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Greenhouse Gas Emissions, Nitrate Leaching, and Biomass Yields from Production of *Miscanthus* × *giganteus* in Illinois, USA

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Abstract Understanding the effects of nitrogen (N) fertilization on *Miscanthus* × *giganteus* greenhouse gas emissions, nitrate leaching, and biomass production is an important consideration when using this grass as a biomass feedstock. The objective of this study was to determine the effect of three N fertilization rates (0, 60, and 120 kg N ha^{-1} using urea as the N source) on nitrous oxide (N_2O) and carbon dioxide (CO_2) emissions, nitrogen leaching, and the biomass yields and N content of *M.* × *giganteus* planted in July 2008, and evaluated from 2009 through early 2011 in Urbana, Illinois, USA. While there was no biomass yield response to N fertilization rates in 2009 and 2010, the amount of N in the harvested biomass in 2010 was significantly greater at the 60 and 120 kg N ha^{-1} N rates. There was no significant CO_2 emission response to N rates in 2009 or 2010. Similarly, N fertilization did not increase cumulative N_2O emissions in 2009, but cumulative N_2O emissions did increase in 2010 with N fertilization. During 2009, nitrate (NO_3^-) leaching at the 50-cm soil depth was not related to fertilization rate, but there was a significant increase in NO_3^- leaching between the 0 and 120 kg N ha^{-1} treatments in 2010 (8.9 and 28.9 kg NO_3–N ha^{-1} year^{-1}, respectively). Overall, N fertilization of *M.* × *giganteus* led to N_2O releases, increased fluxes of inorganic N (primarily NO_3^-) through the soil profile; and increased harvested N without a significant increase in biomass production.

G. D. Behnke · M. B. David (✉)
Department of Natural Resources and Environmental Sciences,
University of Illinois at Urbana-Champaign,
W-503 Turner Hall 1102 S. Goodwin Ave.,
Urbana, IL 61801, USA
e-mail: mbdavid@illinois.edu

T. B. Voigt
Department of Crop Sciences,
University of Illinois at Urbana-Champaign,
S-416 Turner Hall 1102 S. Goodwin Ave.,
Urbana, IL 61801, USA

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Introduction

Worldwide greenhouse gas (GHG) emissions, including those from carbon dioxide (CO_2) and nitrous oxide (N_2O), are large and of concern due to ties to global warming. Governmental policies call for an overall net reduction in atmospheric emissions of carbon dioxide (CO_2), the major GHG emitted from the combustion of fossil fuels. Moreover, the atmospheric concentration of CO_2 is currently increasing at a rate of 1.4 ppmv year^{-1} [1]. The global warming potential of N_2O per molecule has been estimated at about 300 times greater than that of CO_2 [1]; it is the single most important ozone-depleting compound and expected to remain so throughout the twenty-first century [2]. Agricultural soils are important sources of both CO_2 and N_2O, with emissions dependent on crop production systems and fertilizer management [3].

In addition to N_2O gas emissions, N can be lost from soils via nitrate leaching. In the upper Midwest of the USA, many of the best agricultural soils are artificially drained using tile lines, enhancing the loss of NO_3^- [4, 5]. Fertilization and inappropriate timing of fertilizer application can lead to NO_3^--enriched soil water solution and movement of NO_3^- to ground and surface water [6]. If fertilization occurs before the crop can incorporate the N, large amounts of NO_3^- can be leached in short periods around precipitation events in tile-drained soils [4]. For example, Gentry et al. [7] found that in a study on maize over 3 years, record rainfall in only 1 month can contribute to 75% of the NO_3^- loss for an entire year, as much as 148 kg N in 1 day and concentration of 49 mg N L^{-1}. This loss of NO_3^- can lead to local drinking water problems [8] and when transported downstream, hypoxia in the Gulf of Mexico

[9]. McIsaac et al. [10] compared a corn–soybean rotational system to *Miscanthus* × *giganteus* and switchgrass and found the corn–soybean system had significantly greater losses of NO_3^- and total inorganic N measured using a resin lysimeter procedure, suggesting that some biofuel crops may reduce NO_3^- leaching.

The demand for more fuel has caused a need to either import more fossil fuels or increase biofuel production [11]. Biofuel crops such as *M.* × *giganteus* (giant miscanthus) and switchgrass *Panicum virgatum* (switchgrass) can be used as substitutes for fossil fuels to reduce net CO_2 emissions and reduce GHG emissions. Biofuel crops possess a variety of environmental benefits including the reduction of CO_2 emissions produced when substituting biomass for fossil fuel combustion [12]. Depending on the N use efficiency of the crop being grown, however, N_2O emissions can be large enough to cause climate warming instead of the intended climate cooling due to "saved fossil CO_2" [11].

The biomass feedstock, *M.* × *giganteus*, is a naturally occurring sterile triploid hybrid that poses little risk for invasion [13]. It is native to Japan and has *Miscanthus sacchariflorus* and *Miscanthus sinensis* as its parents [14]. *M.* × *giganteus* grows vigorously, can produce large amounts of biomass in temperate regions, and is environmentally adaptable due to its ability to photosynthesize over a wide range of temperatures [15]. It is also a highly efficient N-use grass, enhancing its potential as an energy crop [16]. Studies by Christian and Riche [6] and Christian et al. [17] found that there was no significant yield response to N fertilizer. Likewise, Miguez et al. [18] 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 crops. Heaton et al. [19] found similar results. In a review of several studies compiled by Lewandowski et al. [20], numerous field trials at different locations in Austria, Germany, and Greece confirmed that *M.* × *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 [17, 21].

Nitrogen in harvested *M.* × *giganteus* biomass can vary. Heaton et al. [22] reported that in plots fertilized with 25 kg N ha^{-1}, a range of 5 kg N ha^{-1} in *M.* × *giganteus* biomass harvested in late winter increasing to 187 kg N ha^{-1} in green-harvested *M.* × *giganteus* biomass. Similarly, Jørgensen [23] reported that 45.4 kg N ha^{-1} was removed in the dried biomass at harvest.

