cumulative $N_2O$ emissions across the treatment plots were lower than in 2010 despite having more precipitation in 2009 than in 2010. Precipitation in 2009 was 106 cm and in 2010 it was 81 cm (data from long-term weather station located at the Energy Biosciences Institute farm where plots reside). Maximum air temperature was greater in 2009 compared to 2010; 32.7 and 27.0 °C respectively.

Precipitation a week after fertilization was 0.13 cm in 2009 and 2.41 cm in 2010 (Figure 6). Average soil temperature at 10 cm was 27.6 °C in 2009 and 15.0 °C in 2010 during that week. Soil moisture in 2009 was on average 0.22 $m^3 m^{-3}$ VWC (volumetric water content) compared to 2010 where it was 0.23 $m^3 m^{-3}$ VWC (volumetric water content). Nitrous oxide emitted was 0.028, 0.033, and 0.051 kg $N_2O$-N $ha^{-1}$ for treatments 0, 60, and 120 kg-N $ha^{-1}$ in 2009 and 0.033, 0.076, and 0.203 in 2010 kg $N_2O$-N $ha^{-1}$ for treatments 0, 60, and 120 kg-N $ha^{-1}$.

Nitrous oxide emissions one month after fertilization were 0.101, 0.228, and 0.378 kg $N_2O$-N $ha^{-1}$ for treatments 0, 60, and 120 kg-N $ha^{-1}$ in 2009 and 0.126, 0.282, and 0.822 in 2010 kg $N_2O$-N $ha^{-1}$ for treatments 0, 60, and 120 kg-N $ha^{-1}$ (Figure 6). Precipitation amounts were 11.2 cm in 2009 and 8.6 cm in 2010, leading to soil moisture averages of 0.23 $m^3 m^{-3}$ VWC in 2009 compared to 2010, where it was 0.23 $m^3 m^{-3}$ VWC. Average soil temperature at 10 cm was 24.1 °C in 2009 and 18.3 °C in 2010.

Nitrous oxide produced three months following fertilization was 0.182, 0.437, and 0.599 kg $N_2O$-N $ha^{-1}$ for the 0, 60, and 120 kg-N $ha^{-1}$ treatments in 2009 and 0.201, 0.547, and 2.262 kg $N_2O$-N $ha^{-1}$ for the 0, 60, and 120 kg-N $ha^{-1}$ treatments in 2010 (Figure 6). Precipitation had amounted to 25.2 cm in 2009 and 31.4 cm in 2010 after three months; this corresponded to soil moisture average of 0.214 $m^3 m^{-3}$ VWC in 2009, and similarly 0.216 $m^3 m^{-3}$ VWC in 2010. Soil temperature at 10 cm was 22.7 °C in 2009 and 21.1 °C in 2010.

Peak $N_2O$ values for individual sampling days were 25% lower in 2009 than in 2010 in the 120 kg $ha^{-1}$ treatment plots, 0.087 compared to 0.403 mg $N_2O$-N $ha^{-1}$ $hr^{-1}$, respectively. In 2009, peak $N_2O$ fluxes

on individual sampling days did occur after fertilization, but were not as dramatic as in 2010. In 2010, the peak $N_2O$ flux for on an individual sampling day was correlated to a large precipitation event and the larger fertilizer treatment of 120 kg N ha$^{-1}$; the $N_2O$ flux was 0.403 mg $N_2O$-N ha$^{-1}$ hr$^{-1}$ and following 4.47 cm of precipitation on June 9th, 2010.

In 2009, cumulative $N_2O$ fluxes increased gradually throughout the year, peaking about 39 days after fertilization, for the 60 and 120 kg ha$^{-1}$ fertilizer treatment plots and not influenced as much by fertilization as in 2010, where immediately following fertilization, cumulative fluxes for the 60 and 120 kg ha$^{-1}$ fertilizer treatment plots were large and peaked 49 days following fertilization (Figure 6). Using the control plots as the base, in 2009 the 60 kg N ha$^{-1}$ lost only 0.55 kg $N_2O$-N ha$^{-1}$ and the 120 kg N ha$^{-1}$ lost only 0.60 kg $N_2O$-N ha$^{-1}$. In 2010, the 60 kg N ha$^{-1}$ lost only 0.42 kg $N_2O$-N ha$^{-1}$, while the 120 kg N ha$^{-1}$ lost 2.56 kg $N_2O$-N ha$^{-1}$ (Table 3).

Nonlinear increases in mean daily $N_2O$ fluxes were observed one year, 2010 (Figure 7). Mean daily fluxes were well described by the nonlinear model during 2010 ($r^2 = 0.41$, $p = 0.015$); however, the corresponding standard deviation for each treatment plot was large in both models. Nitrous oxide emissions and soil temperature at 10 cm were significantly related ($r = 0.16$, $p = 0.04$). In addition, soil moisture at 10 cm and $N_2O$ emissions were also significantly related ($r = 0.23$, $p = 0.0034$) (Figure 9).

**Carbon Dioxide**

Cumulative $CO_2$ fluxes were not affected by fertilizer treatment. No significant differences were observed in relation to either mean cumulative $CO_2$ flux or mean daily $CO_2$ flux by fertilizer treatment; in fact, the $CO_2$ emissions were similar across the three fertilizer treatments (Table 4). In 2009, mean cumulative $CO_2$ fluxes ranged from 8.62 Mg-C ha$^{-1}$ in the control plots to 9.11 Mg-C ha$^{-1}$ in the

Table 3. Cumulative N$_2$O flux during 2009 and 2010. Differences are only within the year and measurements.

| Year | Treatment (kg N ha$^{-1}$) | Mean Cumulative N$_2$O Flux (kg N ha$^{-1}$)† |
|------|------|------|
| 2009 | 0 | 0.75 (0.06) ‡ ND)‡ ND |
| | 60 | 1.30 (0.65) |
| | 120 | 1.35 (0.41) |
| 2010 | 0 | 0.35 (0.09)* |
| | 60 | 0.77 (0.39) |
| | 120 | 2.91 (2.08) |

