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Assessing the nitrous oxide mole fraction of soils from perennial biofuel and corn–soybean fields

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ABSTRACT

Little is known about how long-term biofuel production might alter soil nitrogen (N) gas emissions. We conducted a laboratory incubation of surface soils (0–16 cm) from perennial biofuel trial plots (established 2002) at sites in Northern (Dekalb, Mollisols), Central (Urbana, Mollisols), and Southern (Dixon Springs, Alfisols) Illinois, USA. Soils from unfertilized plots of *Miscanthus* (*Miscanthus* × *giganteus)* and switchgrass (*Panicum virgatum*) were compared to fertilized corn–soybean plots during early spring and again in mid-summer. Fresh soils were packed into jars at a bulk density of 1.2 g cm^{-3} and adjusted to a water-filled pore space of 85%. We added about 10 mg NO_3-N kg^{-1} dry soil in each sample, incubated for 24 h, and collected gas samples at 0, 1, 2, and 4 h to measure production of N_2O and N_2 using a C_2H_2 inhibition technique, which allowed calculation of the N_2O mole fraction (N_2O:(N_2O + N_2)). The mean N_2O mole fraction (MF) was significantly higher for the cropped plot (0.83 and 0.99) than that for *Miscanthus* (0.48 and 0.31) and switchgrass (0.45 and 0.22) plots at the Southern site in spring and summer, respectively. There were no significant differences in N_2O MF among treatment plots for the Central and Northern sites. Exchangeable soil nitrate concentrations best explained the N_2O MFs for all treatments in both seasons, and production of perennial biofuel feedstock crops did not exhibit an apparent influence on N_2O MFs. It appeared that soil type combined with fertilizer additions were the major factors controlling the MF of N_2O in our fields, and was much more important than the crop grown or any new soil C added.

1. Introduction

Carbon dioxide (CO_2), nitrous oxide (N_2O), and methane (CH_4) are the major greenhouse gases (GHG) driving global climate changes. Nitrous oxide is often the gas of greatest interest because it has approximately 300 times the global warming potential of CO_2 (IPCC, 2007). Nitrous oxide emission is currently the single most important ozone-depleting substance and is expected to remain the largest throughout the 21st century (Ravishankara et al., 2009). The global increase in N_2O emission is primarily due to fertilizer additions in intensive agricultural production systems (IPCC, 2007). Another source of N_2O emission is the combustion of fossil fuels. The U.S. consumes 25% of the world's total oil production, which is derived mainly from fossil fuels (Greene et al., 2004). A variety of options exist for mitigation of GHG emissions in agriculture and one of these could be substituting fossil fuels with energy produced from agricultural feedstock, i.e., biofuel crops (IPCC, 2007). Crops with less N demand, such as perennial grasses and woody species, may have more favorable climate impacts (Crutzen et al., 2008).

Miscanthus (*Miscanthus* × *giganteus)* and switchgrass (*Panicum virgatum*) are potential perennial biofuel crops, with high biomass production, even higher belowground biomass, and longer growing seasons that take advantage of solar radiation (Kahle et al., 2001; Heaton et al., 2008; Dohleman and Long, 2009). Biofuel feedstock crops can help to reduce C emissions to the atmosphere, reversing the negative impacts of row crop agriculture with excessive fertilization, and providing a substitute for declining petroleum products and increasing energy independence (Greene et al., 2004). Row crops are annual and though productive, require annual energy and financial inputs including tillage and planting, energy intensive N fertilizer, herbicides, and pesticides. As a result, ethanol from maize grain has only a small net positive C balance (Farell et al., 2006). Heaton et al. (2008) reported that *Miscanthus* alone could provide 260% more ethanol per ha than corn grain and suggested that the entire US renewable fuel goals for 2016 could be met today, without impacting US food production, simply by substituting *Miscanthus* on the land producing corn grain for ethanol.

Nitrous oxide emissions are generated by the microbial transformation of N and is often enhanced where available N exceeds

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Table 1
Physical and chemical properties of soils at the three biofuel production sites (0–20 cm).