Simulations conducted by Hughes et al. [24] suggest that by replacing fossil fuel consumption with *M.* × *giganteus* biofuel, CO_2 concentrations could be lowered by up to 323 ppmv by the end of the current century. In another study, Lewandowski et al. [12] wrote that burning *M.* × *giganteus* compared to burning hard coal, can reduce CO_2 emissions by as much as 90%. Their CO_2 balance was highly dependent on efficient N fertilizer application; N fertilizer (production and N_2O emissions) contributed about 43% of the estimated CO_2 emissions (in kg) per ton of dry matter produced [12]. However, the benefits of using energy crops such as *M.* × *giganteus* to replace fossil fuels would be dramatically reduced if they also emit GHGs during production [25]. 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 [25].

Global climate change may potentially be mitigated by the cultivation of bioenergy crops such as *M.* × *giganteus*. However, in the USA, there have been few *M.* × *giganteus* field trials that have studied yield and environmental responses to N fertilization. Therefore, the objective of this study was to evaluate the effects of N fertilization on *M.* × *giganteus* biomass production and harvested N, N_2O, and CO_2 emissions, and inorganic N leaching in Illinois. Our hypothesis was that N fertilization would not increase biomass production in establishing *M.* × *giganteus*, but would increase N losses.

Materials and Methods

Crop Management and Experimental Design

In July, 2008, twelve 10×10 m plots were each planted with 100 greenhouse-produced *M.* × *giganteus* plants produced in 2-cm square pots using commercial soilless potting mix at the University of Illinois Energy Farm, Urbana, Illinois, USA (40.06 N, −88.19 W). Soils at the site had a fine cap of sandy loam sediments throughout the top 30 cm and are classified as Wyanet series (fine-loamy, mixed, active, mesic Typic Argiudolls). The experiment was planted using a randomized complete block design with four replicates and three annual N fertilizer treatments of 0, 60, or 120 kg N ha^{-1}. Urea (46-0-0) was used as the N source and applied on June 23, 2009 and May 6, 2010. Approximately 75% of the grasses failed to survive because of the late planting in July 2008 and severe weather during the 2008–2009 winter. Each plot was replanted in spring 2009 with potted grasses to ensure there were 100 plants in each plot. The study was hand weeded as necessary during the summer of 2009 and 2010 to reduce weed competition and was irrigated during the summer of 2009 to ensure establishment. At the end of each season, final yield data were calculated [26]. Harvest date for the 2009 growing season was 10 January 2010, and 29 November 2010 for the 2010 growing season. Total C and N on harvested plant samples were determined using an Elemental Analyzer (EAS 4010, Costech).

Gas Sampling Procedures

Nitrous oxide measurements were sampled following the GRACEnet chamber-based trace gas flux measurement protocol [27]. Beginning in late April 2009, two 0.031-m^2 polyvinyl chloride (PVC) chamber bases were installed between rows on the west half and east half of each of the 12 plots to allow for settling. A total of 24 chamber bases were used following the vented chamber technique. The chamber tops were made of reflective, white PVC, have a vent tube, sampling ports, and insulation foam to create an airtight seal with the chamber bases. The chamber bases were left in place for the growing season, and were removed before the final biomass harvest.

Nitrous oxide fluxes were measured near noon, when air temperatures were near the average for the day. Before gas sampling, four chamber offsets were measured using a ruler and the data recorded and averaged in order to calculate headspace 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 Precision-Glide® needle syringe at 0-, 10-, 20-, and 30-min intervals through the 20-mm Pharma-Fix® Butyl septa sealed with 20 mm Alum Tops. The gas 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, (Shimadzu® GC 2014 with AOC-5000). Standards were prepared using Scott Specialty Gas® in the laboratory directly before being analyzed on the gas chromatograph. Nitrous oxide emissions were calculated using the regression coefficients obtained from N_2O–N concentrations against sampling time. In addition to collecting gas samples at each chamber, soil temperature at 10 cm and air temperature was recorded during the 30-min interval for each of the 24 rings.

Using the same chamber bases and offsets, CO_2 fluxes were measured using a LI-COR LI-8100 Soil CO_2 Flux System (Lincoln, NE, USA). These measurements were taken twice a week for much of the growing season during the first year at the same time the N_2O measurements were made. The LI-8100 Soil CO_2 Flux System was placed on each chamber base for 90 s; in addition, the LI-8100 Soil CO_2 Flux System also measured soil temperatures at 5 cm using an attached temperature probe. The Soil CO_2 Flux System-collected data was downloaded in the field to a Palm™ LifeDrive™ handheld PDA.

Soil temperature and moisture was measured hourly in each plot over the entire study period using Decagon® Em5b data loggers with ECH2O® ECT soil temperature sensors and ECH2O® EC-5 moisture sensors. The soil–temperature sensors were installed at 10 cm, and two soil moisture sensors were placed in opposite directions at 10 cm along with single moisture sensors at 30 and 50 cm. The ECH2O® EC-5 determines volumetric water content by measuring the dielectric constant of the media using capacitance/frequency domain technology with a 70-MHz frequency.

Soils Analyses

Prior to planting on 17 July 2008, three soil cores were taken from each of the 12 plots using a Giddings probe hydraulic soil sampling and coring. The soil cores were 2 cm in diameter and 100 cm long. Each core was divided into sections from 0–10, 11–20, 21–30, 31–50, and 51–100 cm depths. Each plot's soil characteristics were averaged into the respective corresponding fertilizer treatment and then summarized (Table 1). To determine the soil pH, extractable P, and C to N ratio (C/N), samples were air-dried, ground, and sent to Brookside Laboratories, Inc. (New Knoxville, OH) for analysis. 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 [28]. Particle size distribution (soil texture) was determined at the Soil Pedology Laboratory at the University of Illinois Urbana-Champaign. The silt and clay particles were determined using the hydrometer method [29].

In addition to the 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 kilogram of soil area to be determined.