† Cumulative fluxes were calculated from measured fluxes corrected for temperature variations using a $Q_{10}$ = 2.
‡ Values in parentheses are standard deviations.
ND, means no difference among treatments within a given year ($\alpha$ = 0.05) .
*Treatments are different within a given year ($\alpha$ = 0.05).
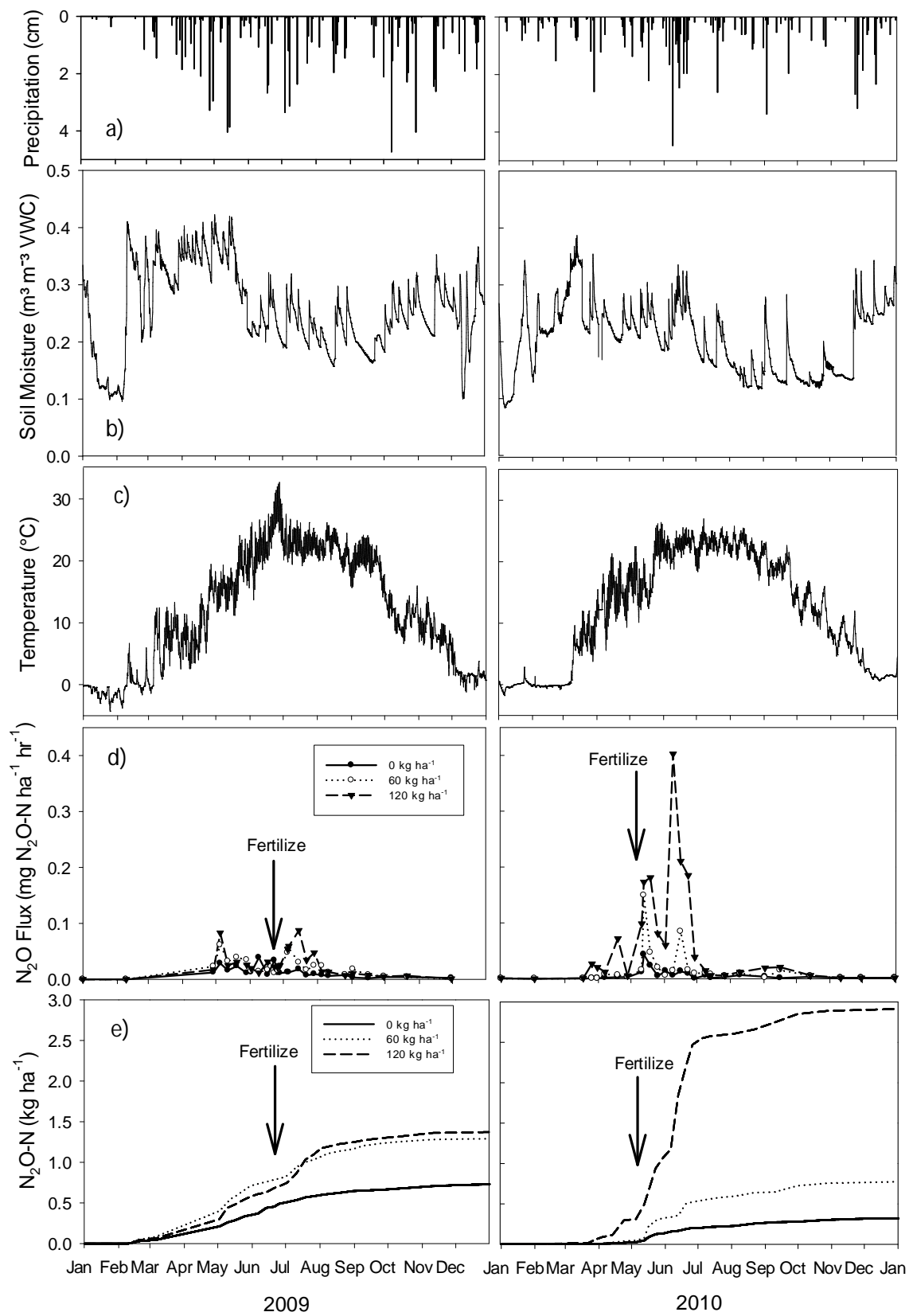
Figure 6. 2009 and 2010 sampling data: Precipitation (a), soil moisture at 10 cm (b), soil temperature at 10 cm (c), individual $N_2O$ sampling dates (d), cumulative $N_2O$ emissions by date (e).
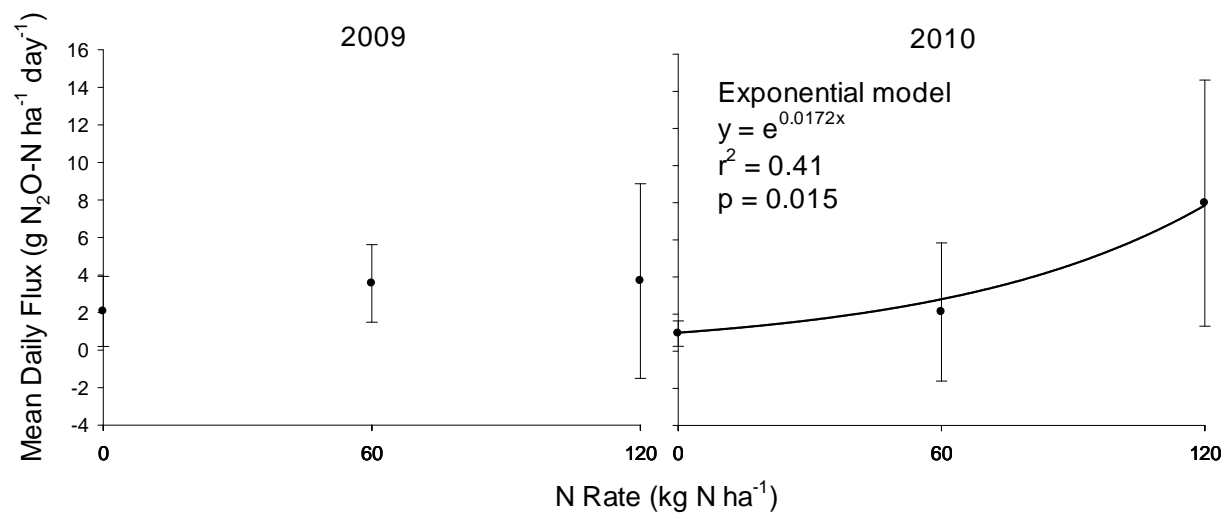
Figure 7. Mean daily $N_2O$ flux during 2009 and 2010. Error bars represent standard deviations of the treatment averages.

Table 4. Cumulative $CO_2$ flux during 2009 and 2010. Differences are only within the year and measurements; however, there was no difference among any treatment or measurement.

| Year | Treatment (kg N ha$^{-1}$) | Cumulative $CO_2$ Flux (Mg-C ha$^{-1}$)[†] |
|------|------|------|
| 2009 | 0 | 8.62 (1.75)[‡] ND |
| | 60 | 9.11 (1.24) |
| | 120 | 8.62 (1.14) |
| 2010 | 0 | 8.90 (0.85) ND |
| | 60 | 9.16 (1.92) |
| | 120 | 8.96 (1.53) |

[†] Cumulative fluxes were calculated from measured fluxes corrected for temperature variations using a $Q_{10} = 2$.

[‡] Values in parentheses are standard deviations. ND, means no difference among treatments within a given year ($\alpha = 0.05$).

Figure 8. 2009 and 2010 sampling data: Precipitation (a), soil temperature at 10 cm (b), individual $CO_2$ sampling dates (c,) Cumulative $CO_2$ emissions (d).