Site name	Northern (Dekalb)	Central (Urbana)	Southern (Dixon Springs)
Location	41°50′40.65″ N 88°51′08.00″ W	40°02′33.18″ N 88°14′16.62″ W	37°27′16.05″ N 88°43′21.60″ W
Soil type/classification	El Paso silty clay loam (fine, smectitic, mesic Aquic Argiudoll)	Flanagan silt loam (fine silty, mixed, superactive, mesic Typic Endoaquoll)	Grantsburg silt loam (fine silty, mixed, active, mesic Oxyaquic Fragiudalf)
pH	6.5	6.0	6.6
Sand (%)	6	5	3
Silt (%)	63	71	78
Clay (%)	31	24	19
PAW (cm)	5.5	4.7	5.5

PAW, plant available water.
Soil pH are the average measured values from both spring and summer samples for each location, and all other information was obtained from http://websoilsurvey.nrcs.usda.gov/app/WebSoilSurvey.aspx.

plant requirements, especially under wet conditions (Smith and Conen, 2004; Oenema et al., 2005). Measurement of N_2O and N_2 during the denitrification process in soils is important in assessing the amount of N_2O production from denitrification and its potential environmental impact (Elmi et al., 2005). The N_2O mole fraction (MF), which is the molar ratio between N_2O and (N_2O+N_2), represents the relative proportion of N_2O in total N emitted from denitrification. The incubation of soils in the presence and absence of acetylene (C_2H_2) permits assay of both denitrification and N_2 fixation and provides information on the MF of N_2O (Yoshinari et al., 1977). It is clear that the lower the N_2O MF, the less greenhouse gas effect there is from denitrification.

Denitrification is highly variable and is regulated by various environmental factors including soil moisture, soil pH, N oxide concentration, and available amount of C in soils (Smith and Tiedje, 1979; Firestone and Davidson, 1989; Hutchinson and Davidson, 1993). However, little is known about how biofuel feedstock crops such as *Miscanthus* and switchgrass might alter the amount and characteristics of soil C over time, leading to differences in N gas emissions and N_2O MF. We hypothesized that an absence of tillage and fertilizer application on highly productive biofuel crops would increase the C concentration, resulting in the complete reduction of nitrate to N_2, and therefore, the N_2O MF would be lower compared to that of conventional row crop agriculture. The goal of our study was to determine the effect of cultivating perennial biofuel crops on N_2O MF. The objectives were to estimate the MF of N_2O on soils cultivated with either corn or soybean and biofuel feedstock crops and to assess the controlling factors for N gas production and N_2O MF with respect to cultivating corn–soybean and perennial biofuel feedstock crops.

2. Materials and methods

2.1. Site description

We conducted a laboratory incubation experiment of soils from three biofuel trial plots that were established in May and June of 2002 at three Agricultural Research and Education Centers located in Northern (Dekalb), Central (Urbana), and Southern (Dixon Springs) Illinois. Replicated side-by-side trials of *Miscanthus* and switchgrass were established along a latitudinal gradient. Prior to establishing the trial plots, these fields had been planted to rotations of maize (*Zea mays* L.), soybean (*Glycine max* L. *Merr.*), and wheat (*Triticum aestivum* L.). Four 10 m × 10 m plots of *Miscanthus* and switchgrass were arranged in a randomized design at each location without fertilizer additions, to examine the response of these crops following the conversion from row cropping (Heaton et al., 2009). Following five years of biofuel production, plots were split to examine N fertilization responses, but we utilized the unfertilized plots. Previous work in Europe has shown that *Miscanthus* generally does not respond to N fertilization (e.g., Himken et al., 1997; Christian et al., 2008), and it is expected that N fertilization will not be needed on fertile soils. Major soil types were Alfisols at the Southern site and Mollisols at the Central and Northern sites. Detailed information on soil series and physical/chemical properties of the study sites is given in Table 1 (Source: http://websoilsurvey.nrcs.usda.gov/app/WebSoilSurvey.aspx).

Representative cropped fields cultivated with either corn or soybean in close proximity to the biofuel trial plots and with the same soils and landscape positions were also selected for soil sampling. The cropped plot at the Northern site was cultivated with corn in both 2008 and 2009, which was fertilized with 202 kg N ha^{-1} of anhydrous ammonia in the fall of both 2007 and 2008, while the Central site was cultivated with corn in 2008 with 168 kg N ha^{-1} of anhydrous ammonia and with soybean in 2009 without fertilizer. At the Southern site, corn was cultivated in both 2008 and 2009 with 180 kg N ha^{-1} in the form of urea ammonium nitrate, side-dressed in late June 2009 due to a late planting date because of wet soil conditions.