Inorganic Nitrogen Leaching

Prior to the 2009 and 2010 growing seasons, four ion exchange resin lysimeters [10, 30, 31] were installed in each of the 12 plots at a depth of 50 cm under undisturbed soil. The year 1 resin lysimeters were in place from April 2009 to April 2010 and year 2 lysimeters were in place from April 2010 to April 2011. The lysimeters were constructed of a 5.1-cm diameter PVC pipe with a coupling section, each with a length of 7 cm. 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. Water draining though the soil profile above the lysimeter carries NO_3^- and NH_4^+ dissolved in the soil water solution, which was 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

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

Treatment	Depth cm	pH	P mg kg^{-1}	Total C g kg^{-1}	Total N g kg^{-1}	C/N	Sand %	Silt %	Clay %
Control	0–10	5.7	34	1.1	0.11	10.4	53	32	16
(0 kg N 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 N 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 N 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

year-old resin was extracted using 1 M KCl. Nitrate–N and NH_4^+–N concentrations in the extract were measured colorimetrically by flow injection analysis with a Lachat QuickChem 8000 (Lachat, Loveland, CO) [10]. 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. To calculate the trimmed means, the maximum and minimum observations were excluded from the calculation.

Data Analysis

To calculate annual N_2O and CO_2 fluxes, field-sampled fluxes were extrapolated to predict flux (F) at 1-h 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 [32]:

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

Here, F_{ref} and T_{ref} are the reference (measured) flux and temperature (in Kelvin), E is the average activation energy of heterotrophic soil respiration (0.65 eV) [33], and k is Boltzmann's constant (8.62×10^{-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 [34]. We choose to use the Arrhenius equation, as opposed to Q_{10}, because of its grounding in physical chemistry and because it correctly predicts the observed decline in Q_{10} with increasing temperature [34].

Statistical analyses were performed using SAS v9.2. 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.

Results

Temperature, Precipitation, and Soil Characteristics

The mean annual temperature was 10.8 °C and the mean annual precipitation was 104 cm between 1971 and 2000 (Illinois State Water Survey historic climate data), and the mean maximum and minimum temperatures from April to November were 22.4 and 10.8 °C, respectively. The precipitation total in 2009 was 106 cm and 81 cm in 2010, measured at a weather station located on the farm.

Soil characteristics were similar throughout the study site (Table 1). Uncharacteristically for this portion of Central Illinois, plots typically had more than 50% sand in the upper 30 cm (Table 1). The percent sand generally decreased, and the pH and the percent silt and clay increased with depth (Table 1). 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 approximately 10 and typically decreased with depth.

Biomass and Harvested Carbon and Nitrogen

Harvested *M.* × *giganteus* yields from the 2009 crop averaged 1.1, 4.1, and 4.0 Mg ha^{-1} for the 0, 60, and 120 kg N ha^{-1}

plots, respectively (Table 2) [26]. Biomass yield averages increased to 14.9, 15.8, and 17.0 Mg ha^{-1} from the 0, 60, and 120 kg N ha^{-1} plots, respectively, in 2010. There was no significant yield response to N fertilization in either 2009 (p=0.14) or 2010 (p=0.17). Carbon and N measurements in the harvested biomass were not taken in 2009 due to 75% of the grasses being first-year plants, but in 2010, the harvested biomass contained 44.9, 53.5, and 66.6 kg N ha^{-1} from the 0, 60, and 120 kg N ha^{-1} plots, respectively, (Table 2) with a strong response to N fertilizer (p=0.0004). There were 6698, 7257, and 7796 kg C ha^{-1} from the 0, 60, and 120 kg N ha^{-1} plots, respectively, in the 2010 harvested biomass with no response to N fertilization (p=0.12). The C/N ratios from the biomass were 153, 137, and 117 kg ha^{-1} for the 0, 60, and 120 kg N ha^{-1} plots, respectively, with no significant response to fertilization (p=0.08).

Gas Emissions

In 2009, mean cumulative N_2O fluxes (Table 3) were not affected by fertilizer treatments (p>0.05). In 2010, however, the cumulative nitrous oxide fluxes were significantly different (p=0.015). 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=0.09). 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.

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 and 81 cm in 2010. Maximum air temperatures were greater in 2009 compared to 2010; 32.7, and 27.0 °C respectively.

Peak N_2O values for individual sampling days were much lower in 2009 than in 2010 in the 120 kg ha^{-1} treatment plots; 0.087 compared to 0.403 mg N_2O–N m^{-2} h^{-1}, respectively (Fig. 1). In 2009, peak N_2O fluxes on individual sampling days occurred following fertilization application, but were not as dramatic as in 2010. In 2010, the peak N_2O flux of 0.403 mg N_2O–N m^{-2} h^{-1} occurred on 9 June in the 120 kg N ha^{-1} plots following 4.47 cm of precipitation.

Table 2 Yield and biomass C and N contents of the harvested *M.* × *giganteus* by treatment in 2009 and 2010 (with standard deviations)

Year	Treatment kg N ha^{-1}	Yield Mg ha^{-1}	Biomass N kg N ha^{-1}	Biomass C kg C ha^{-1}	C/N ratio
2009	0	1.1 (0.7)			
	60	4.1 (3.7)			
	120	4.0 (2.2)			
2010	0	14.9 (2.9)	44.9 (9.0)	6698 (1287)	153 (39)
	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)

Table 3 Mean cumulative N_2O and CO_2 fluxes during 2009 and 2010 (with standard deviations)

Year	Treatment kg N ha^{-1}	Cumulative N_2O flux kg N ha^{-1} year^{-1}	Cumulative CO_2 flux Mg C ha^{-1} year^{-1}
2009	0	0.75 (0.06)	8.62 (1.75)
	60	1.30 (0.65)	9.11 (1.24)
	120	1.35 (0.41)	8.62 (1.14)
2010	0	0.35 (0.09)	8.90 (0.85)
	60	0.77 (0.39)	9.16 (1.92)
	120	2.91 (2.08)	8.96 (1.53)

In 2009, cumulative N_2O fluxes increased gradually following fertilization, peaking about 39 days after fertilization for the 60 and 120 kg N ha^{-1} treatments. In 2010, cumulative fluxes for the 60 and 120 kg N ha^{-1} treatments were large and peaked 49 days following fertilization (Fig. 1). Using the control plots as the base, in 2009 the 60 kg N ha^{-1} treatment lost an additional 0.55 kg N_2O–N ha^{-1} and the 120 kg N ha^{-1} an additional 0.60 kg N_2O–N ha^{-1}. In 2010, the 60 kg N ha^{-1} treatment lost an additional 0.42 kg N_2O–N ha^{-1}, whereas the 120 kg N ha^{-1} loss increased by 2.56 kg N_2O–N ha^{-1} (Table 3).