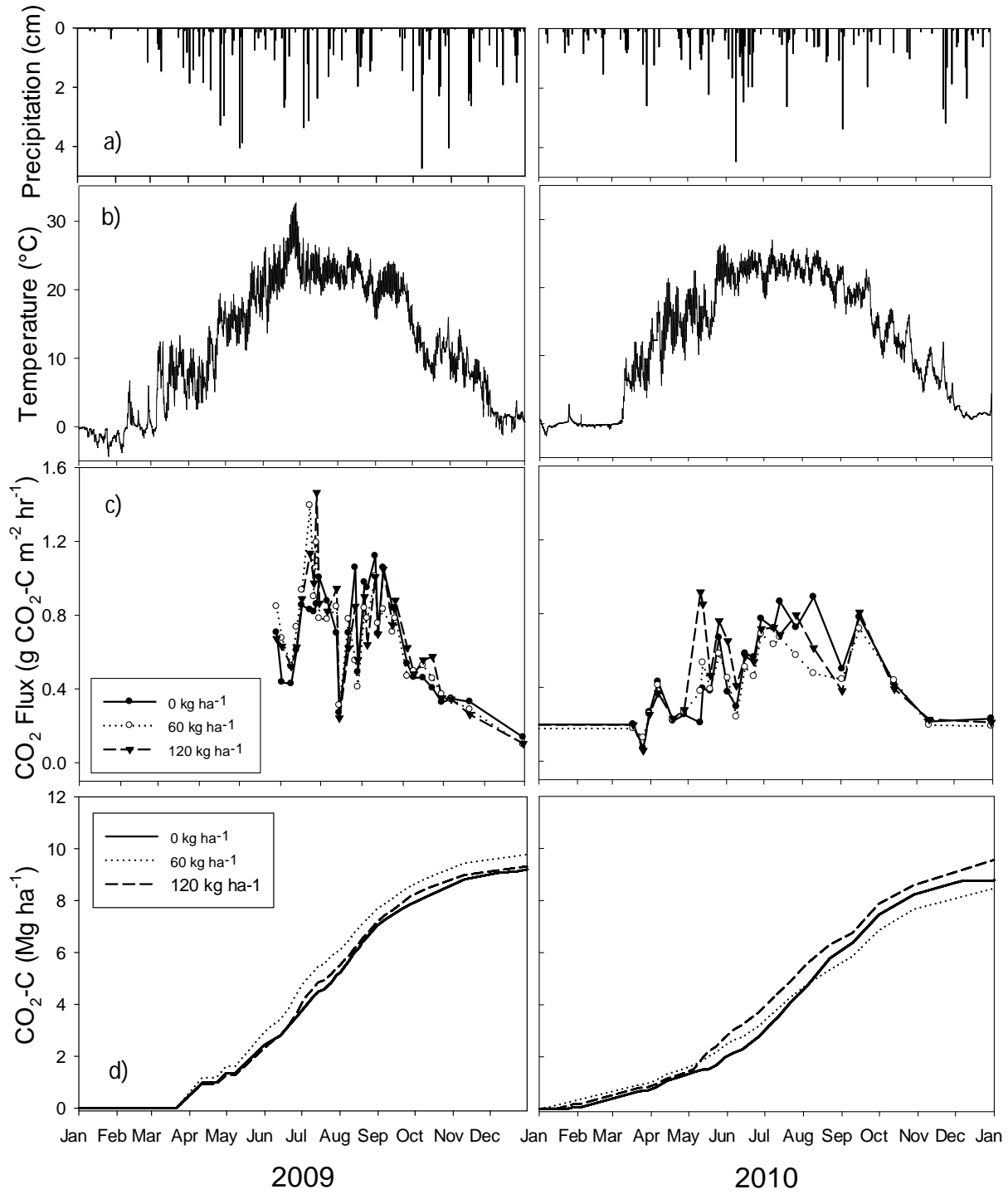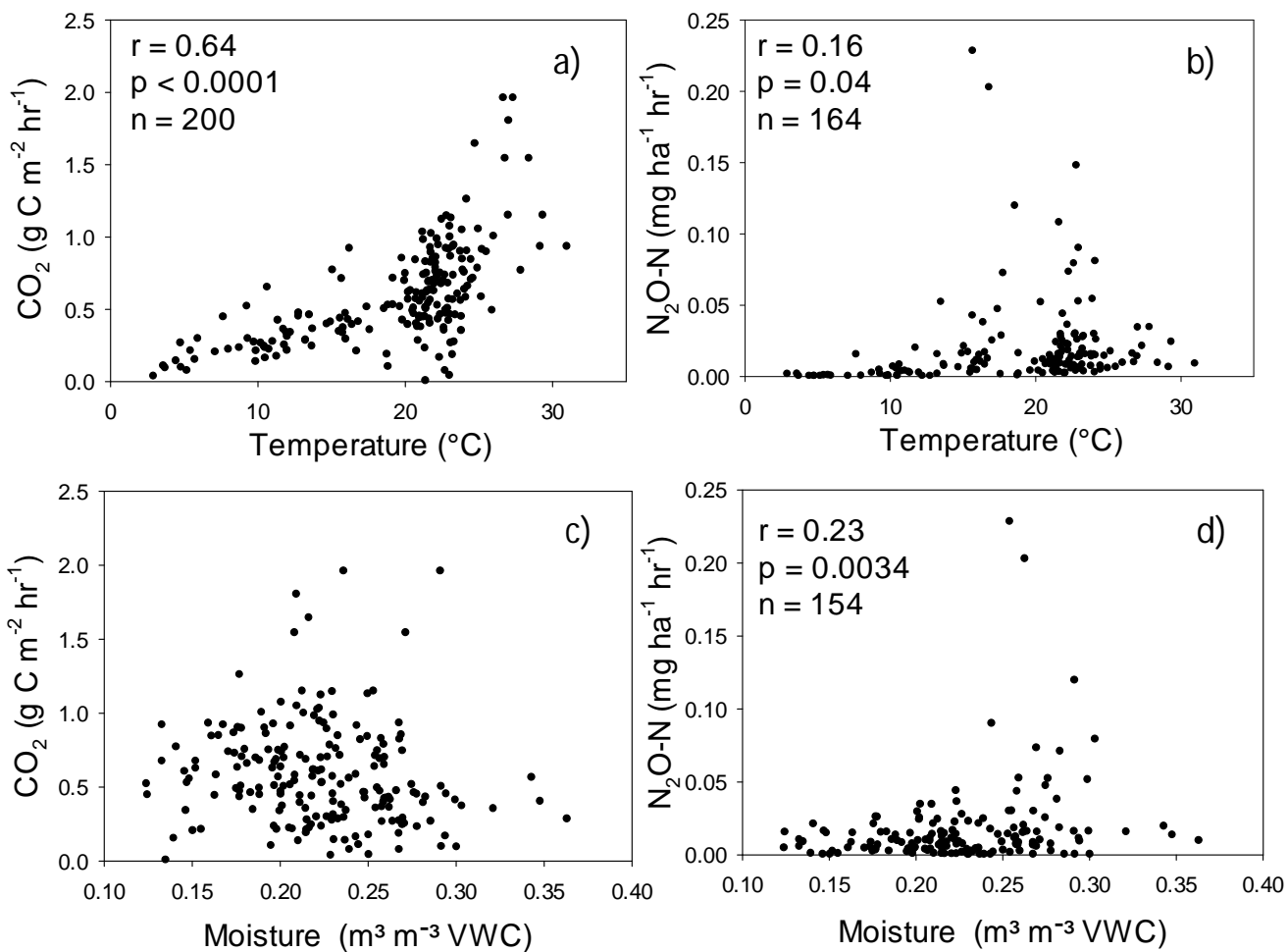Figure 9. $CO_2$ emissions vs. soil temperature at 10 cm (a), $N_2O$ emissions vs. soil temperature at 10 cm (b), $CO_2$ emissions vs. soil moisture at 10 cm (c), and $N_2O$ emissions vs. soil moisture at 10 cm (d). Significance level $\alpha = 0.05$.

60 kg N ha$^{-1}$ treatment plots to 8.62 Mg-C ha$^{-1}$ in the 120 kg N ha$^{-1}$ treatment plots (p = 0.9984) (Table 4).

In 2010, mean cumulative $CO_2$ fluxes ranged from 8.90 Mg-C ha$^{-1}$ in the control plots to 9.16 Mg-C ha$^{-1}$ in

the 60 kg N ha$^{-1}$ treatment plots to 8.96 Mg-C ha$^{-1}$ in the 120 kg N ha$^{-1}$ treatment plots (p = 0.9546).

Mean daily $CO_2$ fluxes were calculated by taking the mean cumulative $CO_2$ flux values and it by 365 to

obtain a daily flux mean. In 2009, mean daily $CO_2$ fluxes ranged from 23.6 kg-C ha$^{-1}$ day$^{-1}$ in the control

plots to 25.0 kg-C ha$^{-1}$ day$^{-1}$ in the 60 kg N ha$^{-1}$ treatment plots to 23.6 kg-C ha$^{-1}$ day$^{-1}$ 120 kg N ha$^{-1}$

treatment plots (p = 0.9984). In 2010, mean daily $CO_2$ fluxes ranged from 24.4 kg-C ha$^{-1}$ day$^{-1}$ in the

control plots to 25.1 kg-C ha$^{-1}$ day$^{-1}$ in the 60 kg N ha$^{-1}$ treatment plots 24.5 kg-C ha$^{-1}$ day$^{-1}$ in the 120 kg

N ha$^{-1}$ treatment plots (p = 0.9546). Carbon dioxide was significantly related to soil temperature at 10

cm (r = 0.64, p < 0.0001). However, $CO_2$ and soil moisture at 10 cm were not related (Figure 9).