2.2. Laboratory incubations

The experiment was conducted in early spring (March) and again during mid-summer (July) of 2009. The three plant types (unfertilized *Miscanthus*, switchgrass and fertilized cropped) were replicated four times, for a total of 12 plots per site. We developed our procedures based on the study by Bergsma et al. (2002). Four undisturbed soil cores (1.9 cm × 16 cm) from five different locations in each plot were taken and thus 20 cores were combined for one composite sample per plot. Soil samples were sieved (<4 mm) in field-moist conditions for all incubations, with subsamples oven-dried for determination of soil moisture content. All incubations were conducted the following day in the laboratory using the field-moist and sieved samples at a room temperature of ~20 °C. We packed each soil (188–204 g based on the sample moisture content) to a volume of approximately 125 mL and to a bulk density of 1.2 g cm^{-3} in 1 L Mason jars by accounting for the moisture content of the field-moist soil in weighing out the soil to fill a specific volume. Three sets of jars were prepared for each soil sample, (i) treated with C_2H_2 (to inhibit the production of N_2), (ii) without C_2H_2 (to measure N_2O production), and (iii) for analyzing soil exchangeable N concentrations. We added deionized water (with added nitrate) ranging from 5 to 20 mL (based on the sample

Table 2
Mean total C and N concentrations for incubated soils collected from biofuel trial plots and cropped fields.

Sampling and site	Carbon ($g\,kg^{-1}$)			Nitrogen ($g\,kg^{-1}$)		
	Miscanthus	Switchgrass	Cropped	*Miscanthus*	Switchgrass	Cropped
2002-Initial						
Central	40.4a	38.8a		2.95a	2.94a	
Southern	15.6a	14.1a		1.45a	1.36a	
Spring						
Northern	31.1A	33.9A	30.5A	2.75A	3.18A	2.65A
Central	43.7Aa	37.0Aa	21.2B	2.99Aa	2.87Aa	1.65B
Southern	15.8Aa	14.2Ba	13.2B	1.34Aa	1.34Aa	1.25A
Summer						
Northern	31.7A	30.5A	29.6A	2.97A	2.97A	2.65A
Central	43.4Aa	37.1Aa	22.0B	3.00Aa	2.95Aa	1.86B
Southern	16.6Aa	14.6Ba	12.9C	1.47Aa	1.42Ba	1.37B

Note: Initial values at planting in 2002 for the Central and Southern sites (Heaton, unpublished data) are also shown. Data for the northern site were not available. Season and site means for C or N with the same capital letter are not significantly different at the 0.05 level. Means by crop and site with the same lower case letter are not significantly different from the initial values at the 0.05 level.

moisture content and the required amount of soil) to each sample to reach a target volumetric water content of 85% water-filled pore space (WFPS), and to provide an exchangeable nitrate concentration of about 10 mg NO_3-N kg^{-1} dry soil. This methodology generally follows Bergsma et al. (2002), where the 85% WFPS is thought to be high enough to promote denitrification for this laboratory incubation. It is likely to occur in the field only following high rainfall events or in areas where fields are ponded during spring rains.

The Mason jars were left overnight with the tops open after adding the N solution to ensure its complete diffusion as well as to create a favorable condition for denitrification. After about 24 h, we closed the jars and 10% of the headspace air was replaced with a syringe by an equal amount of pure C_2H_2 to one set of jars for a 4 h incubation period. Fifteen milliliters of headspace gas samples was then collected at 0, 1, 2, and 4 h of incubation and was transferred to 10 mL vials. C_2H_2 (10% by volume) was added to the C_2H_2-treated jars after removal of an equivalent amount from