Nonlinear increases in mean daily N_2O fluxes were observed in 2010 (Fig. 2). Mean daily fluxes were well described by the nonlinear model during 2010 (r^2=0.41, p=0.015); the corresponding standard deviation for each treatment plot, however, 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; Fig. 3).

Cumulative CO_2 fluxes were not affected by fertilizer treatment in either year (Fig. 4). 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, CO_2 emissions were similar across the three fertilizer treatments (Table 3). 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 60 kg N ha^{-1} treatments to 8.62 Mg C ha^{-1} in the 120 kg N ha^{-1} treatments (p=0.99; Table 3). 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} for the 60 kg N ha^{-1} treatments to 8.96 Mg C ha^{-1} in the 120 kg N ha^{-1} treatments (p=0.95). Carbon dioxide was significantly related to soil temperature at 10 cm (r=0.64, p<0.0001), but CO_2 and soil moisture at 10 cm were not related (Fig. 3).

Soil Inorganic Nitrogen

In 2009, surface soil (0–10 cm) NH_4^+ content was highest following fertilization in each of the treatment plots, with

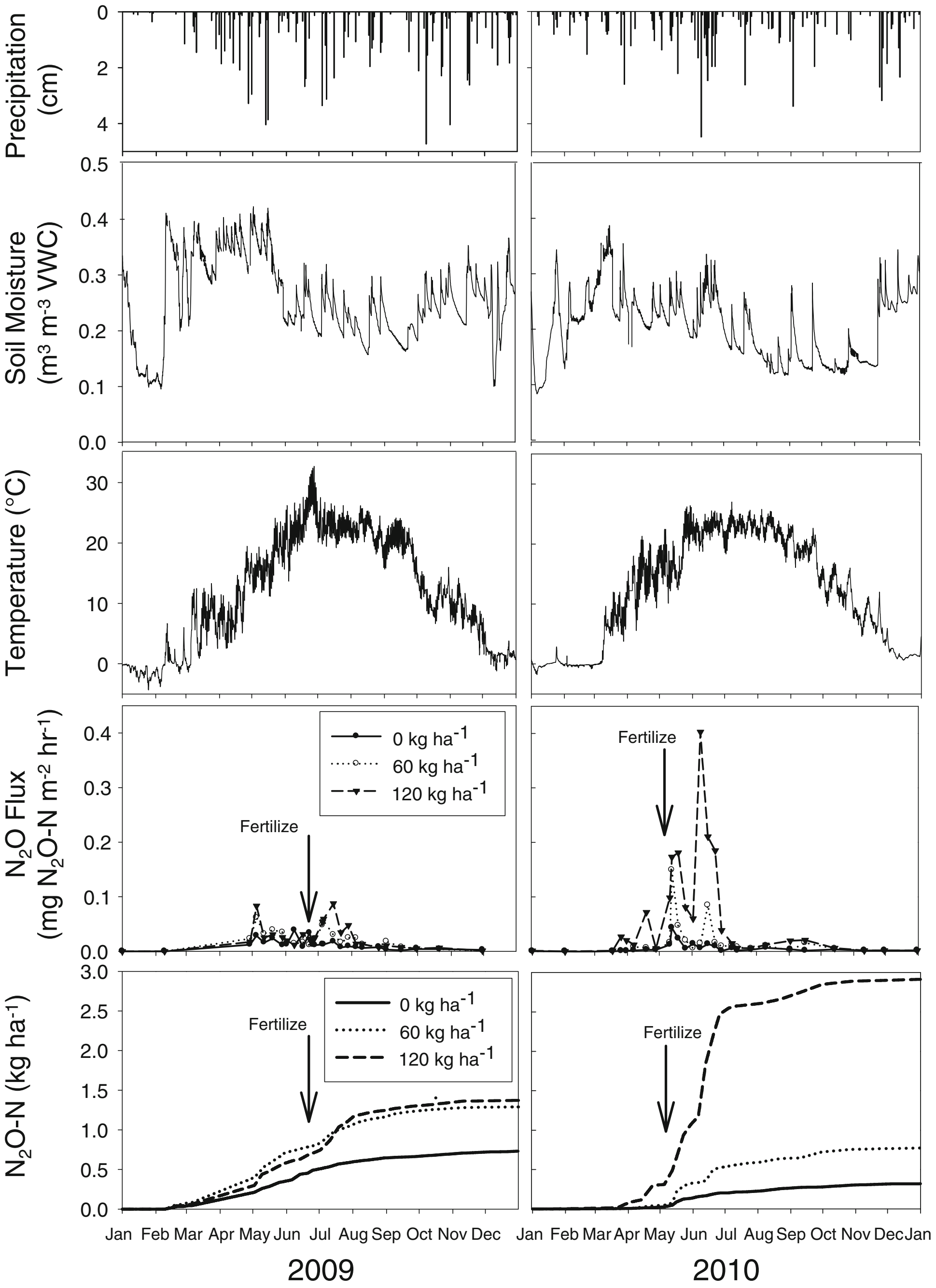

Fig. 1 Daily precipitation, soil moisture at 10 cm, soil temperature at 10 cm, daily N_2O fluxes, and cumulative N_2O emissions by date during 2009 and 2010

the 60 kg N ha^{-1} treatment being larger than 120 kg N ha^{-1} (59 and 22 mg N kg^{-1}, respectively, data on concentration basis not shown; Fig. 5). By the next sampling date soil NH_4^+ in the 120 kg N ha^{-1} was greater 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, when the 60 kg N ha^{-1} treatment had larger peaks near fertilization (May 11, 2010) than the 120 kg N ha^{-1} (27 and 19 mg N kg^{-1}, respectively). The amount of NH_4^+ from the 60 kg N ha^{-1} plots was greater on 13 May 2010 than the 120 kg N ha^{-1} plots (38 and 33 mg N kg^{-1}, respectively). As both years progressed following fertilization, soil NH_4^+ decreased to near 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} treatment. 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 120 kg N ha^{-1} treatments. Soil NO_3^- concentrations in both the 60 and 120 kg N ha^{-1} plots were largest on the day 4.47 cm of precipitation fell, 9 June 2010, also the day of the largest N_2O emissions from the