**Soil Inorganic Nitrogen**

In 2009, surface soil (0-10 cm) $NH_4^+$ content was highest directly following fertilization in each of

the treatment plots, with the 60 kg N ha$^{-1}$ treatment being larger than 120 kg N ha$^{-1}$ (59 and 22 mg N

kg$^{-1}$, respectively) (Figure 10). The next sampling revealed that the 120 kg N ha$^{-1}$ was larger than the 60

kg N ha$^{-1}$ (56 and 30 mg N kg$^{-1}$, respectively). Subsequent sampling following fertilization had decreasing

$NH_4^+$ concentrations for both fertilizer treatments in 2009. The same results were found in 2010, where

the 60 kg N ha$^{-1}$ treatment had larger peaks near fertilization (May 11th, 2010) than the 120 kg N ha$^{-1}$ (27

and 19 mg N kg$^{-1}$, respectively). But even on May 13th, 2010, the 60 kg N ha$^{-1}$ was still larger than the 120

kg N ha$^{-1}$ (38 and 33 mg N kg$^{-1}$, respectively). As each year progressed following fertilization, soil $NH_4^+$

decreased to almost zero in all plots, as it was nitrified and/or taken up by the plants. Soil $NO_3^-$

concentrations in 2009 reached a maximum concentration of 31 mg N kg$^{-1}$ for the 120 kg N ha$^{-1}$. In 2010,

the maximum soil $NO_3^-$ concentration was 29 mg $NO_3$-N kg$^{-1}$ for the 60 kg N ha$^{-1}$ and 59 mg N kg$^{-1}$ for the
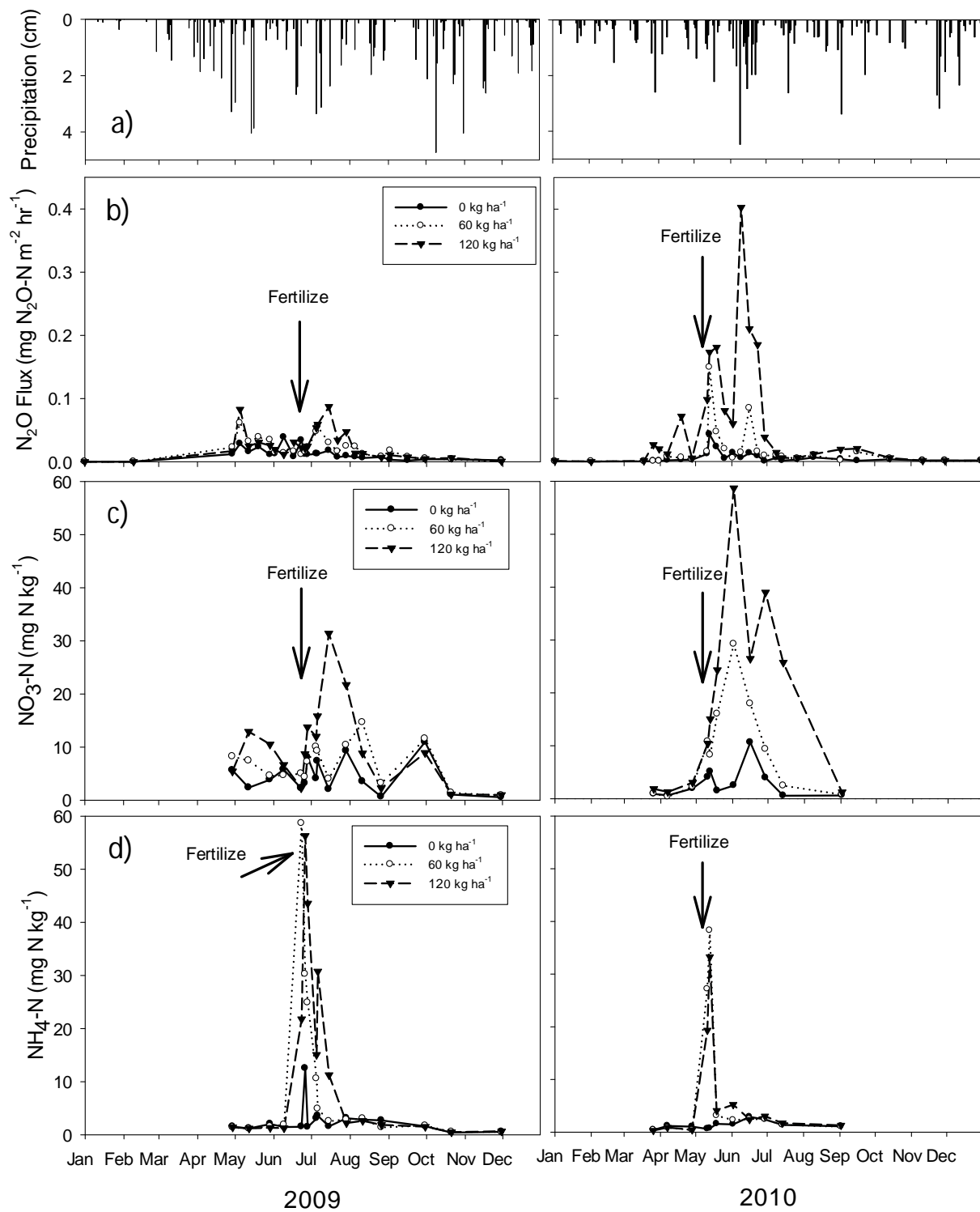
Figure 10. 2009 and 2010 sampling data: Precipitation (a), individual $N_2O$ sampling dates (b), soil nitrate concentration (c), soil ammonium concentration (d).
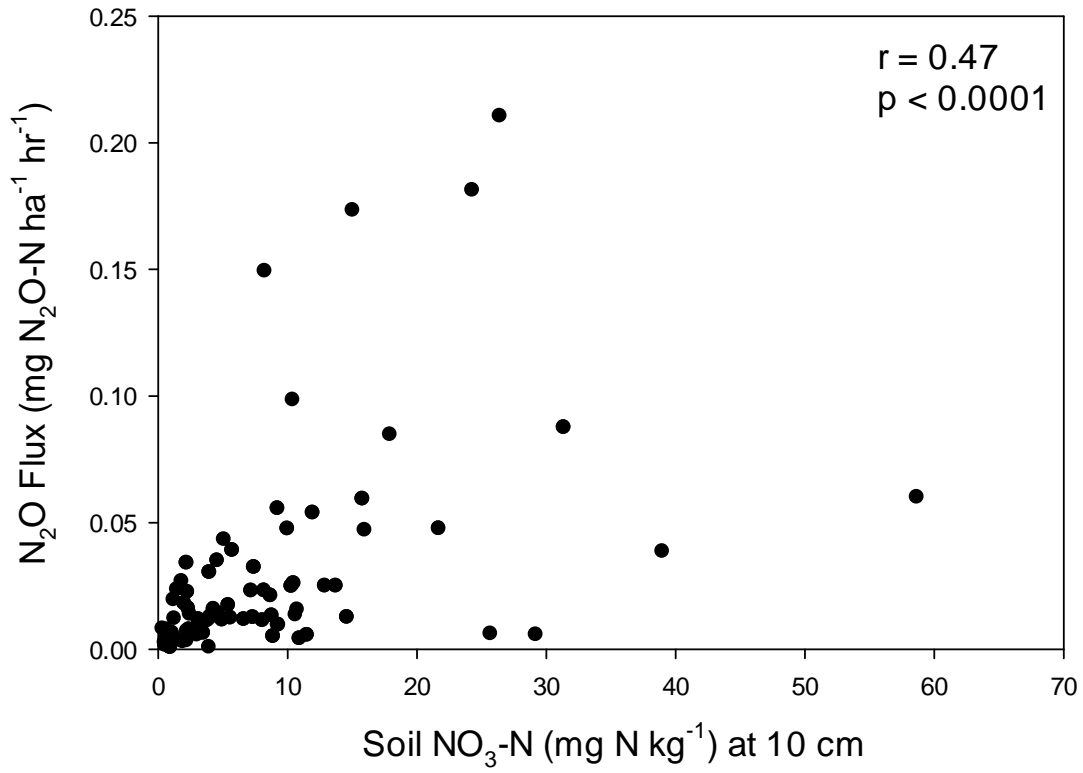
Figure 11. Individual $N_2O$-N emissions vs. soil $NO_3$-N concentrations for both 2009 and 2010.

Table 5. Trimmed mean annual leaching of nitrate, ammonium, and total inorganic nitrogen observed from the ion exchange resin lysimeters at 50 cm soil depth under *M. x giganteus*.

| Year | Treatment (kg N ha$^{-1}$) | Leached NO$_3^-$ (kg N ha$^{-1}$ yr$^{-1}$) | Leached NH$_4^+$ (kg N ha$^{-1}$ yr$^{-1}$) | Leached total Inorganic (kg N ha$^{-1}$ yr$^{-1}$) |
|---|---|---|---|---|
| 1 | 0 | 6.4 (3.2) ‡ ND | 6.8 (0.8) ND | 13.3 (2.8) ND |
|  | 60 | 7.1 (6.7) | 5.6 (0.5) | 12.6 (7.1) |
|  | 120 | 13.3 (7.0) | 7.1 (1.3) | 20.5 (8.0) |
| 2 | 0 | 8.9 (5.9)** | 2.3 (2.1)ND | 9.1 (7.3)** |
|  | 60 | 15.3 (7.1) | 3.0 (1.9) | 18.3 (7.9) |
|  | 120 | 28.9 (6.1) | 6.1 (4.3) | 34.9 (8.5) |

‡ Values in parentheses are standard deviations.
ND, means no difference among treatments within a given year ($\alpha = 0.05$).
*Treatments are different within a given year ($\alpha = 0.05$).
**Treatments are different within a given year ($\alpha = 0.01$).