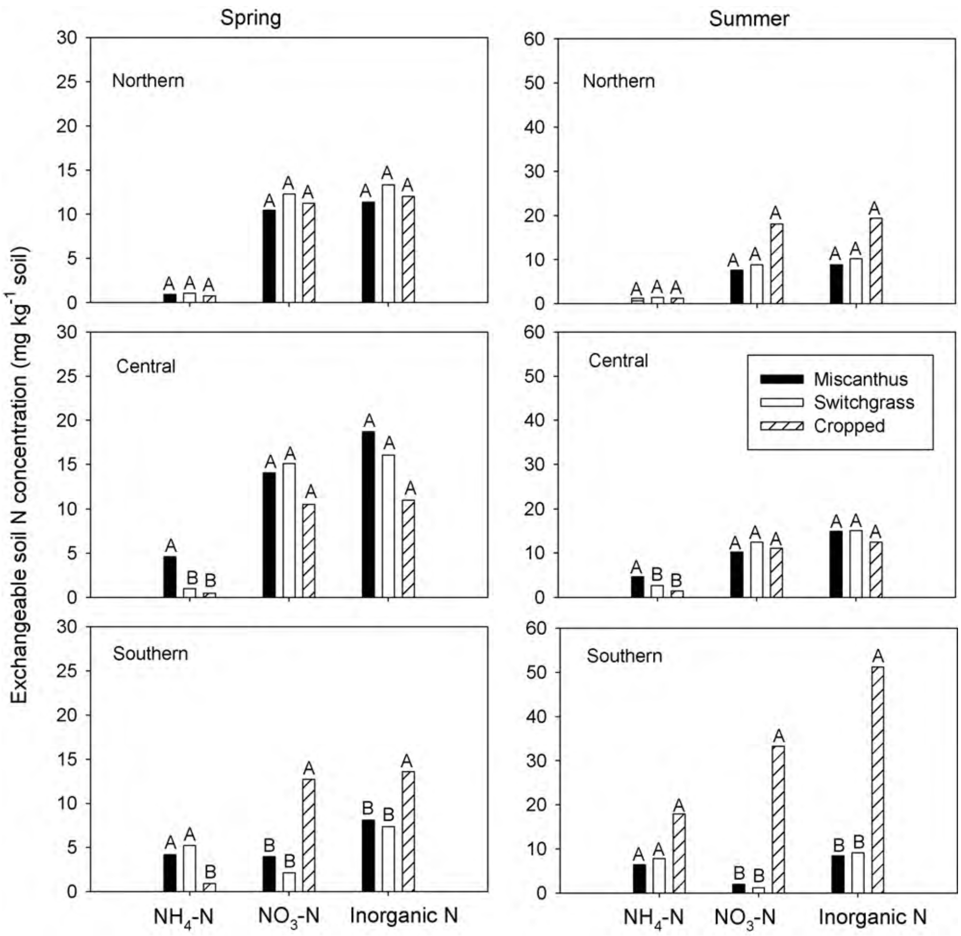

Fig. 1. Mean exchangeable N concentrations in the incubated soils collected from the biofuel trial plots and cropped fields at the Northern, Central, and Southern sites in spring and summer, 2009. Within a site and form of N, bars with the same letter are not significantly different at the 0.05 level. Note the summer *y*-axis is twice the scale of the spring for all sites.