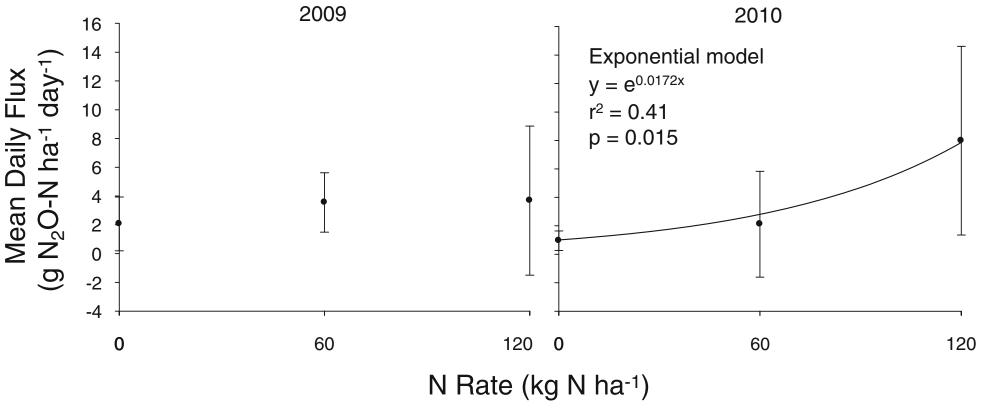

Fig. 2 Mean daily N_2O flux during 2009 and 2010. *Error bars* represent standard deviations of the treatment averages

120 kg N ha^{-1} treatment plot. Following the maximum NO_3^- concentration on 9 June, NO_3^- in the 120 kg N ha^{-1} treatment decreased (26 mg N kg^{-1}), but then increased due to another precipitation event on 29 June (39 mg N kg^{-1}). Following the peak in the 60 kg N ha^{-1} treatment the soil NO_3^- decreased steadily for the rest of the growing season. In the 120 kg N ha^{-1} plots following the 29 June maximum, soil NO_3^- concentration decreased to near zero by the end of the growing season. The control plot had a small maximum concentration on 16 June (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 (Fig. 5). Soil inorganic NO_3^- concentrations were significantly related to N_2O emissions (r=0.47, p<0.0001) for both years combined.

Inorganic Nitrogen Leaching

Mean values of leached NO_3^- from 50 cm in the *M.* × *giganteus* in year 1 were not significantly different among the 0, 60, and 120 kg N ha^{-1} treatments; the values were 6.4, 7.1, and 13.3 kg N ha^{-1} year^{-1}, respectively (p=0.12; Table 4). Likewise, mean NH_4^+ was not significantly different among treatments (p=0.71). Values for NH_4^+ were 6.8, 5.6, and 7.1 kg N ha^{-1} year^{-1} from the 0, 60, and 120 kg N ha^{-1} treatments, respectively. Combining the NO_3^- and the NH_4^+ provides the total inorganic N leached from the plots. In year 1, total inorganic N was not statistically different among treatments (p=0.14); the values were 13.3, 12.6, and 20.5 kg N ha^{-1} year^{-1} from the 0, 60, and 120 kg N ha^{-1} treatments, respectively.

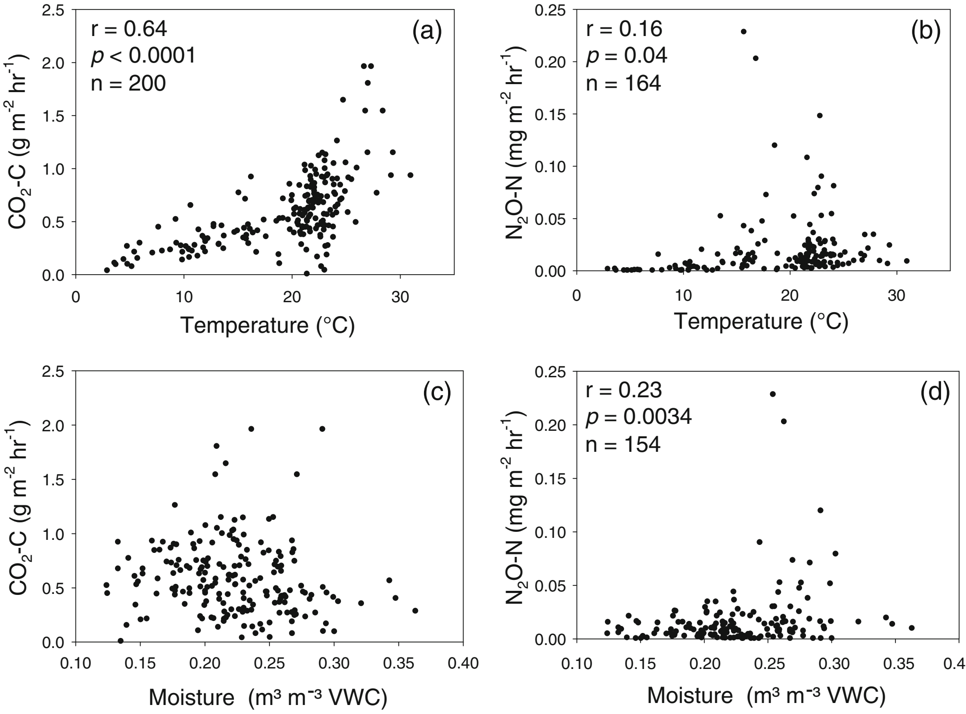

Fig. 3 CO_2 emissions vs. soil temperature at 10 cm (**a**), N_2O emissions vs. soil temperature at 10 cm (**b**), CO_2 emissions vs. soil moisture at 10 cm (**c**), and N_2O emissions vs. soil moisture at 10 cm (**d**)