Table 6. Yield and biomass C and N content of the harvested *M. x giganteus.* The 2009 growing season crop was harvested on January 10th, 2010, and the 2010 growing season crop was harvested on November 29th, 2010.

| Year | Treatment (kg N ha$^{-1}$) | Yield (Mg ha$^{-1}$) | Biomass N (kg ha$^{-1}$) | Biomass C (kg ha$^{-1}$) | Biomass C/N (kg ha$^{-1}$) |
|------|------|------|------|------|------|
| 2009 | 0 | 1.1 (0.7)† ND | | | |
| | 60 | 4.1 (3.7) | | ‡DNR | |
| | 120 | 4.0 (2.2) | | | |
| 2010 | 0 | 14.9 (2.9) ND | 44.9 (9.0)** | 6698 (1287) ND | 153 (39) ND |
| | 60 | 15.8 (1.8) | 53.5 (5.3) | 7257 (851) | 137 (24) |
| | 120 | 17.0 (1.4) | 66.6 (1.3) | 7796 (656) | 117 (9) |

† Values in parentheses are standard deviations.

‡ DNR, data not recorded.

ND, means no difference among treatments within a given year ($\alpha = 0.05$).

* Treatments are different within a given year ($\alpha = 0.05$).

** Treatments are different within a given year ($\alpha = 0.01$).

120 kg N ha$^{-1}$. Soil NO$_3^-$ concentrations in both the 60 and 120 kg N ha$^{-1}$ treatment plots were greatest 34 days after fertilization, the same day as the 4.47 cm of precipitation on June 9$^{th}$, 2010. The greatest concentration of soil NO$_3^-$ corresponded to the largest N$_2$O emissions from the 120 kg N ha$^{-1}$ treatment plot. Following the maximum NO$_3^-$ concentration on June 9$^{th}$, 2010, NO$_3^-$ in the 120 kg N ha$^{-1}$ treatment decreased (26 mg N kg$^{-1}$), but increased due to another precipitation event on June 29$^{th}$, 2010 (39 mg N kg$^{-1}$). Following the peak in the 60 kg N ha$^{-1}$ the soil NO$_3^-$ in the 60 kg N ha$^{-1}$ decreased steadily for the rest of the growing season. In the 120 kg N ha$^{-1}$ plots following the June 29$^{th}$, 2010 maximum, the soil NO$_3^-$ concentration decreased to near zero by the end of the growing season. The control plot experienced a small maximum concentration on June 16$^{th}$, 2010 (11 mg N kg$^{-1}$) following a series of rainfall events, but then steadily decreased to near zero by the end of the growing season (Figure 10).

Soil inorganic NO$_3^-$ concentrations were significantly related to N$_2$O emissions (P < 0.0001) (Figure 11) for both years combined.

**Inorganic Nitrogen Leaching**

The resin lysimeters removed from the 2009 *M.* x *giganteus* crop are represented by Year 1 because they were buried from April of 2009 to April of 2010; year 2 represents resin lysimeters buried in April of 2010 to April of 2011 (Table 5). Mean values of NO$_3^-$ leached from 50 cm in the *M.* x *giganteus* in 2010 were not significantly different among the 0, 60, and 120 kg N ha$^{-1}$; the values were 6.4, 7.1, and 13.3 kg N ha$^{-1}$ yr$^{-1}$, respectively (p = 0.1189). Likewise, mean NH$_4^+$ was not significantly different among treatments (p = 0.7093). Values for NH$_4^+$ were 6.8, 5.6, and 7.1 kg N ha$^{-1}$ yr$^{-1}$ from the 0, 60, and 120 kg N ha$^{-1}$ plots, respectively. Combining the NO$_3^-$ and the NH$_4^+$ provides the total inorganic N leached from the plots. In 2010, total inorganic N was not statistically different among treatments (p = 0.1448); the values were 13.3, 12.6, and 20.5 kg N ha$^{-1}$ yr$^{-1}$ from the 0, 60, and 120 kg N ha$^{-1}$ plots, respectively.

Mean values of $NO_3^-$ leached in 2011 indicated that fertilizer treatment had an effect (p = 0.0005). The $NO_3^-$ values from 2011 were 6.9, 15.3, and 28.8 kg N ha$^{-1}$ yr$^{-1}$ from the 0, 60, and 120 kg N ha$^{-1}$ plots, respectively. Ammonium was not statistically different among treatment plots (p = 0.0954); the values for $NH_4^+$ in 2011 were 2.3, 3.0, and 6.1 kg N ha$^{-1}$ yr$^{-1}$ from the 0, 60, and 120 kg N ha$^{-1}$ plots, respectively. The total inorganic N leached in 2011 also showed an effect to N fertilizer treatment (p = 0.0005); the values were 9.2, 18.3, and 34.9 kg N ha$^{-1}$ yr$^{-1}$ from the 0, 60, and 120 kg N ha$^{-1}$ plots, respectively.

**Yield and Biomass**

Harvested *M.* x *giganteus* yields from the 2009 crop were on average 1.1, 4.1, and 4.0 from the 0, 60, and 120 kg N ha$^{-1}$ plots, respectively (p = 0.1380) (Table 6.). These values were much smaller compared to the 2010 crop, which were 14.9, 15.8, and 17.0 from the 0, 60, and 120 kg N ha$^{-1}$ plots, respectively (P = 0.1698). Biomass C and N data from the harvested 2009 crop were unavailable because the *M.* x *giganteus* was in an early establishment period, so that measurements were not made. The biomass data from the 2010 crop had harvested N contents of 44.9, 53.5, and 66.6 kg N ha$^{-1}$ from the 0, 60, and 120 kg N ha$^{-1}$ plots, respectively (Table 6); the N content from the harvested biomass in 2010 showed an effect to N fertilizer (P = 0.0004). Carbon harvested from 2010 was 6698, 7257, and 7796 kg C ha$^{-1}$ from the 0, 60, and 120 kg N ha$^{-1}$ plots, respectively (P = 0.1218). By dividing the N from the C in biomass gives us the C:N ratios from the biomass, which were 153, 137, and 117 kg ha$^{-1}$ from the 0, 60, and 120 kg N ha$^{-1}$ plots, respectively (P = 0.0755).

**DISCUSSION**

**Soil Type**

Although *M.* x *giganteus* had low $N_2O$ emissions for both years for the 0 and 60 kg N ha$^{-1}$ treatment plots, the 120 kg N ha$^{-1}$ plots did not have as low emissions. The soil present at the DOE site may contribute to its comparable $N_2O$ emissions when comparing $N_2O$ emissions from a corn-soybean rotation on similar sandy loam soils. In a study on corn under loamy and sandy soils, Hoben et al. (2011) observed cumulative $N_2O$-N emissions ranging from 0.75 kg $N_2O$-N ha$^{-1}$ to 2.5 kg $N_2O$-N ha$^{-1}$ in 2007 and 1 kg $N_2O$-N ha$^{-1}$ to 2 kg $N_2O$-N ha$^{-1}$ in 2008 for the 135 kg N ha$^{-1}$ fertilizer treatment (which is the closest to the 120 kg N ha$^{-1}$ used at the DOE site). The DOE plots from the 120 kg N ha$^{-1}$ treatment had 1.35 and 2.91 kg $N_2O$-N ha$^{-1}$, respectively. The soils at the DOE site contain a large amount of sand compared to what is typically found in central Illinois. In a study by De Wever et al. (2000), anaerobic soil incubations revealed that loamy sand soils produce an immediate and high amount $N_2O$ as long as nitrate is available. De Wever et al. (2000) also found that $N_2O$ production was higher under sandy soils compared to loamy soils. Hellebrand et al. (2003) found cumulative $N_2O$ emissions from fertilized (150 kg N ha$^{-1}$) poplar (another proposed biofuel crop) were 1.04, 1.45, and 2.97 kg $N_2O$-N ha$^{-1}$ (averaged for the two poplar plots) for three consecutive years with varying precipitation under sandy soils. The data from Hellebrand et al. (2003) is comparable to the cumulative $N_2O$ emissions from the DOE site for the 120 kg N ha$^{-1}$ fertilization plots.