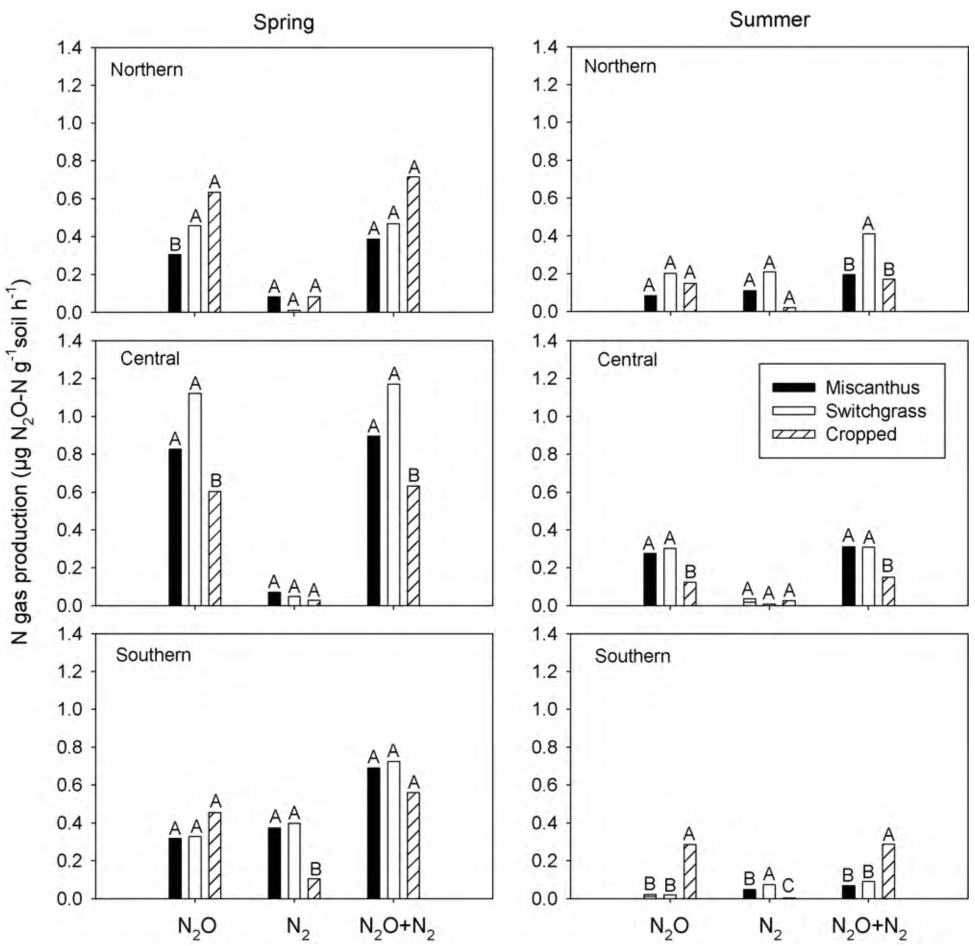

Fig. 2. Mean N gas production in incubated soils collected from the biofuel trial plots and cropped fields at the Northern, Central, and Southern sites in spring and summer, 2009. Within a site and form of N gas, bars with the same letter are not significantly different at the 0.05 level.

the headspace air and atmospheric air was added to the control jars. Three additional jars without soil were used as blanks. After 2 h of incubation, one set of jars was destructively sampled for KCl exchangeable NO_3-N and NH_4-N concentrations on a Lachat QuikChem 8000 flow injection analyzer (Lachat Instruments/Hach Company, Loveland, CO) with minimum detection limits of 0.050 and 0.005 mg N L^{-1}, respectively. Gas samples were analyzed using a Shimadzu gas chromatograph (GC-2014) with an electron capture detector. The N gas flux was calculated using regression coefficients obtained from plotting N_2O concentrations against sampling time, which was followed by calculation of the N_2O mole fraction. N_2 flux was calculated as the difference in N_2O production between C_2H_2-treated and control jars. Oven-dried soil samples were ground and analyzed for total C and N using an elemental analyzer (EAS 4010, Costech). Soil pH (1:2 soil to solution ratio) was determined in deionized water with a digital Orion pH meter (Model 250A).

2.3. Statistical analyses

Data were analyzed by the Statistical Analysis System (SAS) Package (SAS Institute, 2002). Differences in average soil exchangeable N concentrations, N gas production, total C and N concentrations and C:N ratio, and N_2O MFs were evaluated through variance analysis with the PROC GLM procedure. A multiple comparison test (least significant difference) was performed for evaluating the difference in treatments. Simple regression analysis between the measured soil variables and N gas variables was conducted with PROC REG procedure. Pearson's correlation coefficients were determined and significance was accepted at a level of probability of $p < 0.05$.

3. Results

3.1. Soil total C and N concentrations

For *Miscanthus* and switchgrass at the Central and Southern sites, no changes were observed in soil C concentrations (Table 2). A similar pattern was observed for total N. This indicates that during the seven years of growth, only *Miscanthus* trended towards an increase in surface soil C and N in these plots, but the change was not significant. In this study we compared the biofuel soils to nearby cropped fields, and for the Northern site there were no significant differences in total C and N concentrations among the treatments in either season. For the Central site for both seasons, both *Miscanthus* and switchgrass soils had significantly greater total C and N concentrations compared to the cropped field. For the Southern site, *Miscanthus* soils had significantly greater total C than the cropped field in both seasons, whereas switchgrass was only significantly greater in the summer soils.