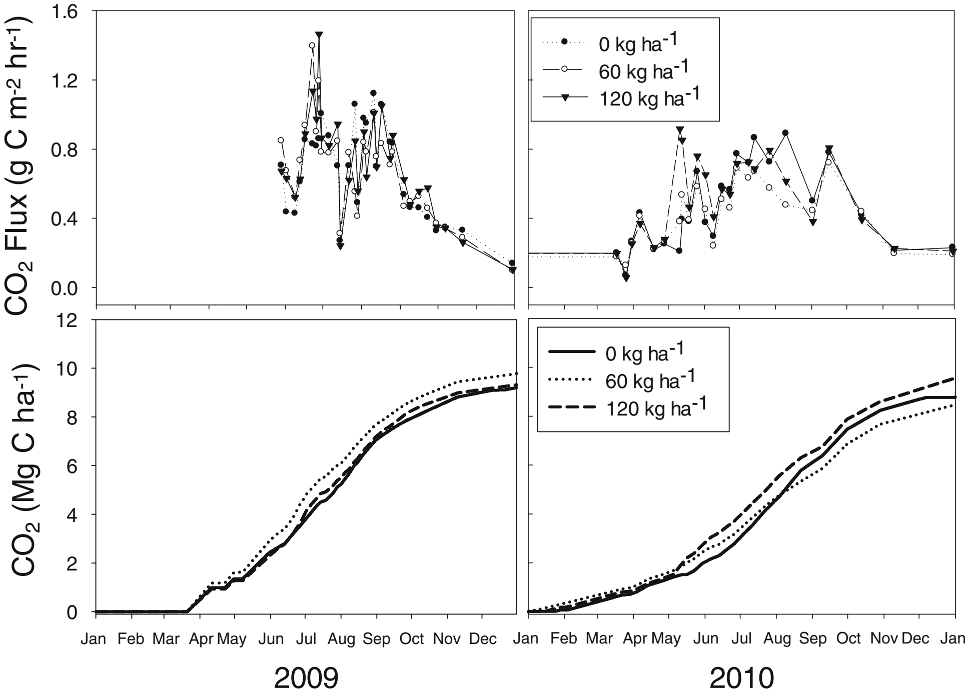

Fig. 4 Daily CO_2 fluxes and cumulative CO_2 emissions during 2009 and 2010

Mean values of NO_3^- leached in year 2 indicated that fertilizer treatment had an effect ($p=0.0005$). The NO_3^- values from year 2 were 6.9, 15.3, and 28.8 kg N ha^{-1} $year^{-1}$ from the 0, 60, and 120 kg N ha^{-1} treatments, respectively. Ammonium was not statistically different among treatments ($p=0.10$); the values for NH_4^+ in 2011 were 2.3, 3.0, and 6.1 kg N ha^{-1} $year^{-1}$ from the 0, 60, and 120 kg N ha^{-1} treatments, respectively. The total inorganic N leached in year 2 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} $year^{-1}$ from the 0, 60, and 120 kg N ha^{-1} treatments, respectively.

Discussion

Biomass and C and N Harvested

Miscanthus × *giganteus* productivity can be great; reported yields in temperate climates range between 5 and 55 Mg ha^{-1} [16]. The 2010 *M.* × *giganteus* yields in this study fall into this range. However, the yields from this study were lower than other trials in the Midwest, probably due to the crop being young and not fully established; 75% were first-year plants in 2009 and second-year plants in 2010. Clifton-Brown and Lewandowski [35] wrote that *M.* × *giganteus* usually becomes fully established and mature in year 3 or beyond. Heaton et al. [36] observed average harvestable yields of *M.* × *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. [16] also indicated that most studies have found that *M.* × *giganteus* does not respond significantly to N fertilization, but again, most of this work was in Europe. Khale et al. [37] indicated that end of the year biomass harvested from *M.* × *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}. In a review of 60 observations of *M.* × *giganteus*, Cadoux et al. [38] 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. [38] 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 this study where harvested biomass yields were 14.9, 15.8, and 17.0 Mg ha^{-1} in the 0, 60, and 120 kg N ha^{-1} fertilizer treatments, respectively.

The average C/N ratio from a study by Heaton et al. [22] was 143 from *M.* × *giganteus* fertilized at 25 kg N ha^{-1}. This C/N ratio was similar to the C/N ratios observed in this study; we observed C/N ratios of 153, 137, and 117 kg ha^{-1} from the 0, 60, and 120 kg N ha^{-1} treatments, respectively. In another study, Cadoux et al. [38] reported 76 kg N ha^{-1} in harvested biomass, an amount similar to the 45, 54, and 67 kg N ha^{-1} from the 0, 60, and 120 kg N ha^{-1} treatment plots. Furthermore, in 2010, N concentration in the harvested biomass was significantly greater in the 120 kg N ha^{-1} treatment, but did not significantly increase the biomass or C harvested compared to the 0 and 60 kg N ha^{-1} treatment plots. As a result, the C/N ratios decreased with additional amounts of N fertilizer, but, ultimately, fertilization removed more N