**Fertilization Rate and $N_2O$ Emissions**

A response to fertilizer treatments was measured for the emissions of $N_2O$, which is similar to the only other study focusing on $N_2O$ emissions from *M.* x *giganteus* (Jørgensen et al., 1997). The authors reported $N_2O$ emissions from two fertilization treatments, 0 and 60 kg ha$^{-1}$ were 0.14 and 1.09 kg N ha$^{-1}$, respectively; these numbers were similar to the DOE study which were 0.75 and 1.30 kg N ha$^{-1}$,

respectively in 2009, and 0.35 and 0.77 kg N ha$^{-1}$, respectively in 2010. Results from several N$_2$O emission studies on row-crop agriculture shows that N$_2$O emissions correlate well with fertilizer N rate; in a review of several studies, Millar et al. (2010) indicated that as fertilizer N amount increased, resulting N$_2$O emission rates increased as well. The observed exponential trend in N$_2$O emissions from 2010 (Figure 7) is in agreement with Hoben et al. (2011). They indicated that N$_2$O increased exponentially with increasing N fertilization amounts. Similar results were observed by Grant et al. (2006), where these authors observed that N$_2$O emissions increased exponentially with increasing fertilizer rates.

**Fertilization Rate and CO$_2$ Emissions**

Unlike N$_2$O emissions, cumulative CO$_2$ emissions were not affected by fertilizer treatments (p = 0.9984 and p = 0.9546 for 2009 and 2010 respectively). No published study was found that tested variable fertilizer N treatments on *M.* x *giganteus* and CO$_2$ emissions, but one study on a wheat-corn-soybean rotation showed no difference among tillage effects, N placement depth effects, soil water content effects, or tillage-N placement interactions with respect to seasonal CO$_2$ emissions (Drury et al., 2006). Drury et al. (2006) found that growing season (May 1$^{st}$ to October 31$^{st}$) CO$_2$ emissions in no till were 5.49 Mg C ha$^{-1}$ in 2000, 5.91 Mg C ha$^{-1}$ in 2001, and 5.37 Mg C ha$^{-1}$ in 2002. This is similar to the DOE *M. x giganteus* study because the plots were also no till and the average was 7.49 Mg C ha$^{-1}$ for the growing season in 2009 and 6.74 Mg C ha$^{-1}$ for the growing season in 2010. Mielnick and Dugas (2000) observed the average annual soil CO$_2$ flux in a tallgrass prairie in Texas was 17 Mg C ha$^{-1}$. Their results were similar to annual fluxes at the Konza Prairie in Kansas of 13-21 Mg C ha$^{-1}$ (Knapp et al., 1998). Mielnick and Dugas (2000) attribute the large annual CO$_2$ fluxes to high annual precipitation (88 cm for the 84 year average) and even distribution of rainfall throughout the year; in addition, greater soil C contents could also explain the high CO$_2$ emissions from the tallgrass prairie. The average cumulative

$CO_2$ flux across all the DOE plots in 2009 and 2010 were smaller than from the tallgrass prairie studies; 8.8 and 9.0 Mg $CO_2$-C ha$^{-1}$, respectively. This is most likely because the authors from the tallgrass prairie studies did not factor in hourly changes in temperature into their annual flux equations, which would most likely account for the larger fluxes because temperature is such a critical factor for $CO_2$ emissions (Figure 9). Another likely reason for the smaller $CO_2$ fluxes from the *M.* x *giganteus* is that it is young and still building soil C.

**Precipitation and Fertilizer Application Timing and Its Effects on $N_2O$ Production**

Soil water amounts in excess of field capacity will promote denitrification and can occur through a variety of circumstances; heavy rain or too much irrigation can lead to denitrification throughout the biologically active soil zone (Smith et al., 1997). In addition, fertilization timing can have an important role in determining the extent of $N_2O$ production (Smith et al., 1997). The peak in $N_2O$ emissions from the 120 kg N ha$^{-1}$ treatment plots in 2010 on June 2$^{th}$, 2010 (Figure 6) can also be attributed to the large precipitation event (4.47 cm of rain) that occurred the day before. This is due to rainfall reducing the $O_2$ content in the soil pore space, a requirement for the production of $N_2O$ by nitrification and denitrification (Jørgensen et al., 1997). In the only $N_2O$ emission study on *M.* x *giganteus*, Jørgensen et al. (1997) observed an immediate peak in $N_2O$ emission following a rainfall event in mid-July.

Parkin and Kaspar (2006) also observed responses to precipitation events on fertilized corn plots; the largest peaks of $N_2O$ occurred in response to rainfall events after fertilizer was applied. In addition, Parkin and Kaspar (2006) also observed $N_2O$ fluxes from the soybean plots after rainfall, but to a lesser extent than the corn plots. Baggs et al. (2003) indicated large fluxes of $N_2O$ after fertilization following rainfall in fertilized rye and bean. Engel et al. (2010) found that for a canola (*Brassica rapa* L.), apart from fertilizer placement effects, $N_2O$ emissions were clearly driven by precipitation events, or the absence of such events. Groffman and Tiedje (1988) found that when soils are dried and then rewetted,

pulses of denitrification and its corresponding $N_2O$ emissions can be large and significant due to enhanced microbial activity. This phenomenon is likely the mechanism for the large $N_2O$ emission recorded at the DOE plots on June 9th, 2010. Groffman and Tiedje (1988) attribute the pulse in denitrification to reduced soil $O_2$ levels from aerobic respiration rather than the increased substrate for the denitrifiers. The large precipitation event on June 9th, 2010 likely left the soil saturated with water. That lack of $O_2$ for respiration led to the denitrification of the soil $NO_3^-$ supplied by fertilization earlier in the spring; the soil $NO_3^-$ concentration was 59 mg N kg^{-1} in the 120 kg N ha^{-1} plot. When the fertilizer was applied on May 6, 2010 rainfall had occurred a few times between the fertilization date and the large rainfall event, but not enough for the soil $NO_3^-$ to be fully converted to $N_2O$ and $N_2$ through denitrification. The average soil moisture from May 6th, 2010 to June 7th, 2010 was 0.23, 0.28, and 0.33 WC for 10, 30, and 50 cm, respectively. The soil moisture on June 8th, 2010 was on average 0.21, 0.26, and 0.33 VWC for 10, 30, and 50 cm, respectively, compared to 0.28, 0.31, and 0.36 on June 9th, 2010 (Figure 6). The precipitation caused an increase in soil moisture, which provided the conditions for rapid denitrification of soil N. This rapid denitrification following rewetting of dry soils by a major precipitation event follows the results of Groffman and Tiedje (1988) and is consistent with Smith and Parsons (1985) who found that denitrification enzymes can persist under drying soils. Groffman and Tiedje (1988) also found that the pulse of denitrification following rewetting of soils can be relatively brief; this is consistent with the results from the DOE study in that the large $N_2O$ emission on June 9th, 2010, resulted in a quick decline of $N_2O$ emissions for the rest of the year, most likely because the $NO_3^-$ needed for microbial denitrification was exhausted.

**Greenhouse Gas Emissions and Abiotic Parameters**

Fertilizer application leads to an increase in soil $N_2O$ emissions by increasing the mineral N pool available for nitrification and denitrification (Engel et al., 2010). Therefore abiotic factors largely control

the amount and timing of $N_2O$ emissions. Temperature was significantly correlated to $N_2O$ emissions ($p = 0.04$); therefore, the general trend observed in this study was as temperature increases, $N_2O$ emissions increased as well. Conversely, as temperature decreased, microbial activity also likely decreased, leaving little to no $N_2O$ emissions because it was simply too cold for microbial activity to allow for nitrification or denitrification. This is supported by Bouwman et al. (1993); the authors concluded that low winter temperatures in temperate regions limit $N_2O$ production. In addition Phillips et al. (2009) concluded that the magnitude of $N_2O$ emissions is likely to vary with temperature since microbial activity is strongly related to temperature.