3.2. Exchangeable soil N concentrations

At the Southern site, mean soil exchangeable NO_3-N concentrations were significantly greater for the cropped plot compared to the *Miscanthus* and switchgrass plots in both spring and summer (Fig. 1). No significant difference was found in overall soil inor-

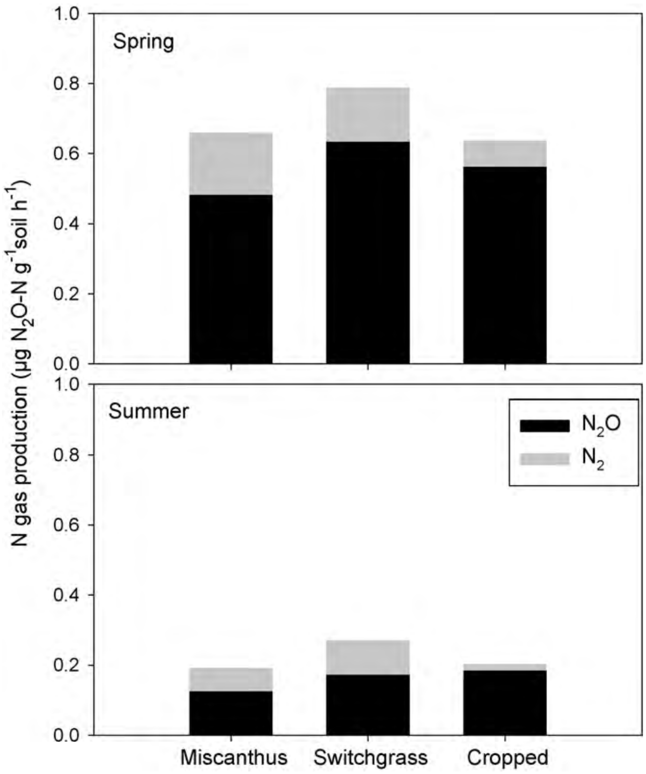

Fig. 3. Combined N gas production in incubated soils collected from different biofuel trial plots and cropped fields in spring and summer, 2009.

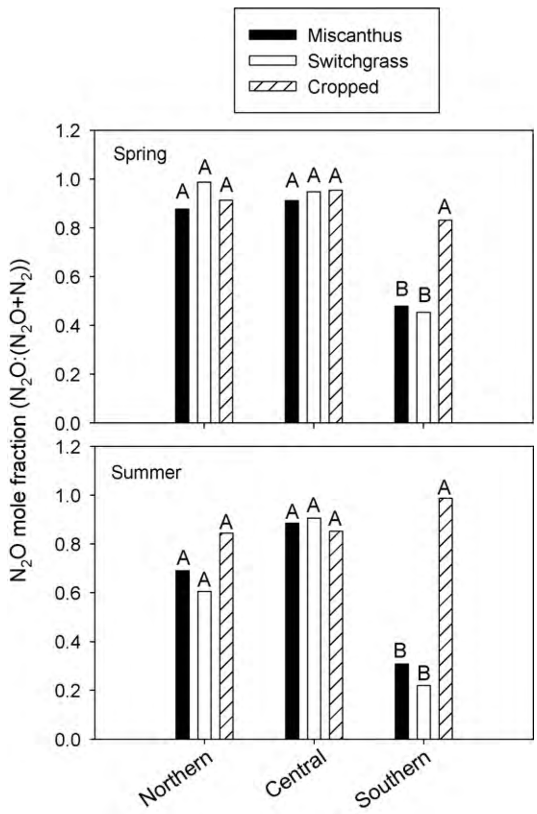

Fig. 4. Mean N_2O mole fractions from incubated soils collected from biofuel trial plots and cropped fields at the Northern, Central, and Southern sites in spring and summer, 2009. Within a site and season, bars with the same letter are not significantly different at the 0.05 level.

ganic N concentrations among the treatment plots at the Central and Northern sites in both seasons. At the Central site, *Miscanthus* soils had greater NH_4-N concentrations compared to the fertilized cropped plot in spring and summer. At the Northern site, no consistent pattern was observed in N concentrations among the treatment plots in both seasons (Fig. 1).

3.3. Nitrogen gas production

There were significant differences in mean N_2 production among the treatment plots only at the Southern site, and the cropped plot had the lowest N_2 production in both seasons (Fig. 2). In spring samples, although there was no consistent pattern in mean N_2O production, soils from the cropped plots produced the greatest N_2O at the Northern and Southern sites, but the mean N_2O production was lowest in the cropped plot compared to switchgrass and *Miscanthus* soils at the Central site (Fig. 2). In the summer, although N_2O production was significantly greater at the cropped plot soil compared to *Miscanthus* and switchgrass at the Southern site, no consistent pattern was found at the Northern and Central sites. However, the cropped plots had lower N_2O production than the *Miscanthus* or switchgrass plot soils at the Central site. This result can be attributed to the fact that soybean was cultivated at the Central site in 2009 when no fertilizer was applied. Combining all three sites, although there was no significant difference in N gas production among the treatment plots, switchgrass soils tended to have higher total denitrification compared to *Miscanthus* and corn plot soils (Fig. 3). Total denitrification measured in soils collected during the summer was two to three times lower than that in the spring; this was likely due to depletion of nitrate by crop uptake.