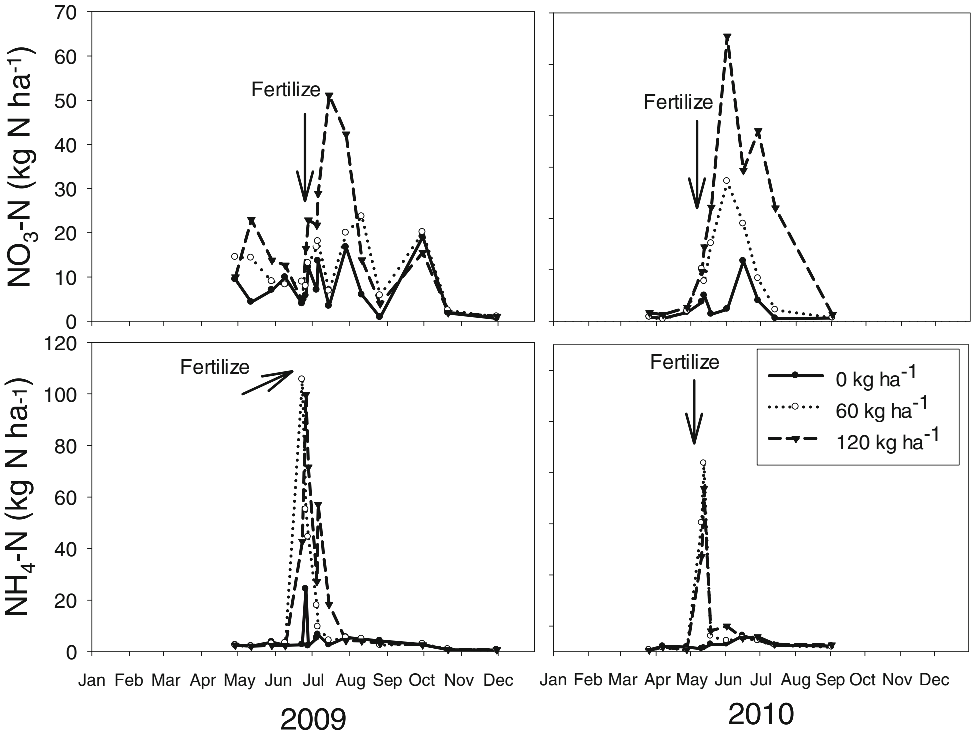

Fig. 5 Soil nitrate and ammonium contents in the upper 10 cm of soil during 2009 and 2010

without producing significantly more biomass. Therefore, an important aspect of our work is that fertilization is not needed for newly planted *M.* × *giganteus* to increase biomass production during the establishment period, supporting the overall hypothesis of this study.

Gas Emissions

Soil type, N fertilization, moisture, and temperature should be considered when discussing gas emissions from crop-producing soils. De Wever et al. [39] found that N_2O production was greater under sandy soils than loamy soils. This study was conducted at a site with a 30-cm cap of sandy loam; emissions of 1.35 and 2.91 kg N_2O–N ha^{-1}, in 2009 and 2010, respectively, were released from the 120 kg N ha^{-1} treatments. These results are similar to those of studies of maize and poplar. Hoben et al. [40] observed cumulative N_2O–N emissions ranging from 0.75 to 2.5 kg N_2O–N ha^{-1} in 2007 and 1 to 2 kg N_2O–N ha^{-1} in 2008 for maize fertilized with 135 kg N ha^{-1}. Also in a sandy soil, Hellebrand et al. [41] found cumulative N_2O emissions of 1.04, 1.45, and 2.97 kg N_2O–N ha^{-1} over 3 years averaged from two poplar plots that received 150 kg N ha^{-1}.

Table 4 Mean annual leaching of nitrate, ammonium, and total inorganic nitrogen measured using ion exchange resin lysimeters at 50 cm soil depth under *M.* × *giganteus* (with standard deviations)

Year	Treatment	NO_3^-	NH_4^+	Inorganic N
			kg N ha^{-1} year^{-1}	
1	0	6.4 (3.2)	6.8 (0.8)	13.3 (2.8)
	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)	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)

As in this study, Jørgensen et al. [25] reported that applying N to *M.* × *giganteus* increased N_2O emissions. Applications of 0 and 60 kg N ha^{-1} produced 0.14 and 1.09 kg N ha^{-1}, respectively [25]. This compares to the N_2O emissions in our study in which the 0 and 60 kg N ha^{-1} treatments produced 0.75 and 1.30 and 0.35 and 0.77 kg N ha^{-1}, respectively, in 2009 and 2010. As in this study (Fig. 2), fertilized row crops also showed that N fertilization can increase N_2O emissions [40, 42, 43].

In both 2009 and 2010, soil NH_4^+–N was, at its greatest concentration after fertilization because urea is readily broken down by soil microbes into NH_4^+–N. Engel et al. [44] 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 [45]. Similar results were observed in this study as NH_4^+–N concentrations were at their greatest directly following fertilization. Recous and Machet [46] 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.

Soil water amounts in excess of field capacity caused by excessive precipitation or irrigation can promote denitrification, and fertilization timing can have an important role in determining the extent of N_2O production [47]. Peak N_2O emissions from the 120 kg N ha^{-1} treatments in this study occurred on 2 June 2010 (Fig. 1), and can be attributed to the 4.47 cm of precipitation that fell the prior day. Soil moisture was significantly correlated to N_2O ($p=0.0034$) emissions. Jørgensen et al. [25] also noted an immediate spike in N_2O emission following a rainfall event in mid-July in *M.* × *giganteus*. Moreover, increased N_2O emissions following precipitation events have been reported by Parkin and Kaspar [48] in fertilized corn, Baggs et al. [49] in fertilized rye and beans, and Engel et al. [44] in canola.

Temperature was significantly correlated to N_2O emissions ($p=0.04$) in this study; as temperature increased, 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. [50]; the authors concluded that low winter temperatures in temperate regions limit N_2O production. In addition, Phillips et al. [51] concluded that the magnitude of N_2O emissions is likely to vary with temperature since microbial activity is strongly related to temperature.

Overall, our N_2O emissions were most affected by precipitation events increasing soil moisture following N fertilization, demonstrating the critical and overwhelming importance of this factor in determining N_2O emissions for the year. Because this is a factor that is not controllable, our work confirms the risk of increased N_2O loss from fertilizer additions due to precipitation events, even with a perennial grass.

Another useful comparison of N_2O emissions from soils is to calculate them as a percentage of applied N fertilizer. The IPCC default value for direct emissions of N_2O in managed systems is 1% of applied fertilizer [52]. Our values ranged from 1.1 to 2.4 % for the two fertilizer rates across both years, showing that in our fertilized *M.* × *giganteus* plots, emissions were well above the IPCC default value.

Unlike N_2O emissions, there were no fertilizer effects on CO_2 emissions in either year. A review of the literature was unable to produce research findings in which N effects on CO_2 emissions from *M.* × *giganteus* was studied, so this is a new and interesting result. Drury et al. [53] found no seasonal CO_2 emissions differences caused by tillage, N placement depth, soil water content, or tillage N placement interactions in a wheat–corn–soybean rotation. Mielnick and Dugas [54] observed the average annual soil CO_2 flux in a tallgrass prairie in Texas was 17 Mg C ha^{-1}, results similar to the findings of Knapp et al. [55] in which annual fluxes of 13–21 Mg C ha^{-1} at the Konza Prairie in Kansas were reported. Mielnick and Dugas [54] attribute the large annual CO_2 fluxes to large volumes of annual precipitation and the even distribution of rainfall throughout the year; in addition, soil C contents could also explain the high CO_2 emissions from the tallgrass prairie. The average cumulative CO_2 flux across our study, 8.8 and 9.0 Mg CO_2–C ha^{-1}, in 2009 and 2010, respectively, were lower than from the tallgrass prairie studies, likely due to the *M.* × *giganteus* being newly established and still building soil C.