Similar trends can be observed for $N_2O$ and soil moisture at 10 cm. As soil moisture increases, $N_2O$ emissions generally increase also. As a result, soil moisture was significantly correlated to $N_2O$ ($p = 0.0034$). At low soil moisture microbial activity is hindered, limiting the amount of $N_2O$ that can be produced. At high soil moisture complete denitrification could be taking place so all the N could be converted to N gas and could explain the low $N_2O$ emissions at high soil moisture content. Conflicting results like these were also observed by Groffman and Tiedje (1988); they concluded that denitrification rates do not have a constant relationship with water content.

Drury et al. (2006) found that soil temperature was the driving factor for the difference in $CO_2$ emissions each year, meaning warmer years will have larger $CO_2$ emissions than cooler years. This phenomenon is confirmed by the significant relationship between $CO_2$ and soil temperature at 10 cm ($p < 0.0001$, Figure 9.). In addition, Parkin and Kaspar (2003) observed that temperature is normally the strongest predictor of $CO_2$ flux. Raich and Potter (1995) also found that temperature is the single most important variable for predicting the soil $CO_2$ flux. Furthermore, Raich and Potter (1995) found that maximum $CO_2$ emissions from soils occur during the summer (approximately May-September). The DOE plots also experienced this pattern because the summer months contributed larger $CO_2$ emissions due

to increased temperatures. 2009 experienced larger maximums in temperature and, as a result, $CO_2$ emissions were larger compared to 2010 (Figure 8). Even though soil temperature and soil moisture was greater in 2009 compared to 2010, cumulative $CO_2$ emissions were about the same in 2010 as 2009. These results further highlight the complexity involved with soil respiration especially because $CO_2$ emissions did not correlate at all with soil moisture at 10 cm. Parkin and Kaspar (2003) also found that soil water content affects $CO_2$ flux, but was not a strong predictor of average diurnal $CO_2$ flux patterns.

Woli et al. (2010) found that for central Illinois soils, soil type and fertilizer additions that increase the soil $NO_3^-$-N concentrations appeared to be the driving force in controlling the mole fraction of $N_2O$ on biofuel production soils. Peak $N_2O$ emissions soon after fertilization can be attributed to the addition of N to the system, especially for the 120 kg N $ha^{-1}$ treatment in 2010; results were similar to Woli et al. (2010) and the $N_2O$ emissions following fertilization can be attributed to higher exchangeable soil $NO_3^-$-N and $NH_4^+$-N concentrations. Both years, soil $NH_4^+$-N was, as expected, at its largest concentration after fertilization because urea was the fertilizer used and is readily broken down by soil microbes into $NH_4^+$-N. Engel et al. (2010) found that the delay in $N_2O$ emission peaks was likely tied to inhibition of urea hydrolysis and nitrification into $NO_3^-$; however, some $N_2O$ is emitted as a result of nitrification (Smith and Cohen, 2004). Similar results were observed at the DOE plots because directly after fertilization, $NH_4^+$-N concentrations were at their highest; then the $NH_4^+$-N is nitrified into $NO_3^-$ by soil microbes, leading to the increased soil $NO_3^-$-N concentrations following fertilization. Recous and Machet (1999) found that after 7 days, only 10% of the applied urea was left in the form of $NH_4^+$-N. They attribute this to quick nitrification of the applied urea. In all of the DOE plots, the C:N ratio is low, meaning that most of the N need by the *M.* x *giganteus* can be supplied from soil N; however at the detriment of the soil N reservoirs. Grant et al. (2006) indicate that a low C:N ratio allows for mineralization of SON. Therefore, fertilizer rates in excess of the crop need, if sustained over time, would cause greater accumulation of soil organic N (SON) and mineral N, and therefore more rapid

mineralization and nitrification, resulting in larger $N_2O$ emissions (Grant et al., 2006). This can be seen clearly with the rapid increase then decrease in $NO_3^-$-N following precipitation events over the growing season. The relationship between soil $NO_3^-$-N concentrations and $N_2O$ flux was significant ($p < 0.0001$) (Figure 11), meaning as soil $NO_3^-$-N concentrations increase $N_2O$ emissions increase as well. After the quick $NH_4^+$-N nitrification, most of the excess N in the soil remains in the form of $NO_3^-$-N and is subject to microbial transformation to $N_2O$ when denitrification occurs. The flux of $N_2O$ and denitrification by soil $NO_3^-$-N amounts is well known (Aulakh et al., 1992; Mulvaney et al., 1997; Smith et al., 1997); Recous and Machet (1999) found that for winter wheat that as fertilizer N remains longer in the soil, more of the N is subject to other microbial processes (immobilization, gaseous emissions, etc.) instead of being taken up by the crop.

**Inorganic Nitrogen Leaching**

In addition to N losses through $N_2O$ production, loss of N can occur through subsurface leaching; the most common form of N that is leached is $NO_3^-$. Additions of N fertilizer are needed to maximize plant productivity, but also add to the N that can be leached from the system. In a study by McIsaac et al. (2010) comparing corn-soybean rotational systems to *M.* x *giganteus* and switchgrass, the corn-soybean system experienced significantly greater losses of $NO_3^-$ and total inorganic N. Their study used the same resin lysimeter procedure conducted at the DOE plots. The *M.* x *giganteus* plots in the study by McIsaac et al. (2010) were not fertilized; their findings for unfertilized *M.* x *giganteus* were slightly smaller compared to the control and 60 kg N ha$^{-1}$ treatment plots in 2010 and just the 0 kg N ha$^{-1}$ treatment plot in 2011. Their observed 4-year average losses from *M.* x *giganteus* were 3.0, 1.8, and 4.8 kg N ha$^{-1}$ yr$^{-1}$ for $NO_3^-$, $NH_4^+$, and total inorganic N, respectively. In year 1 of the study, inorganic N leaching from the 120 kg N ha$^{-1}$ plots was similar to the N losses from the corn-soybean rotation from the McIsaac et al. (2010) study. Losses were on a 5-year average 40.4, 2.4, and 43.0 kg N ha$^{-1}$ yr$^{-1}$ for

$NO_3^-$, $NH_4^+$, and total inorganic N, respectively. In 2011, N losses from all forms of N were larger than the corn-soybean rotation from McIsaac et al. (2010). Christian and Riche (1998) indicated that $NO_3^-$ losses from first year *M. x giganteus* was 154 kg N $ha^{-1}$ $yr^{-1}$, but then quickly decreased to 8 and 3 kg N $ha^{-1}$ $yr^{-1}$ in years 2 and 3, respectively; $NH_4^+$ losses under *M. x giganteus* in all years were less than 1 kg N $ha^{-1}$ $yr^{-1}$.

The loss of N from the 120 kg N $ha^{-1}$ treatment plots in year 2 of the study are also within the range indicated by Gentry et al. (2009) that reported $NO_3^-$ losses from a nearby central Illinois corn and soybean watershed, ranging from 22.7 to 59.9 kg N $ha^{-1}$ $yr^{-1}$. The losses of $NO_3^-$ from the 120 kg N $ha^{-1}$ treatment plots in 2011 are slightly larger compared to a study by David et al. (1997) that observed N losses from drainage tiles near the DOE site ranging from 20.2 to 48.3 kg N $ha^{-1}$ $yr^{-1}$. McIsaac and Hu (2004) found $NO_3^-$ losses from tile-drained watersheds to range between 13.7 to 38.1 kg N $ha^{-1}$ $yr^{-1}$. Nitrate losses from the 120 kg N $ha^{-1}$ treatment plots in 2011 were also larger compared to results from a study by Mitchell et al. (2000) that found $NO_3^-$ losses from corn-soybean to range between 14.0 to 38.0. The 60 kg N $ha^{-1}$ fertilization treatment plots in 2011 experienced smaller $NO_3^-$ losses compared to the 120 kg N $ha^{-1}$ and were on the smaller end of the range of all the above mentioned corn-soybean studies and tile observations with an average $NO_3^-$ loss of 21.9 kg N $ha^{-1}$ $yr^{-1}$. Since the lysimeters were only at a depth of 50 cm, additional root uptake and/or denitrification could have been taking place deeper than the lysimeters were measuring; this deeper uptake or denitrification would reduce the amount of inorganic N reaching tile drains and streams (McIsaac et al., 2010). Even though the quantity of $NO_3^-$ leached under the DOE *M. x giganteus* was much greater compared to the studies by McIsaac et al. (2010) and Christian and Riche (1998), some of the characteristics of *M. x giganteus* suggest that it is capable of retaining N much better; the extensive rooting system, longer growing season, and addition of C to the soil all promotes the retention of $NO_3^-$ (McIsaac et al., 2010).