3.4. Nitrous oxide mole fraction

As noted above, only at the Southern site was the N_2O MF in spring significantly greater in corn plots (0.83) compared to *Miscanthus* (0.48) and switchgrass (0.45) plots (Fig. 4). There was no consistent pattern and significant difference in treatment plots at the Central and Northern sites. In the summer incubations, the N_2O MF was greatest in corn plots (0.99) followed by *Miscanthus* (0.31) and switchgrass (0.22) at the Southern site. Although corn plots had the highest N_2O MF value (0.84) at the Northern site, there was also neither consistent pattern nor significant difference in treatment plots at the Central and Northern sites in the summer (Fig. 4).

3.5. Controlling factors for N gas production and N_2O mole fractions

We performed a correlation analysis using soil pH, exchangeable soil NH_4-N, NO_3-N, and inorganic N concentrations, total N and C concentrations as explanatory variables and N_2O MFs and the production of N_2 and N_2O as the dependent variables (Table 3). Only exchangeable soil nitrate concentration had significant positive correlations with N_2O MF in both spring ($r = 0.92$, $p < 0.001$) and summer ($r = 0.76$, $p < 0.05$). Other variables such as exchangeable soil concentrations of NH_4-N, inorganic N, soil total N and C concentrations also could explain the variation in N_2O MF, but only in the spring. Furthermore, soil pH was negatively correlated with the N_2O MF and N_2O production, but the correlations were significant only in the summer samples ($r = 0.89$, $p < 0.01$ and $r = 0.70$ $p < 0.05$, respectively). The exchangeable soil NO_3-N and inorganic N concentrations had significant positive correlation with N_2O production in the spring ($r = 0.70$, $p < 0.05$ and $r = 0.76$, $p < 0.05$,

Table 3
Pearson's correlation coefficients (r) of measured soil pH and concentrations of N and C with N gas production and N_2O mole fractions over all samples.

Measured variables	N_2O		N_2		N_2O mole fraction	
	Spring	Summer	Spring	Summer	Spring	Summer
Soil pH	−0.66	−0.70*	0.59	0.46	−0.58	−0.89**
Exchangeable soil NH_4-N concentration	0.20	0.08	0.76*	0.33	−0.75*	0.04
Exchangeable soil NO_3-N concentration	0.70*	0.63	−0.92***	−0.38	0.92***	0.76*
Exchangeable soil inorganic N concentration	0.76*	0.48	−0.72*	−0.41	0.73*	0.56
Soil total N concentration	0.49	0.52	−0.67	0.34	0.70*	0.40
Soil total C concentration	0.65	0.53	−0.64	0.12	0.67*	0.45

* Significance at 0.05 level.
** Significance at 0.01 level.
*** Significance at 0.001 level.

respectively). However, there was no correlation between these variables in the summer. Interestingly, there was a high significant inverse correlation between the exchangeable NO_3-N concentration and N_2 production in the spring ($r = -0.92$, $p < 0.001$) but the correlation was not significant in the summer. Contrary to our expectation, total soil C concentration did not exhibit an apparent influence on N_2O MF.

4. Discussion

Although exchangeable nitrate was never totally depleted in any treatment, soils were quite variable in exchangeable nitrate concentrations even with our addition of 10 mg N kg^{-1}. Fertilized cropped plots had the highest concentrations of nitrate at the Southern site (Fig. 1). It is difficult to explain the low nitrate concentrations at the Southern site in the biofuel plots during the summer (considering we added nitrate and the denitrification rate we measured), but other aspects of the N cycle such as immobilization and mineralization may have been affecting concentrations here and in other treatments.

We had hypothesized that the cropped plot would produce the highest N_2O compared to biofuel feedstock crops in each of the three sites. However, this result occurred only at the Southern site in both seasons and at the Northern site in spring. Since N_2O can be formed during oxidation of NH_4 and during reduction of NO_3, higher N_2O production in biofuel soils compared to that in cropped soil at the Central site were attributed to the higher exchangeable soil nitrate and ammonium concentrations.