Drury et al. [53] found that soil temperature was the driving factor for the difference in CO_2 emissions each year with warmer years having greater CO_2 emissions than cooler years. Furthermore, Parkin and Kaspar [56] also observed that temperature is normally the strongest predictor of CO_2 flux, and Raich and Potter [57] reported that temperature is the single most important variable for predicting the soil CO_2 flux with maximum CO_2 emissions from soils occurring during the summer (approximately May–September). There were similar results in our study where we saw a significant relationship between CO_2 and soil temperature at 10 cm ($p<0.0001$, Fig. 3) with greater CO_2 emissions occurring with increased temperatures during the summer months. There were larger maximums in temperature in 2009 and, as a result, CO_2 emissions were greater than in 2010 (Fig. 4). 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 highlighting the complexity of soil respiration.

Inorganic Nitrogen Leaching

In addition to N losses through N_2O production, N loss, primarily in the NO_3^- form, can also occur via subsurface leaching. Additions of N fertilizer are often needed to maximize plant productivity, but can also add to the N that can be leached from the system. In McIsaac et al. [10], the *M.* × *giganteus* plots were not fertilized; their findings for unfertilized *M.* × *giganteus* were slightly smaller compared to the control and 60 kg N ha^{-1} treatment plots in year 1 and the 0 kg N ha^{-1} treatment plot in year 2. Over 4 years, McIsaac et al. [10] observed annual average leaching from *M.* × *giganteus* at a 50-cm depth of 3.0, 1.8, and 4.8 kg N ha^{-1} year^{-1} for NO_3^-, NH_4^+, and total inorganic N, respectively. In year 1 of this study, inorganic N leaching at 50 cm from the 120 kg N ha^{-1} plots was similar to the N leaching from the corn–soybean rotation in which McIsaac et al. [10] reported that N leaching averaged over 5 years were 40.4, 2.4, and 43.0 kg N ha^{-1} year^{-1} for NO_3^-, NH_4^+, and total inorganic N, respectively. In year 2, N leaching from all forms of N were larger than the corn–soybean rotation from McIsaac et al. [10]. Christian and Riche [6] indicated that NO_3^- leaching from first-year *M.* × *giganteus* was 154 kg N ha^{-1} year^{-1}, but then quickly decreased to 8 and 3 kg N ha^{-1} year^{-1} in years 2 and 3, respectively; NH_4^+ leaching under *M.* × *giganteus* in all years were less than 1 kg N ha^{-1} year^{-1}.

The leaching of N at 50 cm from the 120 kg N ha^{-1} treatments in year 2 are also within the range reported by Gentry et al. [5]. They found that NO_3^- losses from a central Illinois corn and soybean watershed ranged from 22.7 to 59.9 kg N ha^{-1} year^{-1}. The leaching of NO_3^- from the 120 kg N ha^{-1} treatment plots in 2011 were slightly larger than reported by David et al. [4]. They observed N losses ranging from 20.2 to 48.3 kg N ha^{-1} year^{-1} from drainage tiles in central Illinois. McIsaac and Hu [58] found NO_3^- losses from tile-drained watersheds to range between 13.7 to 38.1 kg N ha^{-1} year^{-1}. Nitrate leaching at 50 cm from the 120 kg N ha^{-1} treatment plots in year 2 were also greater than the reported NO_3^- losses from a corn–soybean rotation that ranged between 14.0 and 38.0 (Mitchell et al. [59]). The 60 kg N ha^{-1} fertilization treatment plots in 2011 had smaller NO_3^- leaching compared to the 120 kg N ha^{-1} and were on the lower end of the range of all the abovementioned corn–soybean studies and tile observations with an average NO_3^- leaching of 21.9 kg N ha^{-1} year^{-1}. Since the lysimeters were at a depth of 50 cm, additional root uptake and/or denitrification could have been taking place below 50 cm and reduce the amount of inorganic N reaching tile drains and streams [10]. Even though the quantity of NO_3^- leached in this study was much greater than in McIsaac et al. [10] and Christian and Riche [6], given its extensive root system, long growing season, and ability to add C to the soil, all of which promotes the retention of NO_3^-, suggests that *M.* × *giganteus* is capable of retaining N as it becomes fully established [10].

Conclusions

First-year (2009) *M.* × *giganteus* biomass yields were low due to the young, establishing *M.* × *giganteus* and the need to replant 75% of the experiment following 2008–2009 winter kill. There were no biomass yield, N leaching, or gas emission effects related to N fertility treatments.

More interestingly in 2010, however, is that while there were no significant biomass yield responses due to N fertilization, the 120 kg N ha^{-1} treatment increased the amount of N removed in the harvested biomass. Moreover, N_2O emissions in 2010 were significantly greater in the 120 kg N ha^{-1} treatment than in the 0 and 60 kg N ha^{-1} treatments, and the amounts of NO_3^- and total inorganic N leached from the surface soils were significantly increased by fertilizer treatment. A great factor in the N_2O and NO_3^- losses was precipitation amounts and timing.

This study shows the importance of N fertilization practices during the establishment period of *M.* × *giganteus* in Illinois and monitoring the yield and environmental effects at different application rates. The N_2O emissions from *M.* × *giganteus* were greater than the IPCC default value for direct emissions (1%), and ranged from 1.1 to 2.4 % of applied N for this gas with a large global warming potential. 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. Our hypothesis was supported in that there was no increase in biomass of establishing *M.* × *giganteus* due to N fertilization, but there were increased N losses through N_2O emissions and N leaching. We will continue to study the effects of N fertilization in these plots in order to determine the yield and environmental effects of different N application rates as the *M.* × *giganteus* matures.

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