**Yield and Biomass**

The productivity of *M.* x *giganteus* can be great, yielding a range between 5 and 55 Mg ha$^{-1}$; this makes it one of the most productive land plants in temperate climates (Heaton et al., 2010). The yield data from the *M.* x *giganteus* grown at the DOE plots falls into the normal range of biomass yields from many other trials. However, the yield from the DOE trials was lower than other trials in the Midwest, most likely because the crop is still young (3 years old). Heaton et al. (2008b) observed average harvestable yields of *M.* x *giganteus* grown at three locations in Illinois of 30 Mg ha$^{-1}$ without irrigation with only 25 kg N ha$^{-1}$ fertilization in one season. Heaton et al. (2010) also indicated that most studies have found that *M.* x *giganteus* does not respond significantly to N fertilization. Khale et al. (2001) indicated that end of the year biomass harvested from *M.* x *giganteus* grown in Germany ranged from 14.8 to 33.5 Mg ha$^{-1}$ from plots fertilized with 0, 50, and 100 kg N ha$^{-1}$. These high yields are 2-4 times as much as switchgrass; *M.* x *giganteus* produced an average maximum annual biomass of 22 Mg ha$^{-1}$ compared with 10 Mg ha$^{-1}$ from switchgrass (Heaton et al., 2004b). Compared to corn, *M.* x *giganteus* is 60% more productive due to its longer growing season (Dohleman and Long, 2009). In a review of 60 observations on *M.* x *giganteus*, Cadoux et al. (2011) found that, at winter harvest (February), the median dry matter production was 15 Mg ha$^{-1}$. In addition after reviewing numerous studies, Cadoux et al. (2011) gave three recommendations for three N fertilizer amounts; fertilization amounts of 49, 73.5, and 98 kg N ha$^{-1}$ would yield 10, 15, and 20 Mg ha$^{-1}$ of biomass at harvest, respectively. These fertilizer applications and corresponding yield amounts are similar to the results from the DOE study that saw 14.9, 15.8, and 17.0 Mg ha$^{-1}$ of biomass at harvest for 0, 60, and 120 kg N ha$^{-1}$ fertilizer treatments.

The average C:N ratio from a study by Heaton et al. (2009) was 143 from *M.* x *giganteus* fertilized at 25 kg N ha$^{-1}$. This C:N ratio was similar to the C:N ratios observed at the DOE plots; C:N ratios of 153, 137, and 117 kg ha$^{-1}$ were recorded from the 0, 60, and 120 kg N ha$^{-1}$ plots, respectively. These

C:N ratios were greater than what was observed by Heaton et al. (2009) for switchgrass (96). Cadoux et al. (2011) also found that at winter harvest, N content in the biomass was 76 kg ha$^{-1}$; their findings are comparable to the DOE N content at winter harvest in 2010 that had N contents of 44.9, 53.5, and 66.6 kg ha$^{-1}$ from the 0, 60, and 120 kg N ha$^{-1}$ treatment plots. For the *M.* x *giganteus* grown in 2010, the N fertilizer significantly increased the N concentration in the 120 kg N ha$^{-1}$ and therefore the mass of harvested N from that plot, but did not significantly raise the biomass or C harvested compared to the control and 60 kg N ha$^{-1}$ treatment plots. As a result, the C:N ratios decrease with additional amounts of N fertilizer, but, ultimately, fertilization just removed more N but did not produce significantly more biomass.

**CONCLUSIONS**

The beneficial effects of growing biofuel crops are well known: decreased fossil fuel emissions, decreased fertilizer use, C neutrality, and the use of annual cropping species. However, in order to fully understand the effects of growing biofuel crops, such as *M.* x *giganteus*, it is essential to assess the GHG emissions, notably $CO_2$ and $N_2O$, from producing *M.* x *giganteus* in an agricultural setting. In this study I examined the effects of three fertilizer treatments and the gaseous emissions of $CO_2$ and $N_2O$ associated with abiotic factors and their associated biomass yield amounts.

During the two-year study, $N_2O$ emissions in 2010 showed an effect from increased fertilizer N treatment compared to the control. Nitrous oxide emissions were low for both the 0 and 60 kg N ha$^{-1}$ in both 2009 and 2010; the emissions were similar to the only study examining $N_2O$ emissions from *M.* x *giganteus*. The driving factors in the $N_2O$ emissions was primarily precipitation and, to a lesser extent, temperature. In 2010, a large pulse in $N_2O$ emissions occurred in response to a major rainfall event. This rainfall event supplied the needed anoxic conditions for denitrification to occur; therefore since the 120 kg N ha$^{-1}$ treatment had large concentrations of soil $NO_3^-$-N, a large flux of $N_2O$ was emitted.

Carbon dioxide emissions were not affected by fertilizer treatment; emissions were significantly related to soil temperature at 10 cm. Conversely, soil moisture at 10 cm showed no significant relationship to $CO_2$ emissions.

In addition to gaseous $N_2O$ losses, some of the N applied to the experimental site was leached through the soil. In year 1 (spring 2009 to spring 2010) leached inorganic N was not significantly affected by fertilizer treatments. In year 2 (spring 2010 to spring 2011), the $NO_3^-$ and total inorganic N leached from the surface soils were significantly increased by fertilizer treatment. Between 74 and 88% of the inorganic N leached through the system and captured on the resin lysimeters was in the form of $NO_3^-$ throughout the study.

After the first year, 2009, biomass yield was low for all the crops due to the young $M.$ x *giganteus* and previous crop failure. After the second year, 2010, biomass was much larger for all the treatment plots. No significant difference was found in response to fertilization. The larger yield from the 120 kg N ha$^{-1}$ treatment plots did increase the amount of harvested biomass N, which was due to increased N fertilizer. The biomass yield and its corresponding C and N content from 2010, produced an interesting trend: as the fertilization amounts increased, the biomass did not significantly increase; however, the amount of N removed from harvest increased significantly with the additional fertilizer N, so that fertilization led to greater N removal while producing relatively the same biomass.

This study shows the importance of N fertilization practices; fertilizer rate made a difference in the amount of soil $N_2O$ emissions and the amount of inorganic N that leached, but not necessarily on the amount of biomass that is produced under fertilized conditions. Even though the $N_2O$ emissions from the $M.$ x *giganteus* were relatively low compared to other conventionally grown crops, the large global warming potential of $N_2O$ means it cannot be ignored. Furthermore, the amount of inorganic N leached from the 120 kg N ha$^{-1}$ plots was similar to conventionally grown crops without a significant increase in harvestable biomass. Whether this trend continues as the $M.$ x *giganteus* matures needs further study. This study adds to our knowledge surrounding the potential of $M.$ x *giganteus* to act as a new, novel biofuel crop, especially since no study has been published on the effects of fertilization rates on $N_2O$ emissions from $M.$ x *giganteus* grown in Illinois.

As with most scientific studies, there are often some questions that arise after observations and hypotheses are initially made. In future research on $M.$ x *giganteus* grown in Illinois, I would ask: 1) How does urea volatilization affect the $N_2O$ emissions directly following fertilization? 2) How much would a 90 kg N ha$^{-1}$ treatment affect $N_2O$ emissions? 3) How does irrigation during the dry periods in the summer affect the pulse action of $N_2O$ that occurs when the soils are re-wetted after drying for a period

of time? 4) How does snowmelt and soil freezing-thawing affect the pulse of $N_2O$ during the winter here in Illinois? 5) How would a drought affect the $N_2O$ and $CO_2$ emissions? 6) How would manuring the soils instead of fertilization affect the GHG fluxes and other abiotic factors? 7) How does liming affect the soil pH and what is its effect on GHG emissions?

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