Given the fact that perennial biofuel feedstock crops produce remarkably high biomass (Heaton et al., 2008; Dohleman and Long, 2009), we did expect that the quality of some of the surface soil C would have also changed over time due to biofuel production at all sites in response to the belowground C inputs. Furthermore, soil nitrate concentrations were related to N_2O production only in the spring samples and soil total C did not have an apparent influence on N gas production in both seasons (Table 3). Availability of soil C is reported to increase the amount of denitrification while either decreasing (Weier et al., 1993) or increasing (Dendooren et al., 1996; Mathieu et al., 2006) the N_2O MFs. In our study, the N_2O MF increased with an increase in soil C in both spring and summer soils while the relationship in summer was not significant (Table 3). These results suggest that no broad scale (i.e., across all sites) change occurred in soil organic C quality that greatly affected the N_2O MF, even following seven years of perennial biofuel production.

Bergsma et al. (2002) conducted a similar denitrification experiment with different soil moisture histories. They added nitrate, glucose, and deionized water to soil samples for a target WFPS of 85% at a bulk density of 1.2 g cm^{-3} just before the incubation (short-wet) and 48 h before the incubation (long-wet). They incubated the samples for 24 h with or without C_2H_2, and reported that the N_2O MFs did not vary for long-wet (0.34) and short-wet (0.33) treatments in the successional system, while N_2O MFs were 0.36 for the long-wet and 0.90 for the short-wet treatment in the cropped system. Thus a much smaller proportion of N_2O was produced when the soil had been wet for 72 h including the incubation period. In our experiment, we added water and nitrate 24 h prior to the incubation and the WFPS was maintained at a similar percentage, i.e., 85%. The N_2O MFs for cropped soils ranged from 0.83 to as high as 0.99. It seems that keeping the high moisture condition of our incubated soils one or two more days could have resulted in decreased MF values by allowing further reduction of nitrate to N_2 in a prolonged anaerobic condition.

Miller et al. (2008) found that the increase in nitrate within each rate of glucose addition increased the N_2O MF, and concluded that the relative availability of C and nitrate influenced both the amount of denitrification and the N_2O MF. Stevens et al. (1998) observed that the MF of N_2O declined from 0.8 to 0.05, depending on the amount of C content (low N_2O MF in high C content) and reported that N_2O could have been produced simultaneously by nitrification and denitrification, so the production of N_2O from nitrification could have affected the measured MF of N_2O. In our study, the moisture content was constant at 85% WFPS for all samples. Furthermore, we did not amend the soil samples with additional C as we wanted to examine how surface soil C affected the MF following seven years of biofuel production. Ciarlo et al. (2008) found that N fertilization decreased soil pH values, which were inversely correlated with the N_2O MF. Our result is consistent with their findings in that both N_2O production and MF of N_2O were inversely correlated with the soil pH (Table 3) in both seasons (although only significant in the summer). Soil acidity and the relative abundance of electron donors (soil organic C) and acceptors (primarily O_2, NO_3 and sulfate) could also affect the relative proportion of N_2 and N_2O emission from nitrification and denitrification (Firestone, 1982; Firestone and Davidson, 1989).

5. Conclusions

We could not confirm our hypothesis that the N_2O MF would be altered by increased soil C due to biofuel production at any of our sites using our laboratory incubation assay with soil samples from two seasons. At the Southern site, the N_2O MFs were lower in biofuel plots in both seasons compared to that at the cropped plots with tillage and fertilizer application. However, the response at the Southern site seemed to be due to the increased nitrate concentrations in the cropped soil rather than a change in C from the biofuel production altering the N_2O MF, even with our added nitrate. For the Central and Northern site, there was little effect of biofuel production on the N_2O MF of incubated soils compared to the cropped control. Because there was little change in total soil C from biofuel feedstock production, the availability of nitrate for microbial reduction seemed to have the greatest effect on the N_2O MF more than other factors. Therefore, soil type and the addition of fertilizer increasing soil nitrate concentrations appeared to be the major factors controlling the MF of N_2O in response to biofuel production. Overall, our results from three sites and two seasons show a varied response of the N_2O MF dependent primarily on soil type and fertilization (affecting soil nitrate concentrations), with no measurable effect of biofuel crop production, using our short-term, snapshot assay. Certainly long-term field measurements of N gas fluxes in response to biofuel production are needed to confirm these results and therefore our results are limited to the conditions evaluated.

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